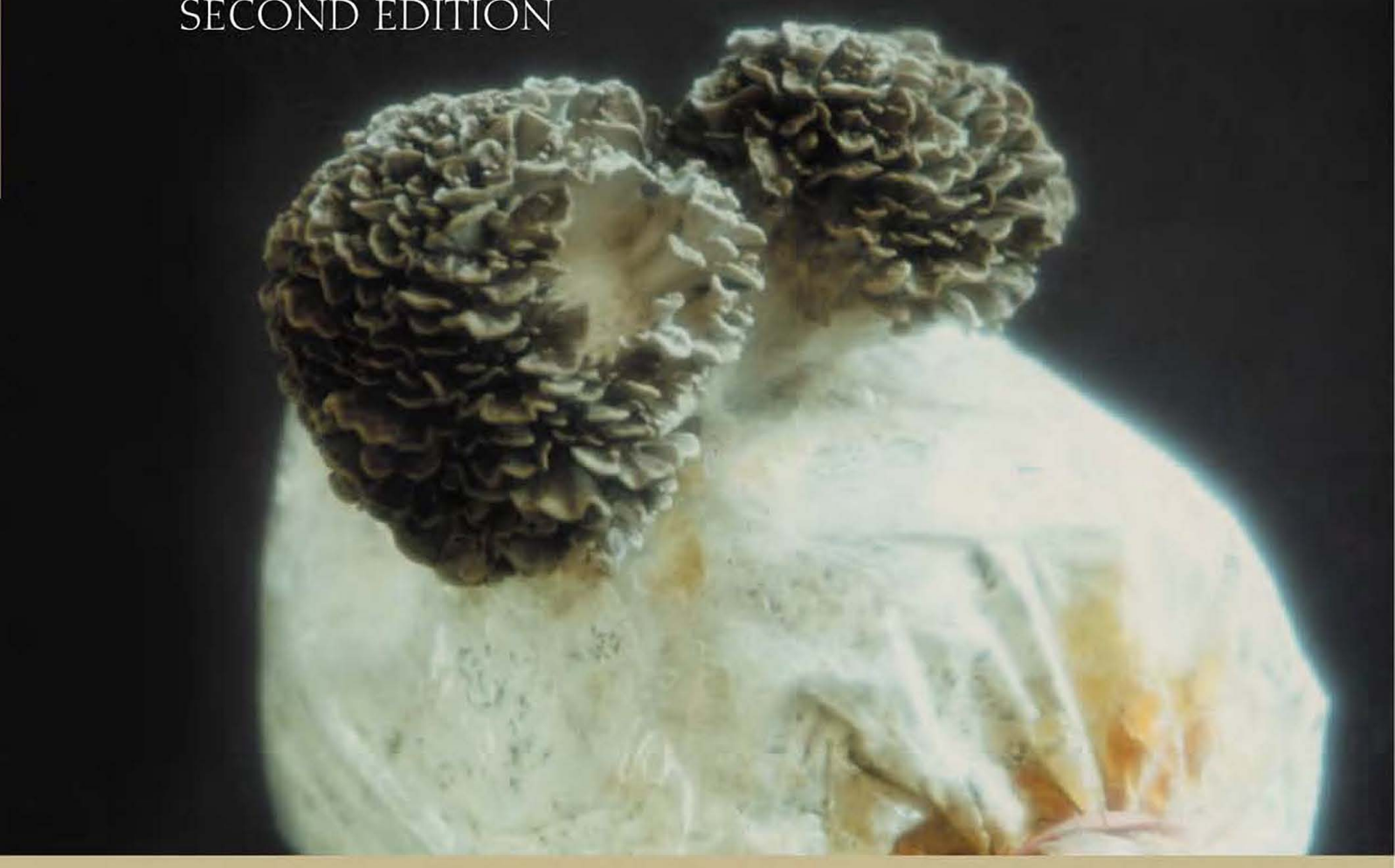
MUSHROOMS

Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact

SECOND EDITION



SHU-TING CHANG AND PHILIP G. MILES

CRC PRESS

کیدارو

فناوری سلامت

Keydaru fanavari salamat

DURASTHUM

محصولاتی طبیعی جهت افزایش توان جسمانی و روانی افراد جامعه

KEYSAN

traditional medicine

سبد محصولات بر پایه پزشکی ایرانی (طب سنتی)
در راستای درمان بیماران

keydaru.ir | keydaru.co

دوراستهوم
DURASTHUM

HEALTHY AGING + LONGEVITY

Total polysacharides per capsule	75µg/mg
Ganoderma lucidum (full spectrum)	220mg
Ganoderma lucidum (water extract)	30 mg

MANAGEMENT OF POLYCYSTIC OVARY SYNDROME (PCOS)

Trigonella foenum-graecum seed extract (standardized to %3.57 trigonelline).	333 mg

کیدارو
فناوری سلامت
Keydaru fanavari salamat

keydaru.ir | keydaru.co

MUSHROOMS

Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact

SECOND EDITION

MUSHROOMS

Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact

SECOND EDITION

SHU-TING CHANG
Department of Biology
The Chinese University of Hong Kong

PHILIP G. MILES
Biological Sciences Department
State University of New York at Buffalo

CRC PRESS

Boca Raton London New York Washington, D.C.

Library of Congress Cataloging-in-Publication Data

Chang, S. T. (Shu-ting), 1930–
Mushrooms : cultivation, nutritional value, medicinal effect, and environmental impact / Shu-Ting Chang and Philip G. Miles. — 2nd ed.
p. cm.
Rev. ed. of: Edible mushrooms and their cultivation / authors, Shu-ting Chang and Philip G. Miles. c1989.
Includes bibliographical references (p.).
ISBN 0-8493-1043-1
1. Mushrooms, Edible. 2. Mushroom culture. I. Miles, Philip G. II. Chang, S. T. (Shu-ting), 1930– Edible mushrooms and their cultivation. III. Title.

SB353.C455 2004
635'.8—dc22 2003065360

International Standard Book Number 0-8493-1043-1
Library of Congress Card Number 2003065360
Printed in the United States of America 1 2 3 4 5 6 7 8 9 0
Printed on acid-free paper

Acknowledgments

From the early planning stages to the final writing, we have received invaluable suggestions and meticulous editorial assistance from Eleanor A. Miles. For preparation of the final version of the manuscript and for her constant encouragement, the authors are also extremely grateful. Throughout the entire endeavor, Curtis Miles generously provided the authors with her computer expertise in preparation of all tables and solving all technical problems associated with the preparation of the manuscript. In addition, she assumed major responsibility for draft preparation when illnesses prevented P.G.M. and E.A.M. from devoting sufficient time to the project. We also thank Patrick Mok of the Chinese University of Hong Kong for his assistance in typing a working copy of an early manuscript, among numerous other services. Important research assistance was received from University at Buffalo, State University of New York students Phillip Barber, Steven Marshall, and Bud Miles.

We are deeply indebted to Professor Wei-Li Lee who generously contributed financial assistance to P.G.M., which was so helpful in providing funds toward preparation expenses and research for the book.

We extend our wholehearted appreciation and gratitude to senior editor John Sulzycki, project coordinator Pat Roberson, project editor Christine Andreasen, typesetter Pamela Morrell, proofreader Steve Menke, and cover designer Elise Weinger of CRC Press, who have been very supportive and extremely valuable in bringing this book to fruition.

Shu-Ting Chang
Philip G. Miles

Preface

The mushroom is the fruiting body of the macrofungi. Approximately 14,000 described species of fungi produce fruiting bodies that are large enough to be considered mushrooms using our definition, which states that "the mushroom is a macrofungus with a distinctive fruiting body that can be either epigeous (aboveground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand." According to this definition, in contrast to other definitions, mushrooms can be Ascomycetes, grow underground, have a nonfleshy texture, and need not be edible. In nature, the role of the mushroom is to produce reproductive spores, to function in the protection of the tissues in which spores are formed, and to provide for spore dissemination. Current studies estimate that 1.5 million species of fungi may actually exist and that there may be 140,000 species that produce fruiting bodies of sufficient size and structure to be considered macrofungi, thus fulfilling our definition of a mushroom.

With a group of this dimension, it is to be expected that there will be great structural variation in mushrooms. Another important feature is that some species are poisonous, an aspect that is treated more extensively in this edition. The edibility of mushrooms has been known to humans since time immemorial, but the intentional cultivation of mushrooms had its beginning in China, around A.D. 600, when *Auricularia auricula* was first cultivated on logs. Today about 7000 species possess varying degrees of edibility, and more than 3000 species may be considered prime edible species, of which only 200 species have been experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, and about 10 species cultivated on an industrial scale. In addition, 2000 species have been suggested to possess medicinal properties. Such medicinal mushrooms produce substances that can improve biological functions and thus the health of the consumer. These products have been called by various names, including dietary supplements, functional foods, phytochemicals, nutraceuticals, and nutriceuticals. Industries providing these substances have expanded in the United States, where the supplement sales were valued at U.S. $3.3 billion in 1990. These sales have increased steadily, and in 2000 there was an estimated value of U.S. $14 billion.

The use of lignocellulosic materials, which provide a sustainable biomass resource for the growth of edible and medicinal mushrooms, is of great environmental importance by recycling organic waste, thereby playing a role in controlling problems of pollution.

As is true for revisions of most scientific books, the main motivations for the second edition of *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact* are inclusion of material and references that have appeared since the publication of the preceding edition and consideration of the comments and suggestions of readers. The current edition includes much new material and a large number of new references. The format and organization are similar to those used in the earlier edition. Both editions provide a treatment of the following topics: overview of mushroom biology and mushroom science; nutritional attributes; medicinal values; overview of biology of fungi; substrate and mycelial growth; sexuality and the genetics of Basidiomycetes; mushroom formation (effects of environmental, nutritional, and chemical factors, as well as genetic factors and breeding); culture preservation; and world production of edible mushrooms. In addition, the chapters on specific edible mushrooms (*Agaricus, Lentinula, Volvariella, Flammulina, Pholiota, Pleurotus, Tremella, Dictyophora, Auricularia, Hericium*) have been enlarged with the inclusion of more recent research findings. Chapters on the medicinal mushrooms *Ganoderma lucidum, Agaricus blazei,* and *Grifola frondosa* have been added, as well as a chapter on the effects of pests and diseases on mushroom cultivation. Finally, the chapter on technology

and mushrooms has been expanded to emphasize the environmental impact of mushrooms and mushroom cultivation.

Mushroom growing processes involve living organisms, and thus it is subject to the numerous interactions that living organisms have with their environment and with one another. Mushroom cultivation methods must be modified and appropriate strains developed for use in environmentally different situations. Thus, we have stressed that it is essential for a grower to have knowledge of the basic principles as well as practical cultivation techniques. A grower not only must know the "how" but also must understand the "why" of the individual steps of the complex events that constitute mushroom cultivation. The fact that there are mushroom species that can be grown in any populated area of the world on waste materials that are available in abundance in both urban and rural areas indicates the great potential for mushrooms to supplement, in a flavorful and nutritious manner, the protein-deficient diet of people everywhere, but especially in developing countries.

Much of the information concerning mushroom cultivation has come from China where the mushroom industry has advanced more rapidly than in any other country in the past two decades. It is hoped that the information and techniques described in this edition will be useful for other developing countries where a good source of protein is urgently needed. Emphasis has been placed on direct and simple methodologies that can be useful in developing countries, rather than on extensively mechanized cultivation procedures. Frequent interpretations have been made by the authors regarding the scientific rationale for the procedures developed.

The use of mushrooms for medicinal purposes continues to expand, and it is hoped that as technology advances for the production of medicinal products, there will be increased activity in medical research and clinical studies to examine the validity of many claims that have been made for various medicinal and tonic uses of these products. Anecdotal accounts are interesting and may be useful, but scientific experimentation is essential. This book is written for growers of edible and medicinal mushrooms and also for university students and researchers of the following specialties: environmentalists concerned with solid state fermentation for conversion of waste materials to food and concomitantly with the avoidance of pollution commonly associated with disposal of wastes; microbiologists interested in thermophilic organisms, as these are important in the composting process; geneticists concerned with strain improvement, especially the breeding of strains of species of edible and medicinal mushrooms that will be suitable for different environmental conditions; horticulturalists interested in the development of efficient cultivation practices; nutritionists involved in the assay and evaluation of mushroom nutrients; pathologists studying mushroom diseases; and medical doctors concerned with the nutritional value of mushrooms as well as with the compounds produced by certain mushrooms that have demonstrated potential in the treatment of various diseases.

The aspects emphasized in this book include cultivation, nutritional value, medicinal effects, and the environmental impact of mushrooms.

As with the preceding edition, this book is not intended to be an encyclopedic review; instead, it is presented with an emphasis on worldwide trends and developments in mushroom biology from an international perspective.

The Authors

Shu-Ting Chang, Ph.D., received the B.Sc. degree in 1953 from National Taiwan University, and he earned an M.S. degree in 1958 and a Ph.D. degree in 1960 from the University of Wisconsin. He was then appointed to the position of Assistant Lecturer in the Biology Department of The Chinese University of Hong Kong, became Lecturer in 1961, Senior Lecturer in 1970, Reader in 1974, Professor in 1978, and Emeritus Professor in 1995. Dr. Chang was Chairman of the Department of Biology from 1983 to 1994. He was Dean of the Faculty of Science from 1975 to 1977 and Director of Student Affairs from 1979 to 1981. Dr. Chang was a Visiting Postdoctoral Fellow at Harvard University in 1966, was a Visiting Fellow at Tokyo University in 1969, and a Visiting Fellow at Australia National University and the Commonwealth Scientific and Industrial Research Organization during 1972–1973 and 1978–1979.

Dr. Chang is a member of the American Association for the Advancement of Science and the Mycological Society of America. He served as Chairman of the Hong Kong Research Council in Biological Education from 1987 to 1989 and was President of the Hong Kong Society of Microbiology from 1982 to 1984. He was President of the International Mushroom Society for the Tropics from 1981 to 1995 and was also Editor-in-Chief of *The Mushroom Journal for the Tropics* during that period. He also was a member of the Editorial Board of *MIRCEN Journal of Applied Microbiology and Biotechnology.* Dr. Chang served as the Executive Secretary of the Headquarters of the UNESCO Regional Network of Microbiology in Southeast Asia from 1984 to 1987, and was a member of the Executive Board of International Union of Microbiological Societies from 1990 to 1994. He is Director of the Center for International Services to Mushroom Biotechnology under UNIDO and an editor of the *International Journal of Medicinal Mushrooms.* He has authored or co-authored six books, co-edited seven books, and authored or co-authored 180 articles in scientific journals.

Dr. Chang is a Fellow of the World Academy of Art and Science, the Institute of Biotechnology, and the World Academy of Productivity Science. He has also been named an Honorary Life Member of the British Mycological Society, and of the International Society for Mushroom Science, U.K. He received the International Cooperation Award for Light Industry in China in 1990 and the Science and Technology Corporation Award from The People's Government, Quingyuan, China in 1994. In 1994, he was named an Officer of the Most Excellent Order of the British Empire (OBE).

Dr. Chang's major research interests are in the areas of fungal genetics, mushroom germplasm conservation, the biology and cultivation of edible mushrooms, and medicinal mushrooms and mushroom nutriceuticals.

Philip G. Miles, Ph.D., received the B.A. degree in 1948 from Yale University with a major in botany. In 1953, he received the Ph.D. degree from Indiana University, with a major in mycology and minors in bacteriology and general botany. He then held appointments as a Research Associate at the University of Chicago and as a Research Fellow at Harvard University with Professor John R. Raper. In 1956, Dr. Miles joined the Biology Department of the University of Buffalo (now the University at Buffalo, State University of New York) as an Assistant Professor, becoming an Associate Professor in 1961, Professor in 1970, and Emeritus Professor in 2002. He served the Biology Department as Co-chairman (1968 to 1969), Chairman (1972 to 1974), and Director of the Division of Biology (1974 to 1976). Dr. Miles also held appointments as Assistant Professor at the Harvard University Summer School in 1958, 1960, and 1962. While on sabbatical leave, Dr. Miles held the following research and teaching appointments: 1963 to 1964, Fulbright Research

Scholar in Japan; 1970 to 1971, Long-Term Visiting Scientist at National Taiwan University and the Institute of Botany, Academia Sinica, under the National Science Foundation–National Science Council of China Cooperative Science Program; 1977 to 1978, Invited Scientist at the Tottori (Japan) Mycological Institute and Visiting Professor at National Taiwan University and The Chinese University of Hong Kong; 1985 to 1986, Exchange Scholar (SUNYAB–Beijing Municipal University Scholar Exchange Program) in Beijing, China, and Honorary Visiting Professor at The Chinese University of Hong Kong.

Dr. Miles is a member of numerous scientific societies including the American Association for the Advancement of Science, the Botanical Society of America, the Genetics Society of America, the Mycological Society of America, the World Society of Mushroom Biology and Mushroom Products, and the honorary society Sigma Xi. He has served on the editorial boards of numerous journals and was the first President of the World Society for Mushroom Biology and Mushroom Products. In 1998, Dr. Miles received an Excellence in Teaching Award from the Chancellor of the State University of New York.

Dr. Miles' research interests have been in the areas of genetics and physiology of sexual mechanisms and morphogenesis of Basidiomycetes, and for these studies he has been the recipient of grants from the National Science Foundation and the National Institutes of Health. Earlier studies were primarily with the experimental organism *Schizophyllum commune;* more recent publications are results of studies of edible mushrooms. He has directed the research of many undergraduate and graduate students, including nine for the Ph.D. degree. He is co-editor of *Genetics and Morphogenesis in the Basidiomycetes* (Academic Press, 1978), and *Genetics and Breeding of Edible Mushrooms* (Gordon & Breach Science Publishers, 1993). Dr. Miles is co-author of *Edible Mushrooms and Their Cultivation* (CRC Press, 1989), and *Mushroom Biology — Concise Basics and Current Developments* (World Scientific Press, 1997).

Contents

1 Overview

I. INTRODUCTION

Fungi have been known from the fossil record as far back in time as the Silurian period, 408 to 438 million years ago — in the Paleozoic era. Fungal diversity had increased by the Pennsylvanian period (286 to 320 million years ago) and included Basidiomycetes and Ascomycetes with some fruiting bodies.[1] That is, mushrooms have been part of the fungal diversity for around 300 million years. Prehistoric humans probably used mushrooms collected in the wild as food and possibly for medicinal purposes. The early civilizations of the Greeks, Egyptians, Romans, Chinese, and Mexicans appreciated mushrooms as a delicacy, knew something about their therapeutic value, and often used them in religious ceremonies. With the widespread intentional cultivation of plants for food, it was inevitable that this choice source of food, the mushroom, would eventually be cultivated and not simply be picked in the wild. However, mushroom cultivation did not come into existence until A.D. 600 when *Auricularia auricula* was first cultivated in China on wood logs. Other wood rotting mushrooms, such as *Flammulina velutipes* (A.D. 800) and *Lentinula edodes* (A.D. 1000), were grown in a similar manner,[15] but the biggest advance in mushroom cultivation came in France about 1600 when *Agaricus bisporus* was cultivated upon a composted substrate. In the Western world, *A. bisporus*, commonly known as champignon or the button mushroom, increased steadily in popularity from that early beginning and is today the mushroom that is produced in the greatest quantity. In the past few decades, however, mushroom species (e.g., *L. edodes* and *Pleurotus* spp.) that have long been popular in Asia and are produced there in large numbers have made and are continuing to make inroads into Western markets. The Shiitake mushroom, *L. edodes*, is the second most important mushroom, ranking just behind *A. bisporus*, and in 1999 the total world production of *L. edodes* was estimated to be very close to that of the button mushroom. It has been estimated that *L. edodes* may become the most popular mushroom grown in the world by 2010.[9,37]

Mushrooms not only provide a nutritious, protein-rich food, but some species also produce medicinally effective products. Cultivated mushrooms are now an important agricultural product worldwide (Figure 1.1). In 1997, the total world production of edible and medicinal mushrooms was estimated to exceed 6 million metric tons, with a value of about U.S. $26 to 30 billion. The bioconversion of lignocellulosic biomass by the mushroom industry to food and useful products is already a significant contribution to the management of agricultural and industrial wastes at regional and national levels. Predictions are that this contribution will continue to increase and will generate a nongreen revolution.[7]

II. WHAT ARE MUSHROOMS?

Historically, mushrooms were classified among the so-called lower plants in the Division Thallophyta by Linnaeus. This was largely due to the relatively simple, anatomically uncomplicated structural attributes (lack of true roots, true stems, true leaves, true flowers, and true seeds). The presence of a cell wall related them to plants rather than to animals. Modern studies have established that mushroom biota, together with other fungi, have features of their own, which are sufficiently and significantly distinct to place them in a separate fungal kingdom, the Kingdom Myceteae. The fungi differ from the plant and animal kingdoms by their possession of a cell wall that is different

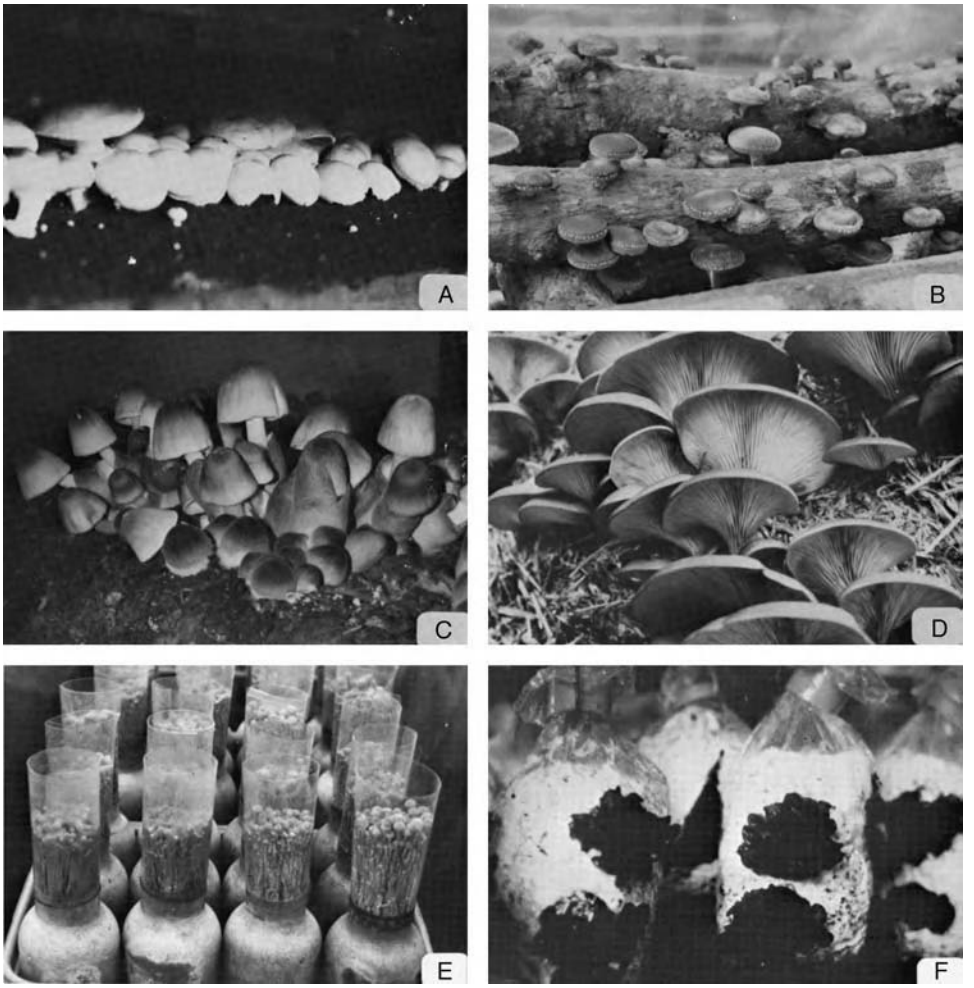


FIGURE 1.1 Cultivated mushrooms responsible for most of the world's production. (A) *Agaricus bisporus*, growing on substrate shelves in mushroom house; (B) *Lentinula edodes*, growing on logs; (C) *Volvariella volvacea*, growing on cotton waste compost in mushroom house; (D) *Pleurotus sajor-caju*, growing on compost made up of mixed paddy straw and cotton waste; (E) *Flammulina velutipes*, growing on sawdust compost in bottles; and (F) *Auricularia auricula*, growing on sawdust and cottonseed hull mixed substrate in plastic bags.

in composition from that of plants and a mode of nutrition that is heterotrophic but, unlike animals, is absorptive (osmotrophic) rather than digestive.

A. Definition

The word mushroom has been used in a variety of ways at different times and in different countries. A broad use of the term mushroom embraces all larger fungi, or all fungi with stalks and caps, or all large fleshy fungi. A more restricted use includes just those larger fungi that are edible and/or of medicinal value. The most extreme use of the term mushroom is its reference to just the edible species of *Agaricus*.[25] In this book the term **mushroom** is broadly defined as follows: "a mushroom is a macrofungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (under ground) and large enough to be seen with the naked eye and to be picked by hand."[16] According to this definition, mushrooms need not be Basidiomycetes, nor aerial, nor fleshy, nor edible. Mushrooms can be Ascomycetes, grow underground, have a nonfleshy texture, and need

FIGURE 1.2 (Color figure follows p. 232.) *Lentinula edodes*, showing pileus and stipe only.

not be edible. We believe that this definition has merit in establishing uniformity of terminology at a time when the number of cultivated species is increasing, when production of established cultivated mushrooms continues to show a steady expansion, and when an increasing number of countries and people are engaged in mushroom cultivation as an agricultural or industrial technology.

B. Characteristics of Mushrooms

The most common type of mushroom is umbrella shaped with pileus (cap) and stipe (stem), e.g., *Lentinula edodes* (Figure 1.2), and some species additionally have an annulus (ring), e.g., *Agaricus bisporus* (Figure 1.3), or a volva (cup), e.g., *Volvariella volvacea* (Figure 1.4), or have both, e.g., *Amanita phalloides* (Figure 1.5). Additionally, some mushrooms are in the form of pliable cups, and others are round like golf balls. Some are in the shape of small clubs; some resemble coral; others are yellow or orange jellylike globs; and some even resemble the human ear. In fact, there is a countless variety of forms. The structure that we call a mushroom is in reality only the fruiting body of the fungus. The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cordlike strands that branch out through the soil, compost, wood log or other lignocellulosic material on which the fungus is growing. After a period of growth, and under favorable conditions, the established (matured) mycelium produces the fruiting structure, which we call the mushroom.

FIGURE 1.3 (Color figure follows p. 232.) *Agaricus bisporus*, showing pileus, gills, stipe, and annulus.

FIGURE 1.4 (Color figure follows p. 232.) *Volvariella volvacea*, showing pileus, gills, stipe, and volva.

FIGURE 1.5 *Amanita phalloides* (extremely poisonous), showing pileus, stipe, annulus, and volva. The skinlike ring (annulus) may disappear with age. The saclike volva is thin, rather fragile, and is usually buried in the ground.

C. Categories of Mushrooms

Mushrooms can be roughly divided into four categories: (1) those that are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus*; (2) mushrooms that are considered to have medicinal applications are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; (3) those that are proved to be or suspected of being poisonous are named poisonous mushrooms, e.g., *Amanita phalloides*; (4) those in a miscellaneous category, which includes a large number of mushrooms whose properties remain less well defined. These may tentatively be grouped together as "other mushrooms." Certainly, this approach of classifying mushrooms is not absolute. Many kinds of mushrooms are not only edible, but also possess tonic and medicinal qualities. The above

categories of mushrooms have been included in *Ainsworth & Bisby's Dictionary of the Fungi,* 8th edition.[27] This grouping certainly is not a perfect one, but it has been useful for estimating numbers of mushroom species.[25] It should be noted that **toadstool** has been used frequently to refer to poisonous mushrooms. Scientifically, the term has no meaning at all. It is suggested that the term toadstool be dropped altogether in order to avoid confusion and to use edible, medicinal, and poisonous mushrooms as described above.

D. Poisonous Mushrooms

Because there is no known test by which to tell if a mushroom is edible or not, a mushroom should never be eaten unless it has been accurately identified and the edibility of the species is known. Even though poisonous mushrooms represent less than 1% of the world's known mushrooms, we cannot ignore the existence of the relatively few dangerous and sometimes fatal species. Mushrooms must be identified by a competent mycological authority. Therefore, if one is not absolutely sure whether a given mushroom is edible or otherwise, it should not be tasted, and the unidentified mushroom should be left alone.

The toxins contained in various species are very different in chemical composition, and thus the effects of poisoning differ considerably according to the species involved. In any case, suspected mushroom poisoning should never be regarded lightly and medical assistance should be sought at once. The following summary of mushroom poisoning is taken from the account by Shepherd and Totterdell.[38]

1. *Amanita*-Type Poisoning

Unquestionably, the *Amanita phalloides* group causes the most dangerous type of mushroom poisoning. The toxins involved belong to the phallotoxin and amatoxin complexes. The phallotoxin phalloidin binds specifically to actin. While the phallotoxins are not active following ingestion, although they are potent when injected intravenously, they have proved useful in experimental studies. In such studies phalloidin, binding to actin, is coupled with fluorescent groups. By this means actin can be localized in the cells. It is the amatoxin such as α-amatine that is involved in amanita poisoning. α-Amatine is a specific inhibitor of RNA polymerase present in all eukaryotes. This blocking of the enzymes associated with the replication of RNA inhibits the formation of new cells. These toxins tend to accumulate in the liver and damage that organ severely. The RNA polymerase of the fungus is not affected. This group has caused the majority of recorded deaths from mushroom poisoning, especially in Europe. The general symptoms of this type of poisoning are severe abdominal pains, nausea, violent vomiting, diarrhea, cold sweats, and excessive thirst. These may last for 48 hours, with dehydration, cramps, and anuria.

2. Muscarine-Type Poisoning

Two toxins, muscarine and ibotenic acid, are involved. They occur in *Amanita muscaria*, *A. pantherina*, and also in a number of *Inocybe* and *Clitocybe* species. Muscarine is known to be responsible for "pupil contraction, blurred vision, lachrymation, salivation, perspiration, reduced heart rate, lowering of blood pressure, and asthmatic-like breathing."[1] Ibotenic acid is responsible for the insecticidal properties of *A. muscaria*, the fly agaric. Both muscarine and ibotenic acids are intoxicants, and there is a long history of different cultures using these compounds from *A. muscaria* for this purpose and in religious rites. The symptoms usually appear soon after eating the mushrooms, with vomiting, diarrhea, and salivation. The most characteristic symptoms are nervous excitement, difficulties in breathing, shivering, and a tendency to collapse.

3. Psychotropic or Hallucinogenic Poisoning

Several different toxins are involved, including psilocin and psilocybin, which are found in species of *Psilocybe, Conocybe,* and *Stropharia*. These compounds are similar in their reaction to *d*-lysergic acid diethylamide (LSD). They act on the central nervous system, producing distortions in vision and of tactile sensations as well as mixed emotional feelings of happiness or depression. Other symptoms are varied, including vomiting, increased rate of heartbeat, and hallucinations, which may last for various lengths of time.

4. *Coprinus* Poisoning

Several *Coprinus* species, such as *C. micaceus* and *C. atramentarius*, when consumed with an alcoholic drink, produce unpleasant but not dangerous symptoms. The symptoms include reddening of the face, increased rate of heartbeat, and, in some cases, vomiting and diarrhea. The mode of action of the chemical in *C. atramentarius* mushrooms is similar to Antabuse, which is a drug used to induce nausea and vomiting in individuals who are trying to overcome an addiction to alcohol.

5. Poisoning from External Sources

The poisoning is not caused by mushrooms themselves but by toxic substances that have accumulated in the mushrooms. The principal causes are (1) heavy metals due to polluting environmental conditions where the mushrooms are harvested that are far in excess of permissible levels, and (2) radioactive contaminants due to the pollution by contaminating radioactive materials in mushroom-hunting areas and subsequent consumption of the collected mushrooms.

III. MAGNITUDE OF MUSHROOM SPECIES

In 2000, the number of known described species of fungi was estimated to be about 74,000.[26] Yet in 1990 the magnitude of fungal diversity, that is, the actual number of species worldwide, was estimated conservatively to be at least 1.5 million species.[24] This figure has been revised after 10 years and is retained as the current working hypothesis for the number of fungi on Earth while waiting for additional data to test its accuracy.[26] Of the 1.5 million estimated fungi, Hawksworth[25] has estimated that 140,000 species produce fruiting bodies of sufficient size and suitable structure to be considered macrofungi, which can be called mushrooms, according to the definition given by Chang and Miles.[16] This implies that we currently know about 14,000 mushroom species, which would account for 10% of the estimated mushroom species. Of these, about 50%, or 7000 species, are considered to possess varying degrees of edibility, and more than 3000 species from 31 genera are regarded as prime edible mushrooms. To date, only 200 of them are experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, and about 10 have reached an industrial scale of production in many countries. Furthermore, about 2000 are medicinal mushrooms with a variety of health attributes.[17] The number of poisonous mushrooms is usually reported to be relatively small (approximately 1%), but there is an estimate that approximately 10% may have poisonous attributes and of these some 30 species are considered to be lethal.[35]

IV. ECOLOGICAL IMPORTANCE OF MUSHROOMS AND FUNGI IN GENERAL

Mushrooms and fungi in general are nongreen organisms lacking chlorophyll. They cannot manufacture their own food from simple inorganic materials, such as water, carbon dioxide, and nitrates, using energy from the sun, as is the case with the green plants. They derive their food from complex organic materials found in dead or living tissues of plants and animals. Those obtaining their

nutrients from dead organic material, e.g., agricultural crop residues, wood of dead trees, animal dung, etc., are referred to as **saprophytic fungi**. Those deriving their food substances from living plants and animals and causing harm to the hosts are called **parasitic fungi**. Such fungi are often of great concern to farmers because they can cause enormous crop damage and even lead to severe food shortages. But there are also some fungi whose members live in a close physiological association with their host plants and animals (e.g., those living inside nests of termites or mushrooms living in association with roots of some grasses or trees such as pines) and in a special type of partnership, whereby each partner enjoys some vital benefits from the other. These are referred to as **mutualistic symbiotic fungi**.

Saprophytic mushrooms are often quite specific in their nutritional and ecological requirements. (1) Some grow on fresh or almost fresh wood residues (e.g., *Lentinula, Pleurotus, Flammulina, Auricularia, Pholiota, Tremella, Agrocybe, Ganoderma*). (2) Some grow on only slightly composted lignocellulosic materials (e.g., *Volvariella, Stropharia, Coprinus*). (3) Some grow on well-composted materials or on animal dung (e.g., *Agaricus*). (4) Some grow on soil and humus (e.g., *Lepiota, Leptista, Morchella, Gyromitra*). Moreover, some saprophytic mushrooms grow only on dead grass and straw. Some grow only on dead wood of specific tree species and shrubs. Some grow only in cool moist climatic conditions, whereas others grow on these organic materials only under warm climatic conditions. Furthermore, wood rotting-mushrooms have been broadly classified as white and brown rot fungi depending on the mode and type of wood contents utilized. White rot fungi utilize cellulose, hemicellulose, and lignin components with the help of cellulase and ligninolytic enzymes. Brown rot fungi only utilize cellulose, and hemicellulose and the lignin part of plant cell walls remain unutilized as a brownish tissue. *Pleurotus* mushrooms are well-known commercially cultivated white rot fungi. Wherever we find them, fungi play significant ecological roles by virtue of their ability to secrete enzymes, which decompose the substrates into simpler organic products that are utilized by the fungi for growth and their metabolic needs. Indeed, the role of mushrooms and other fungi in nutrient recycling processes is of greater ecological importance than most people realize.

Mushrooms that form symbiotic associations with termites are also very important ecologically and economically. The edible mushrooms, which are often harvested from the base of termite nests, do not grow there by mere chance. They are farmed by the worker termites in a special fungus garden inside the termite mounds for their own use and also for their young. The worker termites (which incidentally are blind) collect the wood and other organic matter found near their nests and use it in the cultivation of *Termitomyces* mushroom gardens. In the process, some of the nutrients contained in the wood are incorporated into mushroom mycelia and others are recycled into the soil. About 1800 species of termites are known. Approximately 100 are "fungus growers" (Macrotermitinae). They "grow" about 20 species of the genus *Termitomyces. Termitomyces schimperi* (pat) Heim is the largest species of *Termitomyces* with the cap measuring about 20 cm in diameter.[3] Unlike many other *Termitomyces* species, it lacks an umbo on the upper surface of the cap, which makes the species easily recognizable. *Termitomyces schimperi* is only found in African countries. It is common in northern Namibia and is also found in other tropical African countries (Tanzania, Ethiopia, Zambia, and Malawi), where it grows on termite nests (mounds). This mushroom, eaten by humans and other mammals, provides enzymes needed for decomposition of wood by termites, which, in turn, provide a sterile, moist environment for fungal growth.[2] It is a delicious mushroom and is one of the best-known mushrooms in Africa. So far, this mushroom is only "grown" or "cultivated" by termites, not yet by humans. Further research may lead to the commercial cultivation of this delicious mushroom. The following are suggestions for research on this mushroom:

1. Detailed studies of the mounds, nests, chambers, combs, and especially the pellets (mylospheres)
2. Identification of the fungi associated with the termites with an emphasis on the earliest developmental stages (primordia) of the fruiting body

3. Studies of differences in composition of fresh mylospheres on which *Termitomyces* mycelium grows
4. Experiments with pure cultures of *Termitomyces* with and without mylospheres, possibly with the addition of the intestinal juice of termites or with the addition of termites themselves
5. Studies of the environmental conditions in the chambers where pseudorrhizae of *Termitomyces* are formed

Fungi that live in the soil in symbiotic association with roots of vascular plants in our woodlands and in our forest ecosystems are also very important ecologically and economically. These associations are referred to as **mycorrhizae** (fungus root association). There are some mycorrhizal mushrooms, e.g., Perigold black truffle, *Tuber melanosporum*, and matsutake mushroom, *Tricholoma matsutake*. It is difficult to bring these wild gourmet mushrooms into cultivation because they are the products of a fungus root association. These mushroom species have a mutualistic symbiotic relationship with trees. In these partnerships, the fungi obtain their carbohydrates from the plant roots. The root hosts, in turn, are supplied with inorganic mineral nutrients absorbed from the soil by fungal mycelia. Mycorrhizal fungi in plant roots have, indeed, been demonstrated to strongly stimulate the growth of their hosts. Mycorrhizal associations are important in agroforestry and afforestation practices. Some species that were long assumed to be symbiotic mushrooms, e.g., *Dictyophora* spp., now can be grown on synthetic substrates. There is great potential for the domestication of the so-called mycorrhizal mushrooms into the cultivated species whose ecological roles are still unknown. For example, the Kalahari truffle is the most economically important edible indigenous mushroom in Namibia. Its presence is indicated by a swelling in the sand soil and cracks in the surface, often in a field of pearl millet, which is the key food crop in that area, or near thorn tree species.[39] A mycorrhizal relationship between the mushroom and these plants has been suspected but has not yet been proved. This means that the ecological role is not clear and the real host plants have not been identified with certainty. This hampers the efforts to commercialize production and to determine whether harvesting methods could become sustainable. Claridge et al.[18] have suggested that studies of those hypogeous mushrooms should include efforts (1) to improve understanding of the broad-scale distribution of the hypogeous mushrooms; (2) to identify features (e.g., annual mean moisture index, a surrogate for water stress, topography, water depth, nature of soil, and seasonal variation, etc.) of the habitat that supports the occurrence of the mushroom; and (3) to establish its ecological role. The results would determine whether or not it is truly a symbiotic mushroom. This can enhance the effort to commercialize production or determine whether current harvesting methods are sustainable. To achieve these aims, fruiting bodies of the mushroom should be collected at numerous environmental study sites and an assay made of the distribution of some of the more commonly recorded "host" plants as well as the overall number of species in relation to measured macro- and micro scale habitat variables.

V. COLLECTION AND CLASSIFICATION OF MUSHROOMS

Collection and identification of wild mushrooms are important for the study of mushroom biodiversity and their ecological role. The discovery of new mushroom species will lead to their exploitation by an expanding mushroom industry. The collection and identification of wild mushrooms of species already known will provide the genetic variability required for breeding better mushrooms in higher yield and also provide the phenotypic traits which may be desirable by the mushroom industry or useful for research purposes. Because the mushrooms themselves are the only source of this genetic material, extinction of a single strain or species would mean the potential loss of many thousands of unique genes that might be used for breeding desirable new strains. The process of collection and classification of information pertaining to the morphological, physiological, biochemical, and genetic characteristics of individual mushroom strains and the storage of

this information in computer databases will provide valuable and readily accessible information for breeding programs and academic research.

A. Field Collection

Some basic precautions should be taken to keep the collected materials in proper condition. Most fresh mushrooms are relatively fragile, and they should be protected from vibration and impact by careful packing. When gathering collections for identification, care must be taken to obtain entire, intact specimens. If specimens are available at different developmental stages, all of them should be collected whenever possible. Mushrooms growing on the ground may have important structures at the soil surface or even below it. Collectors should check and record observations. In general, plastic bags are unsuitable for collection of specimens, as they lead to excessive moisture by the swelling of the mushrooms and their rapid deterioration. The specimens are better wrapped in quarter pages of newspaper prepared in advance for the purpose. A permanent marker pen can be used to record such items as date, time, location, smell, substrate (host) color, exudate (if present), habitat, and anything at all unusual about the specimen. Some important characteristics for identification disappear rapidly as the mushroom matures. These characteristics have to be recorded accurately at the time of collection.

As mentioned in the previous section, some mushroom species have a symbiotic relationship with vegetation, particularly trees. Therefore, the substratum (host) should be carefully recorded, as this can be an important feature in identification and in classification, i.e., whether the mushroom grows on dung, wood, bark, living trees, litter, soil, etc. is important information. If the mushroom grows on a living plant or on dead parts recognizable as belonging to a nearby plant, then flowers, fruits, or other parts of the plant should be collected for identification of the host or substrate. Identification of mushrooms may require examination of a "spore print." For specimens with a distinct cap and stem, the cap is removed and placed, fertile-side down, preferably on a microscope slide for determination of size and form or on white paper and black paper for determination of spore color. This preparation is covered with a bowl or similar object to prevent air currents. A thin spore print is often visible after as little as a few minutes, but a useful deposit usually requires longer (2 hours or more).

B. Preserving the Collection

Collected specimens for identification should be examined as quickly as possible and portions should be retained under conditions suitable for returning to the laboratory. Drying in an air-conditioned room, or with a vegetable dehydrator, or with an air dryer is the standard method of preparing permanent collections for a mushroom herbarium. It should be noted that fleshy mushrooms should not be dried at temperatures above 35 to 40°C, because the hyphae and other microscopic structures become too strongly distorted, making later microscopic studies difficult.

C. Precautions in the Use of Keys

Collectors should always remember when using keys that the mushroom in hand might not be included in the book being consulted (or in any other book, for that matter). Once a name has been obtained by using a key, the detailed description provided for the mushroom must be read and compared with the one being identified. If the description does not fit the specimen, the key must be checked again, following a different route. If all of the possible routes are exhausted and a description that fits still cannot be found, it must be assumed that the fungus in hand is not in the books being consulted. With the information gained, other appropriate, available references must be checked, or assistance sought of specialists working with the group in question. Never attempt to force a specimen into a category where it does not fit. More information can be found in Courtecuisse and Duhem[19] and in Chang and Mao.[12]

TABLE 1.1
World Production of Cultivated Edible Mushrooms during the Period 1981 to 1997 (× 1000 MT)

Year	Production	Increase (%)	Average Annual Increase (%)
1981	1257	—	—
1986	2182	73.6	14.7
1990	3763	72.5	18.1
1994	4909	30.5	7.6
1997	6158	25.4	8.5

Source: Data from Chang, S.T., *Int. J. Med. Mushrooms*, 1, 291–300, 1999.

VI. JUSTIFICATION FOR THE TERM *MUSHROOM BIOLOGY*

In many disciplines when knowledge increases and areas of specialization develop within the discipline, it is convenient to indicate that area of specialization with a self-explanatory name. In biology, there are such specializations as neurobiology, bacteriology, plant pathology, pomology, and entomology. These names indicate either a group of organisms, e.g., bacteria, algae, insects, or an approach to the study, e.g., disease, development, and physiology.

Mycology is the science that deals with fungi, of which there are over 74,000 known species. Fungi are important to humans for a variety of reasons. They are the principal causal agents of plant diseases and some significant human diseases as well. Moreover, through their fermentation activities, fungi are major producers of some important products, such as ethyl alcohol, citric acid, and the antibiotic penicillin. It should not be ignored that the value of mushrooms and their products has rapidly increased in recent years as mentioned in a previous section.

The great increase in the number of species brought into cultivation in the 1980s and 1990s corresponds with the dramatic acceleration in total worldwide production of cultivated mushrooms (Table 1.1). The ten most popular species are *Agaricus bisporus/bitorquis, Lentinula edodes, Pleurotus* spp., *Auricularia* spp., *Volvariella volvacea, Flammulina velutipes, Tremella fuciformis, Hypsizygus marmoreus, Pholiota nameko,* and *Grifola frondosa*.[35] In recent years, several new species of edible mushrooms, e.g., *Hericium erinaceus, Dictyophora indusiata, Stropharia rugoso-anulata, Lepista nuda, Agrocybe aegerita, A. cylindraceae, Pleurotus citrinopileatus,* and *Cantharellus cibarius* have also been successfully cultivated. To date, more than 60 mushroom species have been artificially grown with the majority cultivated in East Asian countries. More than 30 species have been cultivated on a commercial scale in China.[33] The amounts produced are expected to increase since good research on the cultivation of these mushrooms, including strain selection, has been undertaken.

Although several terms for this important branch of mycology that deals with mushrooms have been used, and each of these has its merit, when we attend to the matter of definitions, it seems that there is a place for a new term. The new term is **mushroom biology**. Mushroom biology is a new discipline concerned with the scientific study of mushrooms.[16] In Section 1.II.A we defined a mushroom as a macrofungus with a distinctive fruiting body large enough to be seen by the naked eye and to be picked up by hand. This broad definition of mushroom includes both Ascomycetes and Basidiomycetes, hypogeous as well as epigeous species, fleshy and nonfleshy textured macrofungi, edible or non-edible, or poisonous, or medicinal species. The term **mushroom science** already exists, but it is restrictive in that it has been defined as the

discipline that is concerned with the principles and practices of mushroom cultivation and production. It encompasses microbiology, composting technology, environmental engineering, and marketing and management. Dealing solely with cultivation, mushroom science is only one aspect, albeit a very significant one, of mushroom biology. Another aspect of mushroom biology is **mushroom biotechnology**,[6] which, in the context of mushroom products (mushroom derivatives), encompasses microbiology, fermentation technology, processing, and marketing and management. Mushroom biology includes not only these two major applied aspects, mushroom production and mushroom products, but also deals with other basic studies of mushrooms, such as biodiversity, taxonomy, development, nutrition, physiology, genetics, pathology, medicinal and tonic attributes, edibility, and toxicity.

VII. IMPACT OF MUSHROOM BIOLOGY ON HUMAN WELFARE

The 20th century was an explosive time for the accumulation of knowledge. In the 21st century, modern technology for human civilization is expanding every day. However, human beings still face and will continue to face, as a consequence of an increasing world population, three basic problems: shortage of food, pollution of the environment, and diminishing quality of human health. The 20th century began with a world populated by 1.6 billion and ended with 6.0 billion inhabitants; the world population will be nearly 9.0 billion by 2050, according to the latest demographic studies, with most of the growth occurring in the developing countries. The growing world population is increasing by about 80 million people per year. At the present time, about 800 million people in the world are living in poverty. Very importantly, it has been observed that more than 70% of agricultural and forest products have not been put to total productivity and have been wasted in processing. Macrofungi (mushrooms) not only can convert these huge lignocellulosic biomass wastes into human food but also can produce notable biomedicinal products, which have many health benefits. Another significant aspect of mushroom cultivation is its role in decreasing pollution. Mushrooms and their medicinal products have a great potential for supplying healthy food and dietary supplements for domestic consumption as well as for export, provided that the international quality standards and timely supply schedules can be met.

Cultivated mushrooms have now become popular all over the world. In 1999, the world production of cultivated edible mushrooms was estimated to be greater than 7 million tons. The combined total market value for medicinal and edible mushrooms for that year is conservatively estimated at more than U.S. $30 billion. In addition, the contribution of mycorrhizal mushrooms collected in forested ecosystems annually adds several billion U.S. dollars. The world production of mushrooms increased steadily during the last two decades as follows: 1.2 million tons in 1981; 2.2 million tons in 1986; 3.8 million tons in 1990; 4.9 million tons in 1994; 6.2 million tons in 1997 (Table 1.1); and it was estimated to be more than 7 million tons in 1999. China has now become the biggest mushroom producer, consumer, and export country. The following statistics serve to illustrate the dramatic increase in the production of farmed mushrooms in China during the period of 1978 to 2000. In 1978, total production of edible mushrooms in China was estimated to be only 60,000 tons. For the first time production reached more than 1 million tons in 1990, it was 2.6 million tons in 1994, about 4 million tons in 1997, 4.4 million tons in 1998, and 6.6 million tons in 2000 (Table 1.2). Mushroom cultivation and the processing of mushroom products have been beneficial to millions of people in China, India, and other developing countries in terms of financial, social, and health improvement. In addition, cultivation and development of mushroom industries positively generate economic growth and have already had an impact at national and regional levels. This impact is expected to continue to increase and to expand in the future with sustainable research and development of mushroom production and mushroom products generating a nongreen revolution.[7]

TABLE 1.2
The Production of Farmed Mushrooms in China during the Period 1978 to 2000 (× 1000 MT)

Year	Production	Increase (%)	Average Annual Increase (%)
1978	60.0	—	—
1986	586.0	876.7	109.6
1990	1000.0	70.6	17.6
1994	2600.0	160.0	40.0
1996	3500.0	34.6	17.3
1997	4000.0	14.3	14.3
1998	4350.0	8.8	8.8
2000	6630.0	52.4	26.2

Sources: Data from Chang, S.T., *Int. J. Med. Mushrooms*, 1, 291–300, 1999; Huang, N.L., in *Edible Fungi of China* [in Chinese], 104, 3–5, 2000; and Lin, C.M., *The Market of Edible Fungi* [in Chinese], 8, 5, 2002.

VIII. MUSHROOM SCIENCE

A. Definition

Mushroom science is the discipline that is concerned with the principles and practices of mushroom cultivation.[13] To establish the facts from which principles can be derived for future development of the discipline requires systematic investigation — as is true in any branch of science. The systematic investigation must include not only scientific studies but also the practical aspects of mushroom cultivation. The consistent production of successful crops is built on this scientific knowledge and practical experience.

B. Contributing Fields

The practical experience can be obtained only through a period of personal participation, which should include, preferably, a period of formal training in mushroom cultivation as well as careful observations of the practices of commercial mushroom growers. The scientific knowledge is derived basically from the fields of microbiology, fermentation, and environmental engineering.

1. Microbiology

For two main reasons, knowledge of microbiology is essential in mushroom cultivation. In the first place, the mushroom to be produced is a filamentous fungus, which can be studied by microbiological techniques. Second, other microorganisms are important in the composting process, and still others as weeds growing on the substrate or as pathogens to the mushroom being cultivated. In this section we examine the growth requirements of fungi and the basic methods used for preparing a fungal culture for mushroom cultivation.

Fungi are able to synthesize most of the complex compounds that are necessary for living organisms to carry on their life activities. Consequently, they have relatively simple nutritional requirements, and many fungi have been grown in laboratory studies on chemically defined media.

A carbon source is essential in all such media, and because glucose is utilized well by many fungi it is a carbon source commonly used in many media. The carbon compound provides energy for the fungus as well as providing the carbon atoms that constitute the skeleton of the organic molecules that make up the cells. These organic molecules include the lipids of the membranes, the polysaccharides of the cell walls, the nucleic acids, and the proteins.

Nitrogen must also be supplied in the media. It is a constituent of the amino acids (which make up the proteins), purines, pyrimidines, and some vitamins; nitrogen is also a constituent of the polysaccharide chitin, which is a cell wall component of many fungi. Nitrogen is supplied to fungi in media in different forms. Some species are able to grow well with nitrate as the nitrogen source; other species cannot utilize nitrate, but the ammonium ion serves as a nitrogen source. Then, there are other species that cannot utilize either nitrate or the ammonium ion and require an organic nitrogen-containing compound. In some species the requirement for organic nitrogen is for a specific amino acid. Laboratory studies of nitrogen utilization and metabolism by fungi permit the following generalization: a fungus that can utilize nitrate will also utilize the ammonium ion and organic nitrogen compounds, and a species that utilizes the ammonium ion can make use of organic nitrogen compounds.

Fungi also require major elements other than carbon and nitrogen. For example, sulfur, phosphorus, potassium, and magnesium serve the same general functions that they do in higher plants, and these elements are commonly supplied in the media as salts (e.g., magnesium sulfate and potassium phosphate). Equally important, although they are required only in trace amounts, are the so-called minor elements. Included in a minor or trace element solution that is present in various media are salts containing the elements calcium, copper, iron, manganese, molybdenum, and zinc. Although there are general requirements for some of the trace elements, some may be specific for a species and may be required either for vegetative growth or for some particular developmental stage.

There are fungi that have no requirement for exogenous growth factors. Such fungi are able to synthesize their own vitamins. A number of fungi do have vitamin requirements, however, with biotin (vitamin H) and thiamine (vitamin B_1) as the vitamins most commonly required by the filamentous fungi.

The substrate materials must meet all these nutritional requirements. In nature, the organic substrate is commonly composed largely of the insoluble polysaccharide materials of plant cell walls. Such materials are broken down into soluble compounds by extracellular enzymes of the fungus, and these soluble compounds can be transported into the fungal cytoplasm.

In cultivation of edible mushrooms the nutritional requirements that have been mentioned above must be met, but there are other important requirements as well — just as is the case with all intensively cultivated agricultural crops. In the case of higher plant crops, the genetic characteristics of the seeds are of great importance in determination of yield, disease resistance, etc. Similarly, with the edible mushrooms a good quality of spawn, the "seed" of mushroom cultivation, is of prime importance. The spawn is a medium that is impregnated with mycelium, and spawn is made with a pure culture. The establishment of a pure culture requires microbiological knowledge. The pure culture of the edible mushroom may be derived from a single spore culture. Isolation of a single sexual spore from the mushroom and growth of the mycelium following germination of the spore can be used as the pure culture for spawn in the case of homothallic species. In the case of heterothallic species, however, the single-spore progeny differ genetically and it is necessary to mate the mycelium derived from a spore with a compatible mycelium from another single spore to establish a dikaryotic mycelium. In heterothallic species, a dikaryotic mycelium is required for the formation of good fruiting bodies. The single-spore cultures are the basic materials that the mushroom breeders in research laboratories use for improving the mushroom strains (or dikaryotic stocks of heterothallic species) and are not good for spawn production by farmers or commercial spawn producers.

A pure culture can also be obtained as a multispore culture. The multispore culture results from the germination of many spores collected from a fruiting body in which some of the germlings have fused to form dikaryotic mycelia. Multispore cultures of homothallic species, such as *Agaricus bisporus* and *Volvariella volvacea*, may be used as a pure culture for spawn, but such cultures are not suitable for heterothallic species since the dikaryotic mycelium from the multispore culture is genetically different from the fruiting body that produced the spores. A third method for obtaining a pure culture is as a tissue culture from a fruiting body. Any portion of a young fruiting body can be used to establish a tissue culture, but it is preferable to take tissue from the upper part of the stipe, as this area is a region of rapid cell elongation. Fruiting bodies in a stage prior to spore formation are composed of somatic cells of identical genetic makeup; therefore, no genetic variation due to segregation will be present in tissue cultures obtained from these fruiting bodies. If tissue is taken from a mushroom strain or stock of high yield, the pure culture derived from this will be reliable and stable in yield.

Of course, a pure culture for spawn making can also be obtained as a subculture of a previously established pure culture. Such cultures are available in fungal culture collections maintained in laboratories of individuals, of research institutions, or of organizations created for the maintenance and distribution of cultures of microorganisms, such as the American Type Culture Collection.

The principles and techniques of microbiology are also applied to spawn preparation once a suitable pure culture of the mushroom has been obtained. The pure culture is used to inoculate the grain medium or other suitable spawn substrate. When the mycelium has grown out from the inoculum and permeated the substrate, a spawn that can serve as the "seed" for growing mushrooms on suitable compost is formed. All operations from pure culture isolation through spawn preparation must be conducted using sterile technique and performed as rapidly as possible to lessen the opportunity for contamination to occur. Knowledge of microbiology is also required for a proper understanding of composting, which will be treated as a fermentation process (solid state fermentation).

2. Fermentation

Mushrooms are heterotrophic organisms that must obtain all their nutritive requirements from the substrate. In this respect they are unlike the autotrophic higher plants, which obtain water and inorganic nutrients from soil and synthesize organic compounds in leaves through photosynthesis. This means that the compost must make greater contributions for the production of mushrooms than the soil does for higher plant growth. That is, the compost plays a more comprehensive role in production of mushrooms than does the soil for higher plants.

A good substrate for mushroom growth must be suitable both chemically and physically, as well as having the proper condition for microbial activities. A suitable chemical condition is one that allows for the release of some nutrients from the compost substrate during fermentation and pasteurization. A suitable physical condition is one that provides for good aeration and water-holding capacity as well as an anchorage and support for the mushrooms.

The process of composting involves a controlled microbial succession in the substrate. A stable medium, which is suitable for the growth of a particular mushroom, and is less satisfactory for competing microorganisms, is produced from a mixture of organic materials by composting. For both economic and technical reasons composting cannot be carried out under sterile conditions as are most other industrial fermentation processes such as antibiotic production.

Much of our fundamental knowledge of composting comes from experience with *Agaricus* cultivation in which there are commonly two phases in processing the compost — outdoor fermentation and indoor pasteurization. The first phase is the outdoor one, in which the materials to be composted are stacked up in large piles. The stacking in large piles results in the creation of different microenvironmental conditions at various depths of the piles. Because of these different environmental conditions the distribution and metabolic activities of the microorganisms vary within the pile, and during the composting process there is a succession of several generations of

microorganisms including bacteria, actinomycetes, molds, and protozoans. Each succeeding generation of microorganisms has available for its nutrition not only the remaining raw materials of the substrate but also the cellular components and metabolic products of the previous generations of microorganisms.

It has been pointed out by Hayes[28] and also by Wood[41] that different groups of microorganisms dominate at different stages of composting. A mesophilic microflora, which utilizes the available soluble carbohydrates and nitrogen compounds, dominates initially. Increased growth of more living organisms follows this initial phase with a corresponding release of more carbon dioxide, ammonia, and much heat. Thus, at the later stages of composting, the temperature is higher and thermophilic microorganisms become dominant.

In the second phase of composting, the compost is transferred to the growing chambers where it is pasteurized by steam heating to raise the temperature of the compost (which now is thinner) in the beds to a more or less uniform level of approximately 55 to 60°C. This indoor pasteurization process kills the vegetative cells of the microorganisms, and the temperature of the compost is then cooled by air to 25°C before spawning (planting the spawn into the compost). During pasteurization a specific microflora composed of thermophilic microorganisms develops. These thermophilic microorganisms are mainly responsible for the second stage of fermentation, which removes ammonia and makes the compost a selective substrate for *Agaricus* growth.

The mushroom mycelium grows out onto the compost rapidly after spawning and penetrates throughout the compost in a short time. Because the compost has been rendered selective for the mushroom mycelium, which grows rapidly, the growth of other microorganisms is retarded or prevented.

Thus, it is evident that the principles of fermentation are involved in both phases of composting, and the end result is a selective medium for mushroom cultivation.

3. Environmental Engineering

The two distinguishing phases in the growth and development of a cultivated mushroom in this compost are known as the **vegetative** and **reproductive** stages. The vegetative stage is referred to in practice as the spawn running phase, and the reproductive stage as the fructification phase. These two phases mark the transition that takes place from mycelial growth to the formation of a specific morphogenetic structure in fungi.[21,34]

During mycelial colonization of the compost, enzymes are secreted from the mycelium into the compost substrate. Such extracellular enzymes break down the lignocellulosic components of the compost into simpler, soluble organic compounds, which can be absorbed by the hyphae and used for the necessary metabolic requirements of the fungus. Growth of the mycelium results in fusions of the hyphae and a close association of the hyphae with the substrate. Hyphal fusions facilitate the translocation of nutrients within the mycelium, and the close connection of the hyphae with the substrate provides the strong physical support that is necessary for the fruiting body. When this state of development is reached, the mycelium is said to be "established," and it is ready to pass from the vegetative to the reproductive stage. Certain environmental factors may "trigger" this change from vegetative growth to fruiting body formation in mushrooms, and application of environmental engineering practices may be employed by the mushroom grower to bring this about. Such factors as **temperature**, **light**, and **changes in concentration of atmospheric gases** can be significant in the transition from vegetative mycelial growth to fructification (Figure 1.6). A few examples and generalizations are now given.

In regard to temperature, it is known that for most cultivated edible species of mushrooms the optimal temperature for fruiting is lower than the optimal temperature for mycelial growth[30] (Table 1.3).

Although an increase in ambient CO_2 concentration may improve fungal growth by CO_2 fixation systems,[41] this elevated level of CO_2 is also known to inhibit fruiting body initiation and normal development. Consequently, the removal of excess CO_2 and other self-inhibitory volatile metabolites by methods of ventilation is essential for production of mushrooms.

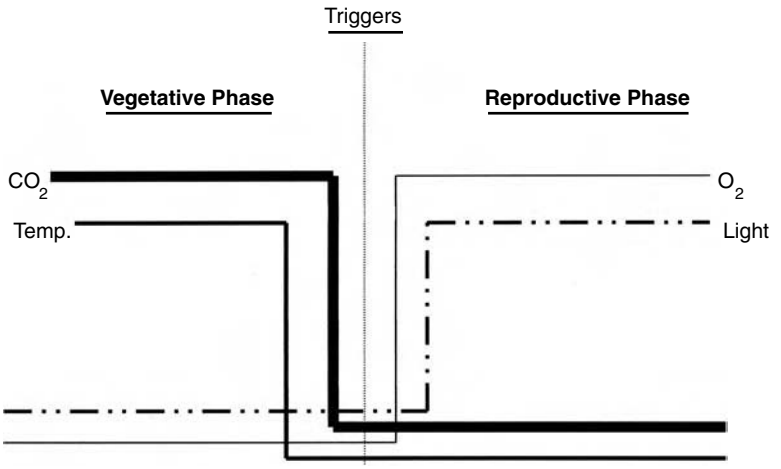


FIGURE 1.6 The two major phases of mushroom growth and development: vegetative phase and reproductive phase. The triggers for the transition from the vegetative phase to the reproductive phase are usually regulated by environmental factors.

TABLE 1.3
Temperature Range and Optima

Species	Mycelial Growth[a]	Fruiting
Agaricus bisporus	3–32 (22–25)	9–12 (15–17)
Agaricus bitorquis	3–35 (28–30)	18–25 (22–24)
Auricularia auricula	15–34 (28)	15–28 (22–25)
Auricularia polytricha	10–36 (20–34)	15–28 (24–27)
Flammulina velutipes	3–34 (18–25)	6–8 (8–12)
Hericium erinaceus	12–33 (21–25)	12–24 (15–22)
Lentinula edodes	5–35 (24)	6–25 (15) Autumn (10) Winter (20) Summer
Pholiota nameko	5–32 (24–26)	8–20 (High temp. strains) 5–15 (Low temp. strains) (7–10)
Pleurotus ostreatus	7–37 (26–28)	25–30 (High temp. strains) 16–22 (Med. temp. strains) 12–15 (Low temp. strains)
Pleurotus sajor-caju	14–32 (25–27)	10–26 (19–21)
Tremella fuciformis	5–38 (25)	20–28 (20–24)
Volvariella volvacea	15–45 (32–35)	22–38 (28–32)

[a] Figures within parentheses are optimal values; values are affected by differences in media, growth conditions, and strains or stocks used. These are representative values obtained from the literature.

Another environmental trigger for mushroom formation in some species of edible fungi is **light**. The requirement for light varies with species, and Eger-Hummel[20] has grouped the mushrooms into four types on the basis of the relationship of light and fruiting. In Type A, light and darkness play no role at any stage of fruiting body development; in Type B, light and darkness play no role

in primordium formation, but light is required at later stages of mushroom development; in Type C, light is required except for a short period at an early stage of development when darkness is essential; in Type D, there is a requirement for light at all stages of fruiting body development.

It has been reported by Gerrits[22] that there is a shift from a preferential use of lignin and protein polymers during mycelial growth to the use of cellulose and hemicellulose when fruiting is initiated. Thus, **nutrients** may serve as a stimulus for fructification. It is commonly found that for fruiting body development, there is a nitrogen requirement that is greater than that for mycelial growth. This additional nitrogen may come from degradation of extracellular protein. In addition to the greater nitrogen requirement for fruiting, other nutrient requirements, including those for vitamins, may be more exacting for reproduction than for vegetative growth, as has been pointed out by Hawker.[23]

Humidity is another environmental factor that must be properly controlled both for good vegetative growth and for fruiting. The moisture content of compost and the humidity of the atmosphere in the growing house are of critical importance for mushroom growth and development.

These various environmental conditions must be regulated to be optimal for the cultivation procedure or state of development of the mushroom, and it is the field of environmental engineering that is called upon to provide this regulation.

C. Mushroom Cultivation Technology

1. Concept

There is tremendous appeal in a process that promises to produce a highly nutritious food of excellent taste from waste materials without making extensive demands on land or having requirements for expensive equipment. Mushroom cultivation is such a process at the conceptual level, and many people have been moved to undertake mushroom growing on a commercial basis for this reason. Unfortunately, although simple in concept, mushroom growing is a complicated business, and when entered into by untrained individuals who are unaware of the various intricacies of the process, pitfalls are frequently encountered that commonly lead to failure of the venture. It is especially distressful to hear of such misfortunes in developing countries where properly developed and managed mushroom farms can make important contributions to the nutrition and economic welfare of the people. It is for this reason that we are outlining and describing the phases of mushroom technology. We want people interested in mushroom growing to be aware that it is a more complicated process than the layperson may realize and to encourage those who are serious about establishing a mushroom farm to consult references about the various phases of mushroom technology and to seek advice from experienced growers.

2. Phases of Mushroom Technology

Mushroom farming involves several different operations, each of which must be performed properly if the enterprise is to be successful. Failure in any phase will result in decreased harvest, at best, or a failure to achieve anything to harvest, at worst. The different phases of mushroom technology that are treated here are (1) the selection of an acceptable mushroom, (2) the requirement for and selection of a fruiting culture, (3) the development of spawn, (4) the preparation of compost, (5) mycelial (spawn) running, and (6) mushroom development. In addition, crop management and marketing are equally important operations.

It has been pointed out previously, e.g., by Chang and Miles,[14] that mushroom cultivation can be a relatively primitive type of farming, or it can be a highly industrialized agricultural activity requiring a sizable capital outlay for mechanized equipment. The straw mushroom, *Volvariella volvacea*, is commonly grown in Southeast Asian countries on small, family-type farms.[4,5,32,40] On the other hand, cultivation of the *Agaricus* mushroom may be highly industrialized with a few farms producing a disproportionately large percentage of a country's yield, as is the case in many Western nations. In either case the main objective of mushroom growers and the researchers who

work in this field is similar, namely, to increase the yield from a given surface area per period of time by use of a high-yielding strain, by shortening the cropping period, or by increasing the number of high-yielding flushes. To bring about this increase in yield requires (1) an understanding of the substrate material and its preparation, (2) the selection of suitable media for spawn making, (3) the breeding of high-quality and high-yielding strains, and (4) improved management of the mushroom beds, including prevention of the development of pests and mushroom diseases.

Thus, when we look beyond the basic concept involved in growing mushrooms, we see that there are a number of interesting operations, each of which can be rate-limiting for total production. The successful grower requires the scientific knowledge that is provided through research and the practical experience obtained by personal participation involving observation of and training in the practices of mushroom cultivation. The practices of mushroom cultivation, or mushroom technology, consist of six major phases. These phases generally occur in the sequence that follows: (1) selection of a mushroom species, (2) selection of a fruiting culture, (3) development of spawn, (4) preparation of compost, (5) spawn running, and (6) mushroom development.

a. Selection of a Mushroom Species

If the mushroom is to be marketed in the fresh condition, then it must be a species that is acceptable on the basis of its taste appeal to the people in the area where it is cultivated. This can be determined for previously cultivated species by examination of import records, if available, or by testing for market acceptability with fresh mushrooms imported for that purpose. Once it has been determined that the species is acceptable to the local consumer, there remain other significant considerations before a decision to cultivate a particular species should be made. For example, are appropriate raw materials for substrate available? Are the environmental conditions such that it will not be excessively costly to maintain the necessary temperatures for mycelial running and for mushroom development? Examination of Table 1.3 indicates that there is considerable variation among edible mushroom species in the temperatures suitable for vegetative growth (spawn running or mycelial running) and mushroom development. Both the temperatures necessary for vegetative growth and that required for fruiting must be considered in selection of an acceptable mushroom, and it should be pointed out that strains or dikaryotic stocks of species may differ in their temperature ranges and optimal values so that even within a single species selections can be made.

b. Selection of a Fruiting Culture

After determination of a mushroom species that is acceptable as a food to the indigenous population, for which suitable substrates are plentiful and the environmental requirements can be met without excessively costly systems of mechanical control, it is necessary to have a fruiting culture. A fruiting culture is one that can be used without further mating to make the spawn for mushroom production. The term **fruiting culture** is defined as a culture that has the genetic capacity to form fruiting bodies under suitable growing conditions. When grown under the proper conditions (conditions that will permit good vegetative growth and fruiting without high costs for equipment and energy), this culture should produce fruiting bodies. In the case of a heterothallic species, the fruiting culture is a dikaryotic mycelium, which was formed by a mating between two compatible single-spore, monokaryotic isolates. In the case of a homothallic species, a single-spore isolate is capable of forming fruit bodies and thus does not need to be mated with other isolates.

Sometimes multispore cultures are used to obtain fruiting cultures of *Agaricus bisporus*, but this is not a suitable technique for heterothallic species. Tissue cultures derived from the stipe or pileus of the mushroom of either homothallic or heterothallic species can be used to establish fruiting cultures. Establishment of tissue cultures is the method used to isolate and propagate sporeless strains. Sporeless strains of species of *Pleurotus* are of great commercial interest, because non-sporeless (normal) strains shed spores early in the development of the fruiting body and continue to produce spores in abundance up to harvesting with the result that the spore density in the air in the mushroom houses becomes very heavy. Unfortunately, it is a common occurrence for

mushroom workers under these conditions to suffer from respiratory tract problems and allergic reactions to these spores. Consequently, there is interest in developing sporeless mutants that will produce fruiting bodies equivalent to those of accepted commercial spore-forming stocks in yield, flavor, texture, fruiting time, etc. Obviously, a strain or stock that does not fruit, or fruits poorly, cannot be considered as a fruiting culture.

c. Development of Spawn

A medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum or "seed" for the substrate in mushroom cultivation is known as mushroom spawn. When the term **pure culture spawn** is used, it means that a strain or stock of unknown origin, free from contaminating organisms, is present. In spawn making, the entire process of preparation should be performed under aseptic conditions.

Failure to achieve a satisfactory harvest may frequently be traced to unsatisfactory spawn. If the spawn has not been made from a genetically suitable fruiting culture, or if a stock has degenerated, or if it is too old, the yield of mushrooms will be less than optimal. The successful spawn manufacturer must have a product that performs consistently well and thus has the confidence of the mushroom grower. For this a stable strain or stock possessing the genetic characteristics required by the growers is an absolute necessity. Ideal environmental conditions and management cannot overcome the limitations of a genetically inferior stock used to make spawn. The principles involved in strain development and selection are treated in Chapter 8, Mushroom Formation: Effect of Genetic Factors; Breeding.

Although the potential of spawn is set by the genetic constitution of the fruiting culture used in its manufacture, the substrate material is also very important. The substrates used in spawn manufacture may be different from the materials used in the cultivation of the mushroom, or they may be the same. Substrates may be used singly or in combination. Some of the substrates used in the spawn making include various grains (rye, wheat, sorghum), rice straw cuttings, cotton waste, rice hulls, cotton seed hulls, etc. Spawns are frequently referred to as grain spawns or straw spawns, which tends to overemphasize the importance of the substrate; whereas, in reality, it is the strain or stock of the mushroom that is of prime importance in determining the merits of a spawn. The spawn substrate serves mainly as the vehicle that carries the vegetative mycelium of the mushroom that is used to inoculate the growing beds. This does not mean that the spawn substrate has no effect on the success or failure of a spawn. The growth pattern of the mycelium may be influenced by the spawn substrate, as is seen by the more rapid growth and filling of the beds (spawn running) with some spawns than with others. A factor that the spawn manufacturer must also consider is the availability of the spawn substrate and, related to this, the cost.

In summary, in the manufacture of spawn, consideration must be given to the genetic capabilities of the fruiting culture: (1) for vegetative growth both in the spawn substrate and in the bed material following inoculation, and (2) for yield and quality of mushrooms produced. Consideration must also be given to the nature of the spawn material, because this influences rapidity and thoroughness of mycelial growth in the spawn container as well as spawn running in the bed following inoculation. Considerations of availability and cost of substrate are also important. Some other obvious features of a good spawn include freedom from contamination, vigorous growth, and good survival in storage.

d. Preparation of Compost

In the *Agaricus* mushroom growing industry, the process that renders horse manure suitable for the growth of mushrooms is known as "composting." This is derived from the general term **compost**, which refers to a mixture consisting largely of decayed organic matter that is used for fertilizing and conditioning land for horticultural purposes. It can be questioned whether the treatment of other substrates for growing should be regarded as composting because the starting materials and lengths of time for growth are not comparable, although the aims are similar, and we use the term

composting in this general way. Consequently, we emphasize the purposes of composting and general changes that are brought about by the process.

A substrate rich in available nutrients does not necessarily constitute a satisfactory medium for growing mushrooms. The reason for this is that the material first must be sterilized, or else bacteria and molds will grow in abundance and curb the growth of mycelium and the development of mushrooms. That is, when **spawn** is inoculated into a raw, unsterilized substrate, the naturally occurring microorganisms quickly gain dominance and retard or even prevent the development of mushroom mycelium. The function of composting is to reverse that situation, i.e., to prepare a medium with characteristics that promote the growth of mushroom mycelium to the practical exclusion of other organisms. To accomplish this, certain chemical properties and physical qualities must be built into the substrate. These features of the substrate are equally important and are interdependent.

When the best food materials that are able to serve the nutritional requirements of the mushroom have been accumulated, then the proper chemical state of the compost has been achieved. These nutrients must be in a form that is readily available to the mushrooms. For example, most mushroom species cannot use nitrates, and thus a substrate with nitrogen available only as nitrate would not be suitable. It is essential that there be nitrogen present in the compost materials in the form of protein. The compost must also be free of toxic substances, which inhibit the growth of spawn. Such toxic substances may be produced by microorganisms present in nature that grow in the substrate during the composting process. Thus, conditions unfavorable to the growth of these toxic-producing organisms must be employed during composting.

In regard to the physical qualities of the substrate, such things as: good aeration by the free admittance of air, ability to hold water without becoming waterlogged (which would curtail aeration), a proper pH, and good drainage are desirable features.

Of course, the compost contains not only the substrate materials but also the microorganisms that bring about the breakdown and decay of the substrate materials during the composting process. These microorganisms are essential for composting. The substrate during composting is never sterile but is literally teeming with millions of bacteria and fungi.

In practice, composting is accomplished by piling up the substrates for a period of time during which various changes take place so that the composted substrate is quite different from the starting material. A substrate consisting of agricultural and chemical materials other than horse manure, when composted, is called a **synthetic compost**. Synthetic composts have been devised with numerous formulations of just about every type of agricultural waste product and residue. Synthetic composts comprise the general type of substrate used for growing most mushrooms and can also be used for *Agaricus*.

Straws and other plant wastes are mainly cellulose, hemicellulose, and lignin — the polysaccharides of plant cell walls. Some bacteria readily attack cellulose and hemicellulose and under suitable conditions easily bring about their decomposition. On the other hand, lignin is quite resistant to decomposition by bacteria. The easily decomposed carbohydrates serve as an excellent source of food for bacteria and fungi. Such carbohydrates diminish after composting as a consequence of the metabolic activities of the microorganisms in the compost, making the substrate less favorable for further growth of these potential competitors to the growth of mushrooms. The metabolic activities of the microorganisms also have some other effects: (1) conversion of simple nitrogenous materials such as nitrates and ammonia to complex proteins, thereby increasing the proteins required later for growth of the mushroom mycelium; and (2) a drop in pH.

Principles of this complicated process of composting are known and guidelines are available, but in practice modifications are necessary to meet various situations that may be encountered. Such modifications might be necessitated by the availability of the raw materials, the microflora in the composting area, the facilities in the growing area, and especially important is the species of mushroom to be cultivated. The highly industrialized technology developed for the cultivation of *Agaricus* cannot be followed unmodified for the commercial production of the straw mushroom *Volvariella volvacea*.

e. Spawn Running

Following composting of the substrate, the compost is put into beds where it is generally pasteurized by steam to kill the vegetative cells of microorganisms. After pasteurization, the bed temperature must be allowed to fall below a certain degree before the spawn is introduced into the bed as inoculum, because high temperature damages the spawn. The amount of spawn used as inoculum per unit surface area is variable, but in general, larger amounts of spawn result in the bed becoming filled out with mycelium more rapidly. The use of greater amounts of spawn increases production cost, however.

Brief mention will be made of the inoculation procedure (spawning) at this time. When the spawn is removed from the container, it is broken into small pieces by crushing and crumbling it with the fingers. Pieces of spawn may then be broadcast over the bed surface and then pressed down firmly against the substrate to assure good contact, or they may be inserted 2 to 2.5 cm deep into the substrate.

Spawn running (mycelial running) is the phase during which mycelium grows out from the spawn and permeates the substrate. Good mycelial growth is essential for mushroom production, and it is essential to maintain proper conditions of the beds and in the mushroom house during spawn running. The proper temperature and humidity for the species must be maintained. The bed surfaces must not be allowed to dry out, but they should be watered lightly with a fine water sprinkler. As the mycelium grows, it generates heat, which contributes to water loss. Improper environmental control (of temperature, moisture, aeration) is a common cause of a poor spawn run. Mushroom house management is as important in this stage as it is in the following phase — mushroom development.

f. Mushroom Development

Under suitable environmental conditions, which are commonly different from those for spawn running, primordia formation occurs, followed by the production of fruiting bodies. Improper aeration, which leads to an increase in CO_2 in the vicinity of the mushroom beds where the mycelium is respiring, may inhibit primordia formation or later stages of mushroom development. Conditions of pH and temperature required for fruiting are also commonly different for fruiting than for mycelial running.

The appearance of mushrooms is commonly in rhythmic cycles called "flushes." Mushrooms are picked at different maturation stages depending on the species and on consumer preference and market value. The method of harvesting also varies with the species. Suitable temperature, humidity, and ventilation conditions must be maintained during the cropping period, because these factors will affect the number of flushes and total yield that will be obtained.

IX. DEVELOPMENT OF MUSHROOM SCIENCE

It should be noted at the onset that mushroom science, by which we mean the study of the principles and practice of mushroom cultivation, is not an exact science. It is a life science, and as such is subject to the limitations imposed on experimentation which living organisms may entail. Thus, a logical, systematic approach involving observation and experimental data, and their scientific evaluation, must be made for the various phases of mushroom cultivation. As noted in the previous section, the major phases of mushroom cultivation are (1) establishment of a fruiting culture, (2) spawn preparation, (3) compost preparation, (4) mycelial running, and (5) fructification.

In the early stages of the development of mushroom science, observations played the principal role. These observations frequently suggested changes in cultivation methods, and those modifications of cultivation procedures that proved to be of merit after experimentation became part of the general practice. Quantitative measurement and objective evaluation are essential aspects of such experimentation. In this way, as in the development of any industry, there were gradual changes with time.

Any applied science, however, must have a firm foundation in information involving the basic sciences. The acquisition of fundamental information and understanding of the causes of the phenomena observed requires that the practitioners of the applied science have a working knowledge of chemistry, physics, and mathematics — and, in this case, a basic knowledge of biology as well. Not only is this necessary for obtaining the correct basic information, but it is also absolutely essential for an understanding of the observations that will permit the formulation of hypotheses that can be tested for validity by experimentation.

Basic research has contributed significantly to the mushroom industry and to mushroom science. For example, microbiological studies have led to the establishment and preservation of pure cultures and the development of spawn for the mushroom industry. The Pasteur Institute, famous for basic microbiological research, produces and sells mushroom spawn, thus providing an example of a mutually beneficial association between basic and applied science.

The following are other examples of the role that basic research has played in the development of mushroom science:

1. Genetic improvement of fruiting cultures by breeding techniques and the preservation of such cultures have resulted mainly from basic investigations.
2. In the area of physiology, basic studies provide a measure of understanding for such things as the degeneration of spawn, the necessity for proper aeration in the growing houses because of inhibition of fruiting and malformation of fruiting bodies in the presence of raised concentrations of CO_2, and the role of light in inhibiting vegetative growth in some species and in triggering fruiting body primordia formation in others.
3. Morphogenetic studies have provided useful information on the development of fruiting bodies — one practical application of which is in the development of sporeless strains of *Pleurotus*. The desirability of using sporeless strains stems from the elimination of spore inhalation by mushroom workers. The spores produced by normal fruiting bodies in large numbers cause allergenic reactions and lung problems in individuals regularly subjected to breathing in an atmosphere containing a high density of spores.

Following initial observations and experimentation in major phases of mushroom cultivation, further growth and development of mushroom science will come about through the establishment of hypotheses, based on observations and experimental data, which can be subjected to testing.

We can anticipate in the future that research will lead to such things as an increase in the number of species that can be grown commercially, the use of a wider variety of substrate materials, decreased cropping periods for a number of species, improved methods of protection against pests, and techniques for preservation.

X. MUSHROOM BIOTECHNOLOGY

It has been pointed out that mushroom biology consists of two main components: (1) mushroom science, which we have considered in the previous section, deals with mushroom production and encompasses the principles and techniques of composting technology and environmental engineering as well as microbiology; and (2) mushroom biotechnology, which is concerned with mushroom products and encompasses the principles and practices of fermentation technology and processing procedures, as well as microbiology. In addition, marketing and management are important segments for both mushroom science and mushroom biotechnology. These segments constitute the key factors in the commercial success of a mushroom-based industry. Mushroom market opportunities must be considered for domestic, regional, and international markets.

There is a long history of the use of mushrooms for medicinal purposes. *Ganoderma* mushrooms have been valued in China for their medicinal properties for over 2000 years. *Ganoderma* is not a fleshy, edible mushroom, so its use was strictly for medicinal or tonic effects, and the medicinal

effects were not coincidental with its being consumed as a food, as is the case with some other mushrooms, such as *Lentinula edodes*. Thus, we know that for more than 2000 years people have been using mushroom-derived compounds for the sole purpose of their medicinal and tonic properties.[11] Currently, there is intense industrial interest in a novel class of compounds extractable from either the mycelium or fruiting body of mushrooms. Between 80 and 85% of all medicinal mushroom products are derived from the fruiting bodies, which have been either commercially farmed or collected from the wild. Only about 15% of all products are based on extracts from mycelia or consist of the actual powdered mycelia plus the substrates used for growing. However, due to increased quality control and year-round production, mycelial-based products might be the trend of the future. These compounds, called mushroom nutriceuticals,[10] exhibit either medicinal or tonic qualities and have immense potential as dietary supplements for use in the prevention and treatment of various human diseases.

XI. NONGREEN REVOLUTION

As the population of the world increases in the 21st century, the amount of food and the level of medical care available to each individual, especially those living in less developed countries, will probably continue to decrease. Environmental pollution will also become a more serious problem. However, the world has an immense amount of lignocellulosic biomass resource, which, like solar energy, is sustainable. This resource has been considered insignificant, or of no commercial and certainly no food value, at least in the original form. It should be noted that significant research funds have been set aside to search for increased productivity in core products, such as the oil in coconuts, cellulose in trees, fiber in sisal, coffee in coffee cherries, and grain in cereal crops. However, few research funds have been reserved to search for the possible reuse of many by-products that are usually considered to be waste materials. This means that the by-products from the processing of core products, usually considered wastes, are not properly reused. When they are carelessly disposed in the surrounding environment by dumping or burning, they lead to environmental pollution and subsequently to health hazards. It should be emphasized that these lignocellulosic wastes are actually a kind of natural resource or raw material. If they are properly managed and utilized, this would lead to economic growth. This approach should be applied to agriculture-based industries in the 21st century. The by-products in processing the core product should be used and treated as raw materials for the production of secondary or tertiary products, such as the use of cereal straw, coffee pulp, spent coffee grounds, and sisal waste for growing mushrooms. After harvesting mushrooms, the spent compost can be used as a feeding material for animals or for growing earthworms. Finally, the residues can then be used as crop fertilizers. In the whole scheme of things, all wastes would be utilized. This is the concept of zero emissions or total productivity.[36]

Mushrooms produce enzymes, which degrade lignocellulosic materials for their own growth and fruiting. This demonstrates the impressive capacity of mushrooms for "biosynthesis," which is different from the "photosynthesis" of green plants. Mushrooms not only can become nutritious protein-rich food, but they also can provide nutriceutical and pharmaceutical products. Mushrooms have a great potential for domestic consumption, as well as for export. The most significant aspect is the recycling of waste by-products during each stage of mushroom production and the creation of a pollution-free environment. Therefore, mushrooms, with their great variety of species, constitute a cost-effective means of both supplementing the nutrition of humankind through the production of edible mushrooms and alleviating the suffering caused by certain kinds of illnesses through the use of medicinal mushrooms and their derivatives as nutriceuticals, and even as pharmaceuticals. By blending advances in basic biological knowledge with those of practical technology, a mushroom-related industry utilizing the lignocellulosic waste materials that are abundantly available in rural and urban areas can have a positive long-term global impact on human nutrition, health, environmental conservation and regeneration, as well as promoting

economic and social changes. Therefore, the significant impact of mushroom cultivation and mushroom derivatives and products on human welfare in the 21st century can be considered globally as a "Nongreen Revolution."[7]

REFERENCES

1. Alexopoulos, C.J., Mims, C.W., and Blackwell, M., *Introductory Mycology*, 4th ed., John Wiley & Sons, New York, 1996.
2. Barnard, P., Biological diversity in Namibia — a country study, Namibian National Biodiversity Task force, Windhoek, Namibia, 112, 1998.
3. Bels, P.J. and Pataragetvit, M., Edible mushroom in Thailand, cultivated by termites, in *Tropical Mushrooms — Biological Nature and Cultivation Methods,* Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 445–461, 1982.
4. Chang, S.T., Cultivation of *Volvariella* mushrooms in Southeast Asia, *Mushroom Newsl. Trop.,* 1(10), 5–10, 1980.
5. Chang, S.T., Mushroom production in Southeast Asia, *Mushroom Newsl. Trop.,* 1(2), 18–22, 1980.
6. Chang, S.T., Mushroom biology: the impact on mushroom production and mushroom products, in *Mushroom Biology and Mushroom Products*, Chang, S.A., Buswell, T.A. and Chiu, S.W., Eds., Chinese University Press, Hong Kong, 1983.
7. Chang, S.T., Global impact of edible and medicinal mushrooms on human welfare in the 21st century: nongreen revolution, *Int. J. Med. Mushrooms,* 1, 1–7, 1999.
8. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing, in China, *Int. J. Med. Mushrooms,* 1, 291–300, 1999.
9. Chang, S.T., The *Lentinula* mushroom industry: past, present and future, in *Proc. Biyang Int. Symp. Technology and Marketing of Lentinula edodes*, Luo, X.C., Ed., Government of Biyang County, China, 9–18, 2002.
10. Chang, S.T. and Buswell, J.A., Mushroom nutriceuticals, *World J. Microb. Biotechnol.*, 12, 473–476, 1996.
11. Chang, S.T. and Buswell, J.A., *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Aphyllophoromycetideae) — a mushrooming medicinal mushroom, *Int. J. Med. Mushrooms*, 1, 139–146, 1999.
12. Chang, S.T. and Mao, X.L., *Hong Kong Mushrooms* [in Chinese], Chinese University Press, Hong Kong, 470 pp., 1995.
13. Chang, S.T., and Miles, P.G., Introduction to mushroom science, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 3–10, 1982.
14. Chang, S.T. and Miles, P.G., A new look at cultivated mushrooms, *BioScience,* 34, 358–362, 1984.
15. Chang, S.T. and Miles, P.G., Historical record of the early cultivation of *Lentinus* in China, *Mushroom J. Trop.*, 7, 31–37, 1987.
16. Chang, S.T. and Miles, P.G., Mushroom biology: a new discipline, *Mycologist,* 6, 64–65, 1992.
17. Chang, S.T. and Mshigeni, K.E., *Mushrooms and Human Health*, University of Namibia, Windhoek, Namibia, 1–7, 2001.
18. Claridge, A.W., Cork, S.J., and Trappe, J.M., Diversity and habital relationships of hypogeous fungi, I. Study design, sampling techniques and general survey results, *Bioder. Conserv.* 9, 151–173, 2000.
19. Courtecuisse, R. and Duhem, B., *Mushrooms and Toadstools of Britain and Europe*, HarperCollins, London, 1994.
20. Eger-Hummel, G., Blue-light photomorphogenesis in mushrooms (Basidiomycetes), in *The Blue Light Syndrome*, Senger, H., Ed., Springer-Verlag, Berlin, 1980.
21. Esser, K., Effect of temperature and light on perithecial development in *Sordaria macrospora*, *Mycologia*, 72, 619–622, 1980.
22. Gerrits, J.P.G., Organic compost constituents and water utilized by the cultivated mushroom during spawn run and cropping, *Mushroom Sci*., 7, 111–126, 1969.
23. Hawker, L., Environmental influences on reproduction, in *The Fungi*, Vol. 2, Ainsworth, G.C. and Sussman, A.S., Eds., Academic Press, New York, 1966.

24. Hawksworth, D.L., The fungal dimension of biodiversity: magnitude, significance, and conservation, *Mycol. Res.,* 95, 641–655, 1991.
25. Hawksworth, D.L., Mushrooms: the extent of the unexplored potential, *Int. J. Med. Mushrooms,* 3, 333–337, 2001.
26. Hawksworth, D.L., The magnitude of fungal diversity: the 1.5 million species estimate revisited, *Mycol. Res*., 105, 1422–1432, 2001.
27. Hawksworth, D.L., Kirk, P.M., Sutton, B.C., and Pegler, D.N., *Ainsworth & Bisby's Dictionary of Fungi*, 8th ed., CAB International, Wallingford, U.K., 1995.
28. Hayes, W.A., Mushroom nutrition and the role of microorganisms in composting, in *Composting,* Hayes, W.A., Ed., W.S. Mancy & Sons, Leeds, U.K., 1977.
29. Huang, N.L., Present situation and prospects of edible fungi industry in China, *Edible Fungi of China* [in Chinese]*,* 104, 3–5, 2000.
30. Kurtzman, R.H., Jr., Mushrooms: single cell protein from cellulose, in *Annual Report* on *Fermentation Processes,* Vol. 3, Perlman, D., Ed., Academic Press, New York, 1979.
31. Lin, C.M., Special report of China Edible Fungi Association, *The Market of Edible Fungi* [in Chinese], 8, 5, 2002.
32. Lou, L.H., Cultivation of edible mushrooms in the tropical and subtropical regions of the Peoples Republic of China, *Mushroom Newsl. Trop.,* 1(2), 14–18, 1980.
33. Mao, X.L., The resource of Chinese mushroom biodiversity and its research and development, *The Market of Edible Fungi* [in Chinese], 8, 8, 2002.
34. Martin, J.F. and Demain, A.L., Fungal development and metabolite formation, in *The Filamentous Fungi*, Vol. 3, Smith, J.E. and Berry, D.R., Eds., Arnold, London, 1978.
35. Miles, P.G. and Chang, S.T., *Mushroom Biology — Concise Basics and Current Developments*, World Scientific, Singapore, 1997.
36. Pauli, G, *Breakthroughs: What Business Can Offer Society,* Epsilon Press, Surrey, U.K., 1996.
37. Royse, D.J. and Collopy, P.D., Genetic diversity in old and new world species of *Lentinula*, in *Proc. Biyang Int. Symp. Technology and Marketing of Lentinula edodes,* Luo, X.C., Ed., Government of Biyang County, China, 19–24, 2002.
38. Shepherd, C.J. and Totterdell, C.J., *Mushrooms and Toadstools of Australia*, Inkata Press, Sydney, Australia, 19–21, 1988.
39. van der Westhuizen, G.C.A. and Eicker, A., *Field Guide — Mushrooms of Southern Africa*, Struik, South Africa, 192–193, 1994.
40. Vedder, P.J.C., *Modern Mushroom Growing*, Educaboek, Culemborg, the Netherlands, 1978.
41. Wood, D.A., Microbial processes in mushroom cultivation; a large scale solid substrate fermentation, *J. Chem. Tech. Biotechnol*., 34B, 232–240, 1984.

2 The Nutritional Attributes of Edible Mushrooms

I. INTRODUCTION

Some mushrooms are a food of good nutritional value, some have medicinal value as dietary supplements, and there are some that have both of these properties. The public has become increasingly concerned about health and nutrition matters in recent years, and this has sparked the commercialization of natural foods consumed as dietary supplements. Mushrooms can be considered a functional food (medical food or nutritional food) in this way. Such functional or medical foods should not claim to cure disease, but there are an increasing number of scientific studies that strongly support some functional foods such as mushrooms as having a role in disease prevention and in some cases of bringing about a suppression or remission of a diseased state.

Since earliest times mushrooms have been treated as a special kind of food. The Greeks believed that mushrooms provided strength for warriors in battle. The Pharaohs prized mushrooms as a delicacy, and the Romans regarded mushrooms as a "Food of the Gods" and served them only on festive occasions. The Chinese treasured mushrooms as a health food, the "elixir of life." The Mexican Indians used mushrooms as hallucinogens in religious ceremonies and in witchcraft, as well as for therapeutic purposes. There is little doubt that early humans tried and tested mushrooms, and by trial and error became familiar with types worth collecting and those to avoid.

The fast-growing mushrooms have received a remarkable amount of interest in recent decades with the realization that they are a good source of delicious food with high nutritional attributes, and that some have medicinal values as well. The sources of energy food are carbohydrates and fats, and the sources of body-building materials are proteins. The accessory food factors, vitamins and inorganic compounds, together with water, are indispensable to good health. However, the greatest difficulty in meeting human nutritional requirements is to supply a sufficient amount of the body-building material — protein.

II. NUTRITIONAL ATTRIBUTES

Mushrooms may be consumed for their palatability and/or nutritional value. Palatability can be judged by color, texture, flavor, and taste, but the determination of nutritional value requires much scientific work. It involves the analysis of the proximate composition and a study of the spectrum of amino and fatty acids, vitamins, minerals, and nucleic acids present. It should be noted that the composition of a given species is affected by the diversity of its genetic makeup, which leads to strain differences, and by environmental conditions, including the nature of the substratum. Crisan and Sands[7] have pointed out that cultivation techniques, as well as the inaccuracies inherent in different methods of analyses, may also introduce variation into the analytical data. Furthermore, mushrooms, like other living organisms, continue to show variation in metabolism in their post-harvest life. This is in addition to the changes in composition relative to the stage of development and the manner of post-harvest storage. Thus it must be emphasized that the available information reported provides only a range of values for reference purposes. Even so, a comprehensive

discussion of several mushrooms, including the data from various workers, will be useful and meaningful for a general comparison. It is worth mentioning here that methods of chemical analysis of mushrooms have been reported by Lau,[15] who emphasizes the principles behind the methods, the procedure, and the precautions that need to be taken to obtain accurate results. Proximate analyses of different species of edible mushrooms and comparison with other food items are listed in Table 2.1 and Table 2.2, respectively.

Quantitative data relating to the nutritive value of mushrooms is sparse. In the absence of feeding trials, alternative methods have been used to determine or predict the nutritional value of foods based on their content of essential amino acids. The Essential Amino Acid Index (EAA Index) is measured according to dietary protein in terms of an essential amino acid pattern based on known adult human dietary requirements. The amino acid score (chemical score) is the amount of the most limiting amino acid in the food protein expressed as a percentage of the same amino acid present in the reference protein. In an attempt to resolve the difficulties inherent in comparisons between those mushrooms containing small amounts of high-quality protein with those containing larger amounts of a protein of lesser nutritional quality, Crisan and Sands[7] proposed in 1987 the use of a nutritional index calculated as follows:

$$\text{Nutritional Index} = \frac{(\text{EAA Index} \times \text{percentage protein})}{100}$$

The EAA Indexes, amino acid scores, and nutritional indexes for various mushrooms and other foods are shown in Table 2.2. In terms of EAA Indexes and amino acid scores, the most nutritive mushrooms (highest values) rank alongside meat and milk in potential nutritive value and considerably higher than most legumes and vegetables. The least nutritive mushrooms rank appreciably lower but are still comparable to some common vegetables.

In addition to their high-quality protein, mushrooms are a relatively good source of the following individual nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterol, and niacin. Fruiting bodies are low in calories, carbohydrates, and calcium, and the commonly cultivated mushrooms are reported to have a total lipid content between 0.6 and 3.1% on a dry weight basis. Unsaturated fatty acids, essential and significant to our diet and to our health, constitute at least 70% of the total fatty acid content.

The desirability of a food product is not necessarily correlated with its nutritional value. Appearance, taste, and aroma are often important in stimulating the appetite and determining preference. Thus, in addition to nutritional value, edible mushrooms possess unique characteristics in terms of color, taste, aroma, and texture, which make them attractive for human consumption.

A. Protein

Published values for the protein content of four popular edible mushrooms, *Agaricus bisporus, Lentinula edodes, Pleurotus* spp., and *Volvariella volvacea*, which are commercially cultivated in various countries, range from 1.75 to 3.63% of their fresh weight.[5] The value can be as high as 5.9%;[8] however, an average value of 3.5 to 4% would appear to be more representative. This means that the protein content of edible mushrooms, in general, is about twice that of asparagus and cabbage, and 4 times and 12 times those of oranges and apples, respectively. On a dry weight basis, mushrooms normally contain 19 to 35% protein (Table 2.1), as compared to 7.3% in rice, 13.2% in wheat, 39.1% in soybean, and 25.2% in milk. Therefore, in amount of crude protein, mushrooms rank below most animal meats but well above most other foods, including milk, which is an animal product. Detailed discussions of mushroom protein in reference to other foods have been given by Hayes and Haddad,[9] Crisan and Sand,[7] Bano and Rajarathnam,[3] and Li and Chang.[17]

TABLE 2.1
Proximate Composition of Cultivated Species of Edible Mushrooms

Species	Moisture	Crude Protein (N × 4.38)	Crude Fat	Carbohydrate		Crude Fiber	Ash	Energy Value	Ref.
				Total	N-free				
Agaricus bisporus	78.3–90.5	23.9–34.8	1.7–8.0	51.3–62.5	44.0–53.5	8.0–10.4	7.7–12.0	328–368	7
Agaricus campestris	89.7	33.2	1.9	56.9	48.8	8.1	8.0	354	7
Auricularia sp. (Philippine var.)	89.1	4.2	8.3	82.8	63.0	19.8	4.7	351	7
Boletus edulis	87.3	29.7	3.1	59.7	51.7	8.0	7.5	362	7
Flammulina velutipes	89.2	17.6	1.9	73.1	69.4	3.7	7.4	378	7
Lentinula edodes	90.0–91.8	13.4–17.5	4.9–8.0	67.5–78.0	59.5–70.7	7.3–8.0	3.7–7.0	387–392	7
Pleurotus eous	92.2	25.0	1.1	59.2	—	12.0	9.1	261	4
Pleurotus florida	91.5	27.0	1.6	58.0	—	11.5	9.3	265	4
Pleurotus ostreatus	73.7–90.8	10.5–30.4	1.6–2.2	57.6–81.8	48.9–74.3	7.5–8.7	6.1–9.8	345–367	7
Pleurotus sajor-caju	90.1	26.6	2.0	50.7	—	13.3	6.5	300	6
Volvariella diplasia	90.4	28.5	2.6	57.4	40.0	17.4	11.5	304	7
Volvariella volvacea	89.1	25.9	2.4	—	45.3	9.3	8.8	276	17

Note: All data are presented as percentage of dry weight, except moisture (percentage of fresh weight) and energy value (Kcal per 100 g dry weight).

TABLE 2.2
Comparison of Nutritive Value of Mushrooms with Various Foods

Essential Amino Acid Indexes		Amino Acid Scores		Nutritional Indexes	
100	Pork; chicken; beef	100	Pork	59	Chicken
99	Milk	98	Chicken; beef	43	Beef
98	Mushrooms (high)	91	Milk	35	Pork
91	Potatoes; kidney beans	89	Mushrooms (high)	31	Soybeans
88	Corn	63	Cabbage	28	Mushrooms (high)
86	Cucumbers	59	Potatoes	26	Spinach
79	Peanuts	53	Peanuts	25	Milk
76	Spinach; soybeans	50	Corn	21	Kidney beans
72	Mushrooms (low); cabbage	46	Kidney beans	20	Peanuts
69	Turnips	42	Cucumbers	17	Cabbage
53	Carrots	33	Turnips	14	Cucumbers
44	Tomatoes	32	Mushrooms (low)	11	Corn
		31	Carrots	10	Turnips
		28	Spinach	9	Potatoes
		23	Soybeans	8	Tomatoes
		18	Tomatoes	6	Carrots
				5	Mushrooms (low)

Note: Ranking based on essential amino acid indexes, amino acid scores, and nutritional indexes as calculated against the FAO reference protein pattern; biological values correlate closely with the EAA indexes. Values for mushrooms represent the mean of the three highest values (high) and three lowest values (low) as determined.[11]

Source: Data from Crisan, E.V. and Sands, A., in *The Biology and Cultivation of Edible Mushrooms,* Academic Press, New York, 1978.

B. Essential Amino Acids

Since estimation of crude protein is an indirect assay for total amino acids that is affected by the varying levels of nonprotein nitrogen present in the sample, the quantitative determination of total amino acids present after acid hydrolysis undoubtedly gives a more accurate evaluation.[22] The protein question also should be examined in reference to quality. Because proteins are made up from over 20 amino acids in varying amounts, they are quantitatively different. The human body can convert some of these amino acids into others, but there are nine essential amino acids (lysine, methionine, tryptophane, threonine, valine, leucine, isoleucine, histidine, and phenylalanine). Furthermore, these nine essential amino acids must be present simultaneously and in correct relative amounts for protein synthesis to occur. If one or more should be in inadequate supply, the utilization of all others in the cellular pool will be reduced in the same proportion. Food products of animal origin always provide a better-balanced and higher-quality protein than do plant foods, which often lack some of the important amino acids; e.g., cereal grains have too little of the essential amino acid lysine, and legumes usually lack the essential amino acids methionine and tryptophane. The proteins of commonly cultivated mushrooms contain all nine amino acids essential for humans (Table 2.3). For all mushrooms listed in Table 2.3, the most abundant essential amino acid is lysine, and the lowest levels among the essential amino acids are those of tryptophane and methionine. Altamura et al.[1] and Hughes et al.[13] have reported that various species of mushrooms contain, in addition to the common amino acids and amides, the less common amino acids and related nitrogenous compounds such as methionine sulfoxides, β-alanine, cystic acid, hydroxyprolines, aminoadipic acid, phosphoserine, cystathione, canavanine, creatinine, citrulline, ornithine, glucosamine, and ethanolamine.

TABLE 2.3
Essential Amino Acid Composition of Edible Mushrooms

Amino Acids	*Agaricus bisporus*	*Lentinula edodes*	*Pleurotus florida*	*Pleurotus ostreatus*	*Pleurotus sajor-caju*	*Volvariella diplasia*	*Volvariella volvacea*	Hen's Egg[a]
Leucine	7.5	7.9	7.5	6.8	7.0	5.0	4.5	8.8
Isoleucine	4.5	4.9	5.2	4.2	4.4	7.8	3.4	6.6
Valine	2.5	3.7	6.9	5.1	5.3	9.7	5.4	7.3
Trytophan	2.0	nd	1.1	1.3	1.2	1.5	1.5	1.6
Lysine	9.1	3.9	9.9	4.5	5.7	6.1	7.1	6.4
Threonine	5.5	5.9	6.1	4.6	5.0	6.0	3.5	5.1
Phenyalanine	4.2	5.9	3.5	3.7	5.0	7.0	2.6	5.8
Methionine	0.9	1.9	3.0	1.5	1.8	1.2	1.1	3.1
Histidine	2.7	1.9	2.8	1.7	2.2	4.2	3.8	2.4
Total essential amino acids[b]	38.9	36.0	46.0	33.4	37.6	48.5	32.9	47.1

Note: Data presented as grams of amino acids per 100 grams of corrected crude protein. nd = not determined.

Sources: Data from Bano, Z. and Rajarathnam, S., in *Tropical Mushrooms – Biological Nature and Cultivation Methods, Chinese University Press, Hong Kong*, 363–380, 1982; and Li, G.S.F. and Chang, S.T., in *Tropical Mushrooms – Biological Nature and Cultivation Methods*, Chinese University Press, Hong Kong, 199–219, 1982.

[a] For comparison.

[b] Excluding arginine and cystine.

C. Fat

The fat content in different species of mushrooms ranges from 1.1 to 8.3% on a dry weight basis, with an average content of 4.0% (Table 2.1). In general, the crude fat of mushrooms has representatives of all classes of lipid compounds including free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, sterol esters, and phospholipids. Huang et al.[12] reported that six commonly cultivated mushrooms have a higher percentage of saponifiable lipid than nonsaponifiable lipid (Table 2.4). The values for saponifiable lipid range from 78.1% in *Auricularia auricula* to 58.8% in *Volvariella volvacea*. The low percentage of saponifiable lipid found in *V. volvacea* is mainly due to the presence of unusually high contents of provitamin D_2 and ergosterol.[11] Expressed in terms of dry weight *A. bisporus* is 2.1% and *V. volvacea* is 1.7% in the saponifiable fraction.

At least 72% of the total fatty acids have been found to be unsaturated in each of these mushrooms (Table 2.5). The high content of unsaturated fatty acids is mainly due to linoleic acid, which, of the total fatty acids, accounts for 76% in *L. edodes*, 70% in *V. volvacea*, and 69% in *A. bisporus* (Table 2.6). These are three of the most commonly cultivated mushrooms. Unsaturated fatty acids are essential in our diet,[10] whereas saturated fatty acids, which are present in high amounts in animal fats, may be harmful to our health. The finding of a high proportion of unsaturated fatty acids and a high percentage of linoleic acid in these mushrooms is a significant factor in regarding mushrooms as a health food.

D. Vitamins

It has been reported that edible mushrooms are a good source for several vitamins including thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin, biotin, and ascorbic acid (vitamin C).[7] For illustrative purposes, the contents of thiamine, niacin, and riboflavin of some edible mushrooms will be given in mg per 100 g dry weight of mushroom. The thiamine content ranges from 0.35 mg in *V. volvacea*, to 1.14 mg in *A. bisporus*, to 1.16 to 4.80 mg in *Pleurotus* spp., to 7.8 mg in *L. edodes*. The niacin

TABLE 2.4
The Total Lipids, Saponifiable Lipids, and Nonsaponifiable Lipids in *V. volvacea* and Other Edible Mushrooms

Mushroom	Percentage of Lipids in Dry Weight				
	Total	Saponifiable		Nonsaponifiable	
Volvariella volvacea	3.0	1.76	(58.8)	1.24	(41.2)
Lentinula edodes					
Dongko (standard grade)	2.1	1.55	(73.7)	0.55	(26.3)
Hongshin (Koshin, Hyangshin)	2.0	1.39	(69.3)	0.61	(30.7)
Kwangtung Hongko (North Mushroom)	1.3	0.96	(73.6)	0.34	(26.4)
Cracky Dongko (best grade)	2.1	1.37	(65.0)	0.73	(35.0)
Agaricus bisporus	3.1	2.12	(68.3)	0.98	(31.7)
Pleurotus sajor-caju	1.6	1.08	(67.8)	0.52	(32.2)
Auricularia auricula	1.3	1.02	(78.1)	0.28	(21.9)
Tremella fuciformis	0.6	—	—	—	—

Note: Figures in parentheses represent the percent of total lipid.

Source: Data from Huang, B.H. et al., *Mushroom Sci*., 12, 1989.

TABLE 2.5
Saturated and Unsaturated Fatty Acids in *V. volvacea* and Other Mushrooms

Mushroom	% Distribution of Fatty Acids			
	Saturated		Unsaturated	
Volvariella volvacea	14.6	(0.44)	85.4	(2.56)
Lentinula edodes				
Dongko (standard grade)	19.9	(0.42)	80.1	(1.68)
Hongshin (Koshin, Hyangshin)	24.0	(0.48)	76.0	(1.52)
Kwangtung Hongko (North Mushroom)	27.9	(0.36)	72.1	(0.94)
Cracky Dongko (best grade)	20.4	(0.43)	79.6	(1.67)
Agaricus bisporus	19.5	(0.60)	80.5	(2.50)
Pleurotus sajor-caju	20.7	(0.33)	79.3	(1.27)
Auricularia auricula	25.8	(0.34)	74.2	(0.96)
Tremella fuciformis	22.8	(0.14)	77.2	(0.46)

Note: Figures in parentheses indicate percent of fatty acid per dry weight.

Source: Data from Huang, B.H. et al., *Mushroom Sci*., 12, 1989.

content varies from 54.9 mg in *L. edodes,* to 55.7 mg in *A. bisporus*, to 64.88 mg in *V. volvacea*, to 46.0 to 108.7 mg in *Pleurotus* spp. The riboflavin content was higher in *A. bisporus* (5.0 mg) and *L. edodes* (4.9 mg) than in *V. volvacea* (1.63 to 2.98 mg).

Lau et al.[18] reported that the ascorbic acid content of edible mushrooms can be easily and rapidly determined by the differential pulse polarographic method, and that among the four cultivated mushrooms whose vitamin contents are being described, *L. edodes* had the highest vitamin C content (9.4 mg/100 g dry sample). The values of the other mushrooms were 7.4 mg for *P. sajor-caju*, 1.8 mg for *A. bisporus*, and 1.4 mg for *V. volvacea*.

TABLE 2.6
Total Fatty Acid Composition of *V. volvacea* and Other Edible Species of Fungi

Mushroom	Fatty Acid (% of total fatty acids)					
	^{C}14:0	^{C}16:0	^{C}16:1	^{C}18:0	^{C}18:1	^{C}18:2
Volvariella volvacea	0.48	10.50	0.62	3.47	12.74	69.91
Lentinula edodes						
Dongko (standard grade)	0.07	15.81	2.51	3.01	5.65	67.79
Hongshin (Koshin, Hyangshin)	0.71	20.94	1.81	1.66	5.23	66.53
Kwangtung Hongko (North Mushroom)	0.83	20.51	3.56	3.21	6.53	53.62
Cracky Dongko (best grade)	0.13	11.31	1.88	2.07	5.27	76.25
Agaricus bisporus	0.86	11.75	1.32	5.36	3.57	69.22
Pleurotus sajor-caju	0.59	16.42	1.42	3.00	12.29	62.94
Auricularia auricula	0.69	17.30	1.12	7.35	31.60	40.39
Tremella fuciformis	0.09	17.20	2.37	3.11	38.83	27.98

Note: ^{C}14:0, myristic acid; ^{C}16:0, palmitic acid; ^{C}16:1, palmitoleic acid, ^{C}18:0, stearic acid; ^{C}18:1, oleic acid; ^{C}18:2, linoleic acid.

Source: Data from Huang, B.H. et al., *Mushroom Sci*., 12, 1989.

The presence of sterols in fungi has been reviewed by Weete and Weber.[23] γ-Ergosterol, provitamin-D_2, is the common sterol found in Basidiomycetes.[24] Huang et al.[11] reported the isolation and identification of sterols in the mushroom *V. volvacea* as well as in some other edible species (Table 2.7). Among the six species of Basidiomycetes studied, *V. volvacea* had the highest provitamin-D_2 content on a dry weight basis (0.47%), followed by *L. edodes* (0.27%) and *A. bisporus* (0.23%), and *Tremella fuciformis* had the least (0.01%). The mature stage of *V. volvacea* had a higher content of provitamin-D_2 than the egg stage. As expected, a higher content of provitamin-D_2 was found in the cap than the stalk, both in the mature and egg stages of *V. volvacea*. Since the

TABLE 2.7
Lipid and Sterol Content of *V. volvacea* and Other Species of Edible Fungi

Species	Total Lipid (% of dry material)	Sterols (% of dry material)[a]		
		I	II	III
Volvariella volvacea	3.0	0.47	0.05	0.35
Lentinula edodes				
Dongko (standard grade)	3.1	0.21	0.07	0.07
Hongshin (Koshin, Hyangshin)	2.0	0.15	0.08	0.06
Kwangtung Hongko (North Mushroom)	1.3	0.06	0.03	0.02
Cracky Dongko (best grade)	2.1	0.27	0.07	0.06
Agaricus bisporus	3.1	0.23	—	—
Pleurotus sajor-caju	1.6	0.13	0.09	0.07
Auricularia auricula	1.3	0.07	—	—
Tremella fuciformis	0.6	0.01	0.02	0.03

[a] I, provitamin-D_2; II, provitamin-D_4; III, γ-ergosterol.

Source: Data from Huang, B.H. et al., *Mycologia,* 77, 959–963, 1985.

stalk supports more weight than the cap, it would be expected that the stalk would have a greater percent of structural polysaccharides. The increase of these structural polysaccharides would, therefore, account for a lower percent of other cell constituents, e.g., sterols. The ergosterol present in mushrooms can be converted to vitamin D with ultraviolet irradiation.

E. Carbohydrate and Fiber

According to Crisan and Sands,[7] pentoses, methylpentoses, hexoses, as well as disaccharides, amino sugars, sugar alcohols, and sugar acids are constituents of mushroom carbohydrates. *Pleurotus* species contain carbohydrates,[3] ranging from 46.6 to 81.8% (as compared to 60% in *A. bisporus* on a dry weight basis). Recently, much interest has arisen in characterizing the components of water-soluble polysaccharides obtained from the fruiting bodies of mushrooms[25] because of their ability to inhibit the growth of tumors. A major fraction of the acidic polysaccharide designated as H51 is reported to have strong antitumor activity, and structurally this component consists of a skeleton of β(1,3)-linked glucose residues, probably having branches of galactose and mannose residues and also containing acidic sugars. The fiber content ranges from 7.4 to 27.6% in *Pleurotus* species (as compared to 10.4% in *A. bisporus*, and to 4 to 20% in *V. volvacea*). Fiber is considered to be an important ingredient in a balanced and healthy diet. Anderson and Ward[2] reported that feeding patients with diabetes with high-fiber diets reduces their daily insulin requirement and stabilizes their blood glucose profile, possibly by decreasing the rate of glucose absorption and/or delaying gastric emptying.

F. Minerals

Mushrooms are a good source of minerals. The minerals present in the substrate are taken up by the growing mycelium and translocated to the sporophores. As in higher plants, the mineral of highest content is potassium (K), followed by phosphorus (P), sodium (Na), calcium (Ca), and magnesium (Mg). These are considered to be the major mineral constituents, and copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), and cadmium (Cd) make up the minor mineral elements.[3,4,18]

It is calculated that the concentration of K, P, Na, Ca, and Mg constitute about 56 to 70% of the total ash content.[17] K is particularly abundant and accounts for nearly 45% of the total ash content. Na and Ca are present in approximately equal concentrations in all the mushrooms except for *L. edodes* in which Ca is present in an especially large amount.

The Cu content was higher in all species of *Pleurotus* examined than in the other edible mushrooms. The Cu content varied from 12.2 to 21.9 ppm for the *Pleurotus* species.

Ca and lead (Pb) contents varied from 0.3 to 0.5 ppm and from 1.5 to 3.2 ppm, respectively, in all species of *Pleurotus*.[4] Of all the heavy metals, the Zn content was highest in all species of *Pleurotus*. This is especially noteworthy since the Zn content of the straw substrate was low. Also to be noted is the fact that the concentrations of Pb, Cd, Cu, and Zn in the cultivated mushrooms are well under the prescribed limits of the Fruit Product Order and Prevention of Food Adulteration Act of 1954.[3]

G. Nucleic Acids

Microorganisms are characterized by a high nucleic acid content. Viikari and Linko[21] have reported that the nucleic acid content of microorganisms is in the range of 8 to 25% (dry weight), whereas those of algae and fungi are known to be 3 to 8% and 3.2 to 4.7% (dry weight), respectively.[14] For easy comparison, these are listed in Table 2.8 together with the nucleic acid content of some conventional foods. The practical safe limit of nucleic acid intake of most adult populations suggested by the Protein Advisory Group of the United Nations System[19] is a daily maximum of 4 g; only half the amount should be from a microbial source. This is because humans lack the urate

TABLE 2.8
Nucleic Acid Content of Some Edible Mushrooms and Foods

Sample	Nucleic Acid Content		Ref.
Yeast	6.0–12.0%	dry weight	14
Bacteria	8.0–16.0%	dry weight	14
Algae	3.0–8.0%	dry weight	14
Cereals	1.1–4.0	g/100 g protein	14
Meat and fish	2.2–5.7	g/100 g protein	14
Microorganisms	8.0–25.0	g/100 g protein	21
Mushrooms	2.7–4.1%	dry weight	15

TABLE 2.9
Nucleic Acid Content of Four Edible Mushrooms

Mushrooms	Percent Moisture	Percent Nucleic Acid Content (dry weight basis)[a]		
		DNA	RNA	Total
Agaricus bisporus	89.07	0.17 ± 0.01	2.49 ± 0.08	2.66
Pleurotus cystidiosus	92.63	0.37 ± 0.02	2.56 ± 0.10	2.93
Pleurotus sajor-caju	87.45	0.21 ± 0.02	3.85 ± 0.05	4.06
Volvariella volvacea	89.21	0.29 ± 0.01	3.59 ± 0.20	3.88

[a] $X \pm$ S.D.: mean ± standard deviation ($n = 6$).

Source: Data from Li, G.S.F. and Chang, S.T., *Eur. J. Appl. Microbiol. Biotechnol.*, 15, 237–240, 1982.

oxidase enzyme for the oxidation of uric acid, which is the sparingly soluble metabolic product of the purine bases guanine and adenine.[20] It is suspected that high plasma concentration of uric acid may result in precipitation of urate in tissues and joints and also leads to stone formation in the kidney and bladder. In 1967, the First International Conference on Single Cell Protein called attention to the problem of the high nucleic acid content of any rapidly growing cell. As given in Table 2.9, *Pleurotus sajor-caju* contains the highest amount of nucleic acids among the four edible mushrooms studied — 4.06% on a dry weight basis.[18] This is equivalent to 0.51% on a wet weight basis. Accordingly, it is safe to consume as much as 392.5 g of fresh *P. sajor-caju* daily. The limit could be even higher in the case of the other mushrooms with a lower nucleic acid content, or around 20% more after being cooked. Therefore, the content of nucleic acids in edible mushrooms should not be a limit to their use as a daily vegetable.

H. General Considerations

Analyses of the proximate composition of the commonly cultivated mushrooms reveal that edible mushrooms are rich in crude protein and carbohydrates, moderate in crude fiber and ash, and low in fat content. The energy values are low. They are a good source of essential amino acids, vitamins, and minerals. Potassium and phosphorus are the two dominant elements in the mineral portion. The mushrooms contain a substantial amount of thiamine, riboflavin, niacin, and provitamin D_2. In 100 g of crude protein (calculated as 4.38 × total nitrogen) there are 32 to 48 g of the nine essential amino acids. Of these, lysine is the most abundant, while tryptophane and

methionine are low. The nucleic acid content is within the range of other filamentous fungi and is much lower than that of the rapidly growing bacteria. It is considered to be safe to eat mushrooms as a daily vegetable.

REFERENCES

1. Altamura, M.R., Robbins, F.M., Andreotti, R.E., Long, L., Jr., and Hasselstrom, T., Mushroom ninhydrin-positive compounds, amino acids, related compounds and other nitrogen substances found in cultivated mushroom, *Agaricus compestris, J. Agric. Food Chem*., 15, 1040–1043, 1967.
2. Anderson, J.W. and Ward, K., High-carbohydrate high-fiber diets for insulin-treated men with diabetes mellitus, *Am. J. Clin. Nutr*., 32, 2312–2321, 1979.
3. Bano, Z. and Rajarathnam, S., *Pleurotus* mushroom as a nutritious food, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 363–380, 1982.
4. Bano, Z., Bhagya, S., and Srinivasan, K.S., Essential amino acid composition and proximate analysis of the mushrooms *Pleurotus eous* and *P. florida, Mushroom Newsl. Trop.*, 1(3), 6–10, 1981.
5. Chang, S.T., Mushrooms as human food, *BioScience*, 30, 399–401, 1980.
6. Chang, S.T., Lau, O.W., and Cho, K.Y., The cultivation and nutritional value of *Pleurotus sajor-caju, Eur. J. Appl. Microbiol. Biotechnol*., 12, 58–62, 1981.
7. Crisan, E.V. and Sands, A., Nutritional value, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 137–168, 1978.
8. Flegg, P.B. and Maw, G.A., Mushrooms and their possible contribution to world protein needs, *Mushroom J*., 48, 396–405, 1976.
9. Hayes, W.A. and Haddad, N., The food value of the cultivated mushroom and its importance to the mushroom industry, *Mushroom J*., 40, 104–110, 1976.
10. Holman, R.T., Significance of essential fatty acids in human nutrition, in *Lipids,* Vol. I, Paoletti, R., Poscellati, G., and Jasina, G., Eds., Raven Press, New York, 215–226, 1976.
11. Huang, B.H., Yung, K.H., and Chang, S.T., The sterol composition of *Volvariella volvacea* and other edible mushrooms, *Mycologia*, 77, 959–963, 1985.
12. Huang, B.H., Yung, K.H., and Chang, S.T., Fatty acid composition of *Volvariella volvacea* and other edible mushrooms, *Mushroom Sci*., 12.
13. Hughes, D.H., Lynch, D.L., and Somers, G.F., Chromatographic identification of the amino acids and carbohydrates in the cultivated mushroom *Agaricus campestris* L. ex. Fries, *J. Agric. Food Chem*., 6, 850–853, 1958.
14. Kihlberg, R., The microbe as a source of food, in *Annual Review of Microbiology*, Clifton, L.E., Raffel, S., and Starr, M.P., Eds., Annual Reviews, Palo Alto, CA, 427–466, 1968.
15. Lau, O.W., Methods of chemical analysis of mushrooms, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 87–116, 1982.
16. Lau, O.W., Shiu, K.K., and Chang, S.T., Determination of ascorbic acid in vegetables and fruits by differential pulse polarography, *J. Sci. Food Agric*., 36, 733–739, 1985.
17. Li, G.S.F. and Chang, S.T., Nutritive value of *Volvariella volvacea*, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 199–219, 1982.
18. Li, G.S.F. and Chang, S.T., The nucleic acid content of some edible mushrooms, *Eur. J. Appl. Microbiol. Biotechnol*., 15, 237–240, 1982.
19. Protein Advisory Group, Single-cell protein guideline No. 4, FAO/WHO/UNICEF Protein Advisory Group, United Nations, New York, 1970.
20. Scrimshaw, N.S., Single-cell protein for human consumption: an overview, in *Single Cell Protein*, Vol. 2, Tannenbaum, S.R. and Wang, D.I.C., Eds., MIT Press, Cambridge, MA, 4–12, 1975.
21. Viikari, L. and Linko, M., Reduction of nucleic acid content of SCP, *Proc. Biochem*., 12, 17–19, 1977.
22. Weaver, J.C., Kroger, M., and Kneebone, L.R., Comparative protein studies (Kjeldahl, dye binding, amino acid analysis) of nine strains of *Agaricus bisporus* (Lange) Imbach mushrooms, *J. Food Sci*., 42, 364–366, 1977.

23. Weete, J.D. and Weber, D.J., *Lipid Biochemistry of Fungi and Other Organisms*, Plenum Press, New York, 1980.
24. Yokokawa, H. and Mitsuhashi, T., The sterol composition of mushrooms, *Phytochemistry*, 20, 1349–1351, 1981.
25. Yoshioka, Y., Emori, M., Ikekawa, J., and Fukuoka, F., Isolation, purification and structure of components from acidic polysaccharides of *Pleurotus ostreatus* (Fr.) Quel., *Carbohydrate Res*., 43, 305–320, 1975.

3 Medicinal Value

I. INTRODUCTION

Mushrooms have long been considered to have medicinal value. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as a source of food. Humankind has constantly searched for new substances that can improve biological functions and thereby make people fitter and healthier. Recently, Western society has placed a great emphasis on plants, herbs, and foods as sources of these health enhancers. About 3.5 billion people worldwide, well over half of the world's population, rely on plant-based medicines and dietary supplements for their primary health care. These products have variously been called vitamins, dietary supplements, phytochemicals, nutraceuticals, and nutriceuticals. Dietary supplements[81] are ingredients extracted from foods, herbs, plants, and fungal species that are not used as a regular food but which boost the immune system or otherwise help maintain health. Phytochemical (phytonutrient) is a more recent evolution of the term that emphasizes the plant source of such protective disease-preventing compounds. **Nutraceuticals**, proposed by DeFelice in 1979 and quoted by Brower in 1988,[3] are foods that provide medical or health benefits, including the prevention and treatment of disease. The term nutraceutical has been redefined by Zeisel[81] as a diet supplement that delivers a concentrated form of a presumed bioactive agent from a food at dosage levels exceeding those that could be obtained from normal food. Nutraceuticals are present in a nonfood matrix and are used to enhance health. A mushroom **nutriceutical**[6] is a refined and partially defined extract from either the mycelium or the fruiting body of a mushroom, which is consumed in the form of capsules or tablets as a dietary supplement (not in the form of a food) and which has potential therapeutic application. During the past decade, there has been a major expansion in the industries involved in providing these substances, especially in the United States. In 1990, U.S. dietary supplement sales were valued at U.S. $3.3 billion; in 1992, U.S. $3.7 billion; in 1994, U.S. $5.0 billion; in 1996, U.S. $6.5 billion; in 1998, U.S. $12.0 billion; and in the year 2000, it was estimated to have reached U.S. $14.0 billion.[81]

In 1994, worldwide sales of medicinal mushrooms, mushroom extracts and various derived products were estimated at U.S. $3.8 billion.[5] By 1999, this figure had risen to U.S. $6.0 billion.[73] In regional terms, Asia and Europe accounted for approximately 99% of this market with North America contributing less than 0.1% to the overall total. In 1994, the U.S. market for mushroom-based dietary supplements had an estimated value of U.S. $35 million. However, since that time, demand in North America for medicinal mushrooms and derived products has increased by between 20 to 40% annually depending on the species. The use of mushroom extracts in nutriceutical products and sports drinks constitutes the main area of expansion.

II. MEDICINAL MUSHROOMS

Of the 14,000 to 15,000 species of mushrooms in the world,[21] around 700 have known medicinal properties. However, it has been estimated that there are about 1800 species of mushrooms that have potential medicinal attributes. Thus, mushrooms have vast prospects as sources of medicinals. These have been investigated in the last decade in *in vivo* and *in vitro* model systems. Many bioactive substances with immunomodulating effects have been isolated recently from mushrooms. These

include polysaccharides, high-molecular-weight polysaccharides, low-molecular-weight protein-bound polysaccharides, glycoproteins (lectins), triterpenoids, and fungal immunomodulatory proteins (Fips).[7,23,82] Many, if not all mushrooms, contain biologically active polysaccharides, which have antitumor and immunostimulating properties. As shown in Table 3.1, almost all the main taxonomic families of higher fungi have mushroom species that contain biologically active polysaccharides. The data reveal that there are 660 species from 37 taxa containing antitumor or immunostimulating polysaccharides. Fruiting bodies, submerged cultivated mycelial biomass, and liquid cultivated broth are the sources of the bioactive compounds. The majority (77.3%) of those compounds are extracted from fruiting bodies by hot water or by hot water combined with ethanol under various degrees of temperatures. The second source (20.8%) of those bioactive polysaccharides comes from cultivated mycelium, and the third origin (2.0%) is derived from liquid cultivated broth. So far, there are only two common medicinal mushrooms, *Ganoderma lucidum* and *Agaricus blazei,* which have been demonstrated to have the bioactive polysaccharides derived from these three sources — fruiting body, mycelium, and culture filtrate (Table 3.2).

Although bioactive polysaccharides are widespread among mushrooms, different species can produce polysaccharides with different types of polysaccharides having different properties. For example, the protein-bound polysaccharide PSK (trade name, Krestin) was developed in Japan from the cultured mycelium of *Coriolus versicolor* CM-101 strain. It is composed of 62% polysaccharides and 38% protein. The mean molecular weight is 94 kDa. The main component of the carbohydrate moiety is glucose, with galactose, mannose, xylose, and fucose as minor components,[51] whereas polysaccharide-peptide (PSP) in China was developed in submerged mycelium of the strain Cov-1 of the same species. It possesses a molecular weight of approximately 100 kDa and is composed of 90% polysaccharides and 10% peptides.[27] The carbohydrate moiety of PSP is mannose, xylose, galactose, arabinose, and rhamnose; however, PSK contains no arabinose or rhamnose, but contains fucose.[77] The protein moiety both of PSK and PSP is rich in acidic amino acids (such as aspartic acid, glutamic acid, etc.). Moreover, different origins (fruiting body, mycelium, liquid growth) of polysaccharides isolated from the same strain can have quite different chemical structures and functions. For example, the structures of polysaccharides isolated from *Agaricus blazei* are mainly β-glucan protein in the fruiting body, glucomannan protein in the mycelium, and mannan protein in the filtrate.[44]

Yang et al.[75] demonstrated that the exopolymer obtained from a liquid culture broth of *Ganoderma lucidum* enhanced the swimming endurance capacity of mice, but neither the material extracted from the fruiting body nor the endopolymer produced from cultured mycelium of the same species showed any positive response in this regard. The results also indicated the potential of *G. lucidum* exopolymer in exhibiting hypoglycemic, hypolipidemic, endurance enhancing, and immunomodulating activities in the experimental animals. Yang et al.[76] reported that the administration of the exopolymer (200 mg/kg body weight) obtained from submerged broth of *Lentinula edodes* reduced the plasma glucose level by as much as 21.5% and increased plasma insulin by 22.1%, as compared to the control group. It also lowered the plasma total cholesterol and triglyceride levels by 25.1 and 44.5%, respectively. As mentioned in the above section, more than 77% of all medicinal mushroom products are derived from the fruiting bodies, which have been either commercially farmed or collected from the wild. Only about 20% of all products are based on extracts from cultivated mycelia, and about 2% are from submerged broth used for growing mycelia. Mycelia formed by growing pure cultures in submerged conditions are of constant composition, and the submerged culture is the best technique for obtaining consistent and safe mushroom products. In addition, increased quality control of the mushroom products and year-round production under controlled conditions make mycelial-based products, including exopolymers from culture broth, the wave of the future.

Most of the knowledge about the medicinal properties of mushrooms comes from literature from the Far East, where such mushrooms as *G. lucidum* (Curt.: Fr.) P. Karst., *L. edodes* (Berk.) Sing., *Coriolus versicolor* (L.: Fr.) Quel., and *Tremella fuciformis* Berk. have been collected,

TABLE 3.1
Antitumor or Immunostimulating Polysaccharides Isolated from Mushrooms

Taxa	No. of Species	Origins of Bioactive Polysaccharides: Fruiting Body	Mycelial Biomass	Liquid Broth	Remarks
Auriculariales	3	3	3	—	
Dacrymycetetales	2	2	2	—	
Tremellales	9	7	9	—	
Cantharellaceae	7	7	1	—	
Clavariaceae	11	11	1	—	
Clavulinaceae	1	1	1	—	
Sparassidaceae	1	1	1	—	
Ramariaceae	5	5	—	—	
Hydnaceae	1	1	1	—	
Hericiaceae	4	4	3	—	
Corticiaceae	28	27	21	—	
Coniophoraceae	1	1	1	—	
Thelephoraceae	12	12	—	—	
Hymenochaetaceae	31	27	29	3	
Fistulinaceae	2	1	2	—	
Ganodermataceae	7	5	5	3	*G. lucidum* has all three origins
Polyporaceae	83	72	68	6	
Schizophyleaceae	1	—	—	1	
Lycoperdaceae	2	—	2	—	
Phallaceae	3	2	1	—	
Boletaceae	31	31	—	—	
Paxillaceae	4	4	2	—	
Strobilomycetaceae	4	4	—	—	
Gomphidiaceae	2	2	—	—	
Hygrophoraceae	38	38	—	—	
Pleurotaceae	4	4	1	1	*L. edodes* has all three origins
Tricholomataceae	113	112	9	1	
Entolomatacea	23	23	—	—	
Cortinariaceae	57	57	—	—	
Bolbitiaceae	15	15	1	—	
Strophariaceae	19	19	1	—	
Crepidotaceae	4	3	2	—	
Amanitaceae	24	24	—	—	
Pluteaceae	10	10	1	—	
Agaricaceae	32	32	2	1	*A. blazei* has all three origins
Coprinaceae	25	25	—	—	
Russulaceae	41	41	—	—	
Total 37	660	633	170	16	
Percentage (%)	—	77.2	21.8	2	

Sources: Data from Reshetnikov, S.V. et al., *Int. J. Med. Mushrooms*, 3, 361–394, 2001; Wasser, S.P. and Weis, A.L., *Int. J. Med. Mushrooms*, 1, 31–62, 1999; Liu, F. et al., *World J. Microb. Biotechnol*., 11, 486–490, 1995; Yang, B.K. et al., *J. Microbiol. Biotechnol*., 11, 902–905, 2001; Mizuno, T. et al., *Food Rev. Int*., 11, 23–61, 1995; and Yang, B.K. et al., *Biosci. Biotechnol. Biochem*., 66, 937–942, 2002.

TABLE 3.2
Bioactive Polysaccharides Isolated from Different Origins of Several Common Mushrooms

Species	Fruiting Body	Cultivated Mycelium	Culture Broth	Ref.
Lentinula edodes	++	+	+	10, 42, 76
Coriolus versicolor	+	++	—	66, 77, 81
Schizophyllum commune	—	—	++	28, 31
Ganoderma lucidum	++	+	+	47, 75, 82
Agaricus blazei	++	+	+	44
Hericium erinaceus	++	+	—	43
Volvariella volvacea	+	—	—	42, 47
Grifola frondosa	+	—	—	44, 83
Flammulina velutipes	++	+	—	44

Note: ++ High bioactive effects; + moderate bioactive effects; — not available.

cultivated, and used for thousands of years. In the past two decades, several pharmacologically active substances have been identified. Biologically active polysaccharides are the best-known mushroom-derived substances, which are particularly effective in retarding the progress of various cancers and other diseases through immune stimulation rather than direct cytocidal effects, and in alleviating the side effects of chemotherapy and radiation treatment through cell-level regenerative effects. Mizuno et al.[44] and Hobbs[22] have summarized these studies. The first three biologically active compounds developed from medicinal mushrooms were polysaccharides, and all of them are β-glucans. These are **krestin** (PSK) from *C. versicolor* mycelia containing (1→4), (1→3) or (1→4), (1→6)-β-D-glucan,[66] **lentinan**,[9,32] a high-molecular-weight (1→3)-β-D-glucan from *L. edodes* fruiting bodies; and **schizophyllan**,[28,31] a high-molecular-weight (1→3), (1→6)-β-D-glucan prepared from *Schizophyllum commune* culture filtrates. Another popular edible mushroom, *Flammulina velutipes* (Curt.:Fr.) P. Karst., also has high antitumor activity. Polysaccharides and a low-molecular-weight protein-bound polysaccharide (EA6) were isolated from this mushroom.[23] It was demonstrated that EA6 was active against tumors when administered orally, but was not so effective by intraperitoneal injection. These polysaccharides are of different chemical composition and the biochemical mechanisms for mediating their biological activity are still not clearly demonstrated. However, numerous studies have shown that regular consumption of certain mushroom species as either a regular food or as extracted compounds (nutriceuticals) is effective in both preventing and treating specific diseases, mainly through immunopotentiation and antioxidant activity. Thus, the intake of mushrooms and their extractable bioactive compounds appears to be effective in cancer prevention and growth inhibition. Another important fact is the certainty that mushroom extracts, compared with other drugs, show a very low toxicity when regularly consumed, even in high dosages. While the historical and traditional usage of the medicinal mushrooms, especially in the Far East, is extremely extensive,[22] attention is given in the following sections to a few of the modern approaches that have been verified in the last three decades by accurate scientific and medicinal studies.

III. EFFECTS OF MEDICINAL MUSHROOMS

A. Hematological Effects

Lectins are proteins or glycoproteins with specific binding sites for sugars. They are not antibodies or enzymes[16] but have a specific affinity toward glycosylated materials, and they have become

useful reagents in studies of cell surface structures.[34] Some lectins have been shown to have antitumor and immunomodulatory activities.[15,70–72] Furthermore, lectin–carbohydrate interactions represent a common and important feature of molecular events underlying the immune response.[55] The term lectin is used interchangeably with such terms as agglutinins, hemagglutinins, and phytohemagglutinins. The interaction between the fungal lectin and the surface glycoproteins of red blood cells is an example of the hematological activities of edible mushrooms, and some fungal lectins have been characterized and purified as described below.

The lectin isolated from *Agaricus campestris* is a tetramer with an estimated molecular weight of 64,000.[49] Its hemagglutination reaction is not inhibited by sugar but is inhibited by a sonic suspension of red cell ghosts.[50] The lectin purified from *Flammulina velutipes* was estimated to have a molecular weight of 20,000 and to be mitogenic with respect to mouse spleen lymphocytes in addition to its hemagglutination activities. This lectin does not contain carbohydrate, half-cystine, methionine, or histidine.[65]

Volvatoxin A is a lectin isolated from *Volvariella volvacea* and has been shown to reduce hemolytic activity toward Group O red blood cells.[36] This lectin is composed of two subunits with molecular weight of 50,000 and 24,000, with the hemolytic activity associated with the smaller subunit only. Another lectin was also isolated from *V. volvacea*. It has a molecular weight of 26,000 and has shown moderate hemagglutination toward Group O red blood cells.[35] This lectin is composed of two identical subunits and does not contain half-cystine, methionine, or histidine.

Pleurotolysin is a lectin from *Pleurotus ostreatus* and is a hemolytic agent for mammalian red blood cells *in vitro*. It is a dimer with a molecular weight of 24,100 and an isoelectric point at pH 6.4.[2]

B. Antiviral Effects

Cochran was the first to report that antiviral substances were present in mushrooms.[17] This encouraged the screening of aqueous extracts of some Japanese mushrooms. As a result of this program, it was found that an aqueous extract of the donko mushroom (*Lentinula edodes*) fruiting body, as well as the spores, contained antiviral activity against influenza A/SW15 virus infection in mice.[67] The antiviral influenzal activity was mediated by the induction of interferon on the host. A phenol fraction of the mushroom extract was capable of conferring the antiviral activity. These results suggested that perhaps the RNA fraction of the mushroom extract was inducing interferon since double-stranded RNA (ds-RNA) has been documented as capable of inducing interferon.[30]

Suzuki et al.[58] confirmed that the interferon-inducing activity is due to the ds-RNA from the spore extract of *L. edodes*. The origin of this ds-RNA was ascertained to be derived from the mycophages attached to the spore and the fruiting body.[60,69] It was further discovered that viruslike particles were present in several mushrooms — not only in *L. edodes*.[45]

Human immunodeficiency virus (HIV) was isolated as the etiological agent of acquired immunodeficiency disease syndrome (AIDS). Anti-HIV activity, an inhibitory effect on HIV replication *in vitro*, was reported from an extract of the culture medium of *L. edodes* mycelia.[67] Lentinan, a polysaccharide isolated from the fruiting body of this mushroom, has no ability to block HIV infection. However, sulfated lentinan completely prevented HIV-induced cytopathic effect.[78] PSK from *Coriolus versicolor* has also been reported to possess antiviral activities against ectromelia virus and cytomegalovirus infections.[66] Anti-HIV activities were reported in a water-soluble extract of *Ganoderma lucidum*.[29] Most recently, el-Mekkawy et al.[12] isolated the anti-HIV compounds and reported them as ganoderiol F and ganodermanontriol. Min et al.[41] also isolated anti-HIV components as ganoderic acid β, ganodermanondiol, ganoderanotriol, and ganolucidic acid A, and lucidumol B. Zhuang and Mizuno[83] reported that sulfated *Grifola frondosa* extract was able to prevent as much as 97% HIV-infected T-helper lymphocytes from being destroyed *in vitro*. This is important because measuring the T-helper cell count is one way to trace the progression of HIV to full-blown AIDS.[68]

C. Antitumor Effects

The viruslike particles from *Lentinula edodes* are even able to suppress the Ehrlich ascites carcinoma in mice.[61] Antitumor activity is not unique to *L. edodes*, as it is also present in other mushrooms.[24,31] However, since the late 1970s, most research effort has focused on studies of *L. edodes*. Much literature has accumulated about the antitumor activity of *L. edodes*, with most information concerned with the identification and purification of the responsible ingredients, but not with the mechanism of action.

Chihara et al.[9,10] reported that a water-soluble polysaccharide fraction from a fruiting body of *L. edodes* could inhibit the growth of mouse Sarcoma 180 in mice, and even complete regression was observed in Swiss albino mice. The dosages for bioassay ranged from 1 to 10 mg/kg body weight and were given intraperitoneally.

Fujii et al.[14] isolated and characterized a new antitumor polysaccharide, KS-2, which was excreted from the cultured mycelia of *L. edodes*. KS-2 suppressed Sarcoma 180 as well as Ehrlich ascites carcinoma in mice when given orally or intraperitoneally; moreover, it could induce interferon production in mice.

Sugano et al.[57] even detected the anticarcinogenic actions of water-soluble and alcohol-insoluble fractions from the culture medium of *L. edodes* mycelia. The alcohol-insoluble fraction was composed of a xylose-containing polysaccharide and protein. Ehrlich ascites carcinoma in mice was used as a bioassay system in this study.

Antitumor polysaccharides from mushrooms have been extensively studied during the last 15 years. These polysaccharides are found in the fruiting bodies, cultured mycelia, and culture broth of mushrooms as described in Table 3.1 and Table 3.2. They vary in chemical composition, structure, and antitumor activity as shown in Table 3.3. Many mushroom polysaccharides are present mainly as glucans with different types of glycosidic linkages such as (1→3), (1→6)-β-glucans, and (1→3)-α-glucans, but some are true heteroglycans. The others mostly bind to protein residues as PSK and PSP. The main sources for antitumor polysaccharides are from mushroom cell walls that consist of chitin and cellulose. However, mushroom chitin does not have any antitumor activity.[44]

In general, high-molecular-weight glucans appear to be more effective than those with low molecular weight. However, obvious variations of antitumor polysaccharides are also noted. Antitumor polysaccharides may have other chemical structures, such as hetero-β-glucan, heteroglycan,

TABLE 3.3
Bioactive Polysaccharides Isolated from Mushrooms Having Antitumor and Anticancer Effects

Species	Tumor or Cancer Treated	Polysaccharide Origin[a]	Polysaccharide Structure	Ref.
Coriolus versicolor	Cancer of digestive organ, lung and breast	CM	(1→4), (1→3) or (1→4), (1→6)-β-D-glucan	25, 66
Lentinula edodes	Cancer of stomach	FB	(1→3)-β-D-glucan	10, 32
Schizophyllum commune	Cervical cancer	CB	(1→3), (1→6)-β-D-glucan[b]	31
Ganoderma lucidum	Antitumor	FB, CM		44, 47
Agaricus blazei	Antitumor	FB, CM	(1→6)-β-glucan	44
Grifola frondosa	Antitumor	FB	(1→6), (1→3)-β-D-glucan	83
	Antitumor	CM	(1→3), (1→6)-β-D-glucan	

[a] CM, culture mycelium; FB, fruiting body; CB, culture broth.

[b] (1→3)-β-D-Glucans including glucurono-β-D-glucan, arabinoxylo-β-D-flucan, xylo-β-D-glucan, mano-β-D-glucan, and xylomanno-β-D-glucan, as well as their protein complexes.

β-glucan-protein, and heteroglycan-protein complexes.[47] In conclusion, the antitumor components of mushrooms vary in their chemical nature and include polysaccharides, proteins, glycoproteins, and triterpenoids. It does appear that the inclusion of cultivated mushrooms, particularly *L. edodes*, *Grifola frondosa*, *Agaricus blazei,* and *Pleurotus* spp., in the diet is likely to provide some protection against some diseases, particularly some tumors.

D. Antioxidant Activity

As noted previously, recent years have seen a steady expansion of the mushroom industry as it relates to the medicinal properties of mushrooms. The ability of mushroom-derived preparations (MDPs) to prevent oxidative damage to cellular DNA has been evaluated using the single-cell gel electrophoresis ("Comet") assay.[56] MDPs were obtained from fruiting bodies of nine common mushrooms. These showed wide variation in their ability to protect against oxidative DNA damage with the highest protection afforded by an MDP obtained by cold water extraction of *Agaricus bisporus* fruiting bodies (Ab-cold) and with the next highest protection obtained by hot water (100°C) extract of *Ganoderma lucidum* (Gl-hot). Jones and Janardhanan[26] reported also that the methanol and aqueous extracts of *G. lucidum* showed a marked free radical scavenging activity.

These findings indicate that some edible mushrooms consist of a valuable source of biologically active compounds with potential for protecting cellular DNA from oxidative damage. Such protective compounds have possible commercial value as dietary supplements for offsetting adverse biological effects associated with coronary heart disease, cancer, and age-related neurodegenerative diseases. They might also facilitate the development of treatments for the repair of indiscriminate cellular DNA damage that occurs during certain forms of chemotherapy and radiotherapy.[4]

E. Cardiovascular and Renal Effects

Volvatoxin A is a cardiotoxic protein from *Volvariella volvacea*.[36] In isolated toad hearts the toxic protein caused ventricular systolic arrest at a dose of 0.1 mg/ml; in cats an intravenous injection of the protein produced changes in the electrocardiogram (ECG) at a dose of 0.7 mg/kg body weight. It depressed the ST segment and inverted the T wave, but no significant changes in blood pressure were noted.

Chronic ingestion of *Lentinula edodes* was reported to reduce the serum cholesterol level in human subjects[59] (hypertension is attributable to a high serum cholesterol level). An antiplatelet substance was isolated from the aqueous extract of *Auricularia polytricha*. This antiplatelet substance could inhibit platelet aggregation.[20] This substance was later identified to be adenosine,[39] and was suggested to be responsible for the low incidence of arteriosclerosis among Asians who consumed *A. polytricha* regularly.

An aqueous extract from *Pleurotus sajor-caju* was associated with a hypotensive action that could reduce the glomerular filtration rate (GFR) in rats.[62] The hypotensive action was mediated by interfering with the renin–angiotensin system like a converting enzyme inhibitor. The clinical implication of a GFR-reducing effect is to reduce the rate of nephron deterioration and thus extend the life span of patients with chronic renal failure, but the mechanism of action is unknown. Bailey et al.[1] reported that *Coprinus comatus* can lower blood glucose in mice. Normal mice were fed a diet containing powdered dried fruiting bodies of *C. comatus* (33.3% w/w). Plasma glucose concentrations were reduced after 11 days, and intraperitoneal glucose tolerance was improved.

Furthermore, the aqueous extract of *V. volvacea*[11] has been reported to produce a hypotensive effect in normotensive rats. Feeding powdered maitake (*Grifola frondosa*) mushrooms to spontaneous hypertensive rats resulted in a lowering of the blood pressure.[33] It has also been reported that when the dried powder of two other edible mushrooms*, A. auricula* and *Tremella fuciformis*, was fed to the rats, they effectively lowered both the serum total cholesterol and the low-density lipoprotein (LDL) cholesterol levels.[8] Because the mushroom did not affect the concentration of

serum high-density lipoprotein (HDL) "good" cholesterol, the reduction of serum total cholesterol by the mushroom diets is attributable to the fall in the LDL "bad" cholesterol.

F. Carcinogenicity of Mushrooms

The chronic toxicity of mycotoxin is closely related to hepatocarcinogenicity — e.g., aflatoxin b from *Aspergillus flavus*.[45] Chronic toxicity is rare in edible mushrooms but not non-existent, and *Agaricus bisporus* is included in this group. This commonly eaten cultivated mushroom of commerce in the Western hemisphere contains a chemical that has a nitrogen–nitrogen bond-containing-chemical called agaritine. The breakdown product of agaritine, 4-(hydroxymethyl) benzenediazonium tetrafluoroborate, is gastric tumorigenic in Swiss albino mice when given orally at a dose of 400 μg/g of body weight.[64]

G. Allergic Reaction to Spores

Allergic reactions to spores from *Pleurotus ostreatus* and other species of *Pleurotus* were reported in the early 1970s, both in West Germany[79,80] and in Great Britain.[52,54] Several years later *Pleurotus* spores as allergens were further studied.[18,80]

Recently, the incidence of *Pleurotus* growers developing respiratory problems was documented in Canada.[13] Severe symptoms reoccurred within 30 minutes to 1 hour on each of three subsequent exposures to spores of *P. ostreatus,* but not to mushroom compost or other mushroom species. The symptoms generally included fatigue, mild headaches or sinus pressure, coughing, mild difficulty in breathing, pain in the limbs and joints, and a generalized malaise or ill feeling. These influenza-like symptoms included a fever of 39 to 40°C, which lasted from 1 to 2 days up to 1 week, but then disappeared without treatment.[46] Similar symptoms associated with a *Pleurotus* spore allergy were also reported in China.

By use of the radio-allergo-sorbent test (RAST) to determine the level of antibody present in the blood of allergic persons, Halmepuro et al.[19] suggested that *P. ostreatus* spore allergens share antigenic determinants with those present in *P. ostreatus* cap or mycelia. More details of the role of basidiospores in the allergic reactions have recently been reported.[38,46,53]

IV. GENERAL CONSIDERATIONS

There is no doubt that mushroom-based products can serve as superior dietary supplements, particularly, the *Ganoderma* products, which have been used as a dietary supplement or medicinal food in China for more than 2000 years. Recently, the products of medicinal mushrooms have been demonstrated to enhance the immune system and promote the natural defense system. They are also good for patients who have received treatment with radiotherapy or chemotherapy, as they may help to reduce the side effects from such therapies. These include (1) increasing the number of leucocytes in the blood, and enhancing the immune functions (it has been known that chemotherapy treatment can markedly deplete the number of platelets in the blood and put patients at risk of excessive bruising, internal bleeding, brain hemorrhage, and sometimes death); (2) increasing and improving appetite; (3) reducing pain; (4) anti-emetic properties; (5) stopping hair loss; (6) inducing tumor regression; (7) potential antioxidant and genoprotective properties, and (8) general health-improving effects. Exactly how these products work is still a matter of conjecture, but numerous trials (both laboratory and human) have shown, again and again, that they are effective in complementing conventional medicines in fighting diseases. People unfamiliar with the field may ask, "If those mushrooms have such beneficial effects, can chemists isolate the active component so that it can be marketed as a drug?" If the answer is "yes," the main focus is on a single active component of the mushroom, e.g., lentinan, which is close to drug standard, and on certain diseases, e.g., cancers or heart diseases only. The products are prescription drugs. If the answer is

"no," then the main focus is on a group of compounds, and on people's quality of life. The products are dietary supplements and are not single active components. Instead, there are many of them. They all contribute to the beneficial effects of the mushroom. For example, the protection against cancer afforded by *Ganoderma* products could be attributed to several compounds present in *Ganoderma*: the tetero-glucans, lectins, terpenoids, steroids, nucleic acids, and immunomodulatory proteins, such as Ling Zhi-8. It seems that these compounds, and possibly other compounds yet to be identified in *Ganoderma*, contribute in concert to the anticancer, antitumor, antiviral, antibacterial, and immunomodulating properties of *Ganoderma*. This means that the synergistic effects of several components in an extract are responsible for the therapeutic or prophylactic properties rather than a single active chemical ingredient. It could be tentatively concluded that mushroom products (mushroom nutriceuticals) are of multifunctional value.

At the end of 1999, the number of children orphaned by AIDS in Sub-Saharan Africa, stood at 12.1 million, compared to 1.1 million children orphaned by AIDS in the rest of the world. In several countries in the SADC region (e.g., Botswana, Lesotho, Namibia, South Africa, and Zimbabwe), some 20 to 30% of 15- to 25-year-olds are living with HIV/AIDS. With the well-established fact that *Ganoderma* and other medicinal mushrooms (e.g., *Lentinula edodes* and *Grifola frondosa*) can be farmed on available natural substrates and are known to be very effective in promoting the body's immune systems, including them as a dietary supplement to HIV-positive individuals and AIDS victims is a reasonable approach. The financial challenge involved in cultivation and preparation of the products is manageable, as the world market for these mushrooms is very lucrative.

The current intense industrial interest worldwide in medicinal mushroom materials has resulted in a huge increase in the number of products reaching the retail market in recent years. The medicinal effect and health benefits of qualified mushroom products should not be doubted. However, a major problem associated with mushroom-based dietary supplements is their wide variability and the current lack of standard production and testing protocols necessary to guarantee product quality.[6,40] Unfortunately, less reputable manufacturing companies have exploited the fact that quality control regulations are virtually nonexistent in order to market poorly defined products of questionable efficacy. However, it is generally recognized that the situation is changing and that companies, in particular those marketing their products in the major growth areas of North America and Western Europe, will shortly be required to (1) back up efficacy claims with hard scientific data and (2) provide clear evidence of product quality control. Therefore, there is serious need for improvement in both quality and regulatory controls in the area.[96] Both are essential to increase and maintain consumer confidence, protect public health, and to meet current and future quality and safety criteria set by the regulatory authorities.

The research achievements in medicinal mushrooms during the last two decades give the impression, and the confidence, that medicinal mushrooms have much to offer to the health-care system for humans in the 21st century. In cases where modern medicines may not provide a complete remedy, complementation by mushroom nutriceuticals may augment the success of the treatment. Prevention of diseases is beneficial to everyone and deserves the same attention that is given to the curing of diseases. Efforts directed to disease prevention can have positive financial and social impact and, on the individual basis, can maintain or even improve quality of life and human dignity.

REFERENCES

1. Bailey, C.J., Turner, S.L., Jakeman, K.J., and Hayes, W.A., Effect of *Coprinus comatus* on plasma glucose concentration in mice, *Planta Med.*, 50, 525–526, 1984.
2. Bernheimer, A.W. and Avigad, L.S., A cytolytic protein from the edible mushroom, *Pleurotus ostreatus, Biochem. Biophys. Acta*, 585, 451–461, 1979.
3. Brower, V., Nutraceuticals: poised for a healthy slice of the healthcare market? *Nat. Biotechnol.*, 16, 728–731, 1988.

4. Buswell, J.A. and Chang, S.T., Potential antioxidant/genoprotective properties of *Agaricus bisporus*, *Mushroom J.*, 625, 6, 2002.
5. Chang, S.T., Mushroom research and development-equality and mutual benefit, in *Mushroom Biology and Mushroom Products*, Royse, D.J., Ed., Pennsylvania State University, University Park, chap. 1, 1996.
6. Chang, S.T. and Buswell, J.A., Mushroom nutriceuticals, *World J. Microb. Biotechnol.*, 12, 473–476, 1996.
7. Chang, S.T. and Buswell, J.A., *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae) — a mushrooming medicinal mushroom, *Int. J. Med. Mushrooms,* 1, 139–146, 1999.
8. Cheung, P.C.K., The hypocholesterolemic effect of two edible mushrooms: *Auricularia auricula* and *Tremella fuciformis* in hypercholesteremic rats, *Nutr. Res.*, 16, 1721–1725, 1996.
9. Chihara, G., Hamuro, J., Maeda, Y., Arai, Y., and Fukuoka, F., Fractionation and purification of the polysaccharides with marked antitumor activity especially Lentinan, from *Lentinus edodes, Cancer Res*., 30, 2776–2781, 1970.
10. Chihara, G., Maeda, Y., Humuro, J., Sasaki, T., and Fukuoka, F., Inhibition of Mouse Sarcoma 180 by polysaccharides from *Lentinus edodes, Nature,* 222, 687–688, 1969.
11. Chiu, K.W., Lam, A.H.W., and Pang, P.K.T., Cardiovascular active substances from the straw mushroom, *Volvariella volvacea*, *Phytother. Res*., 9, 93–99, 1995.
12. el-Mekkawy, S., Meselhy, M.R., Nakamura, N., Tezuka, Y., Hattori, M., Kakiuchi, N., Shimotohno, K., Kawahata, T., and Otake, T., Anti-HIV-protease substances from *Ganoderma lucidum*, *Phytochemistry*, 49, 1651–1657, 1998.
13. Florence, S., Toogood, J.H., and Anderson, J., Mushroom pickers' lung associated with *Pleurotus ostreatus* (PO), *J. Allergy Clin. Immunol*., 77, 169 (abstr.), 1986.
14. Fujii, T., Maeda, H., Suzuki, F., and Ishida, N., Isolation and characterization of a new antitumor polysaccharide, KS-2, extracted from culture media of *Lentinus edodes, J. Antibiot*., 31, 1079–1090, 1978.
15. Ganguly, C. and Das, S., Plant lectins as inhibitors of tumour growth and modulators of host immune response, *Chemotherapy*, 40, 272–278, 1994.
16. Goldstein, I.J., Hughes, R.C., Monsigny, M., Osawa, T., and Sharon, N., What should be called a lectin? *Nature*, 285, 66, 1991.
17. Goulet, N.R., Cochran, K.W., and Brown, C., Differential and specific inhibition of ECHO viruses by plant extracts, *Proc. Soc. Exp. Biol. Med*., 103, 96–98, 1960.
18. Grabbe, K. and Zadrazil, F., Ein Betrag zur Notwendigkeit des Arbeitsschutzes bei der Kultivierung höherer Pilze, *Mushroom Sci*., 9(2), 227–238, 1977.
19. Halmepuro, L., Weissman, D., Olson, J., Salvaggio, J.E., and Lehrer, S.B., Characterization of *Pleurotus ostreatus* spore antigens/allergens, *J. Allergy Clin. Immunol*., 77, 201, 1986.
20. Hammerschmidt, D.C., Szechwan purpura, *N. Engl. J. Med*., 302, 1191–1193, 1980.
21. Hawksworth, D.L., Mushrooms: the extent of the unexplored potential, *Int. J. Med. Mushrooms,* 3, 333–337, 2001.
22. Hobbs, C., *Medicinal Mushrooms*, Interweave Press, Loveland, CO, 1995.
23. Ikekawa, T., Beneficial effects of edible and medicinal mushrooms on health care, *Int. J. Med. Mushrooms*, 3, 291–298, 2001.
24. Ikekawa, T., Nakanishi, M., Vehara, N., Chihara, G., and Fukuoka, F., Antitumor action of some Basidiomycetes especially *Phellinus linteus, Gann*, 59, 155–157, 1968.
25. Ishikawa, K., Majima, T., and Ebina, T., Anti-tumour effect of a Coriolus preparation, PSK: induction of macrophage chemotactic factor (MCF) in spleens of tumour bearing mice, *Biotherapy*, 5, 251–258, 1992.
26. Jones, S. and Janardhanan, K.K., Antioxidant and antitumor activity of *Ganoderma lucidum* (Curt.:Fr.) P. Karst.-Reishi (Aphyllophoromycetideae) from South India, *Int. J. Med. Mushrooms*, 2, 195–200, 2000.
27. Jong, S.C. and Yang, X.T., PSP-A powerful biological response modifier from the mushroom *Coriolus versicolor*, in *Advanced Research in PSP*, Yang, Q.Y., Ed., Hong Kong Association for Health Care Ltd., Hong Kong, Intro. II, 1999.
28. Kikumoto, S., Miyajima, T., Kimura, K., Okubo, S., and Komatsu, N., Polysaccharide produced by *Schizophyllum commune*. Part II: Chemical structure of an extracellular polysaccharide, *Nogei Kagaku*, 45, 162–168 [in Japanese], 1971.

29. Kim, H.W., Shim, M.J., Choi, E.C., and Kim, B.K., Inhibition of cytopathic effect of human immunodeficiency virus-I by water-soluble extract of *Ganoderma lucidum*, *Arch. Pharmacol. Res*., 20, 425–431, 1997.
30. Kleinschmid, T.W.J., Biochemistry of interferon and its inducers, *Annu. Rev. Biochem*., 41, 576–597, 1972.
31. Komatsu, N., Okubo, S., Kikumoto, S., Kimura, K., Saito, G., and Sakai, S., Host-mediated antitumor action of Schizophyllan a glucan produced by *Schizophyllum commune, Gann*, 60, 137–144, 1969.
32. Kosaka, A. and Yamashita, A., Lentinan as an effective immunomodulator against breast cancer, with special reference to clinical applications, *Int. J. Immunother,* 9, 111–116, 1993.
33. Kyoko, A., Nanba, H., Otsuka, M., and Kuroda, H., Blood pressure lowering activity present in the fruit body of *Grifola frondosa* (Maitake) 1, *Chem. Pharmacol. Bull.*, 36, 1000–1006, 1988.
34. Liener, I.E., Protease inhibitors and lectins, *Int. Rev. Biochem*., 27, 97–122, 1979.
35. Lin, J.Y. and Chou, T.B., Isolation and characterization of a lectin from edible mushroom, *Volvariella volvacea, J. Biol. Chem*., 96, 35–40, 1984.
36. Lin, J.Y., Teng, T.W., Shi, G.Y., and Teng, T.C., Isolation of a new cardiotoxic protein from edible mushroom *Volvariella volvacea, Nature*, 246, 524–525, 1973.
37. Liu, F., Ooi, V.E.C., and Chang, S.T., Anti-tumour components of the culture filtrates from *Tricholoma* sp., *World J. Microb. Biotechnol.*, 11, 486–490, 1995.
38. Lopez, M., Butcher, B.J., Salvaggio, J.E., Olson, J.A. Reed, M.A., McCants, M.L., and Lehrer, S.B., Basidiomycete allergy: what is the best source of antigen? *Int. Arch. Allergy Appl. Immunol*., 77, 169–170, 1985.
39. Markhija, A.N. and Bailley, J.M., Identification of the antiplatelet substance in Chinese black tree fungus (letter), *N. Engl. J. Med*., 304, 175, 1981.
40. Miles, P.G. and Chang, S.T., *Mushroom Biology — Concise Basics and Current Developments*, World Scientific, Singapore, 164, 1997.
41. Min, C.N., Nakamura, N., Miyashiro, H., Bae, K.W., and Hattori, M., Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-I protease, *Chem. Pharm.. Bull.*, 46, 1607–1612, 1998.
42. Mizuno, T., Bioactive biomolecules of mushrooms: food function and medicinal effect of mushroom fungi, *Food Rev. Int.*, 11, 7–21, 1995.
43. Mizuno, T., Bioactive substances in *Hericium erinaceus* (Bull.:Fr.) Pers. (Yamabushitake), and its medicinal utilization, *Int. J. Med. Mushrooms*, 1, 105–119, 1999.
44. Mizuno, T., Saito, H., Nishitoba, T., and Kawagishi, H., Antitumour-active substances from mushrooms, *Food Rev. Int.,* 11, 23–61, 1995.
45. Mori, K., Kuida, K., Hosokawa, D., and Takehara, M., Virus-like particles in several mushrooms, *Mushroom Sci*., 10(Part I), 773–787, 1978.
46. Olson, J.A., *Pleurotus* spores as allergens, *Mushroom J*., 172, 115–117, 1987.
47. Ooi, V.E.C. and Liu, F., A review of pharmacological activities of mushroom polysaccharides, *Int. J. Med. Mushrooms*, 1, 195–206, 1999.
48. Reshetnikov, S.V., Wasser, S.P., and Tan, K.K., Higher Basidiomycota as a source of antitumor and immunostimulating polysaccharides (Review), *Int. J. Med. Mushrooms*, 3, 361–394, 2001.
49. Sage, H.J. and Connett, S.L., Studies on a hemagglutinin from the meadow mushroom, *J. Biol. Chem*., 244, 4713–4719, 1969.
50. Sage, H.J. and Vazquez, J., Studies on a hemagglutination from the mushroom *Agaricus compestris, J. Biol. Chem*., 242, 120–125, 1967.
51. Sakagami, H. and Takeda, M., Diverse biological activity of PSK (Krestin), a protein-bound polysaccharide from *Coriolus versicolor* (Fr.) Quel., in *Mushroom Biology and Mushroom Products*, Chang, S.T., Buswell, J.A., and Chiu, S.W., Eds., Chinese University Press, Hong Kong, chap. 25, 1993.
52. Sakula, A., Allergy to the spores of *Pleurotus florida, Lancet*, 1, 137, 1974.
53. Santilli, J., Jr., Rockwell, W.S., and Collins, R.P., The significance of the spores of the Basidiomycetes (mushrooms and their allies) in bronchial asthma and allergic rhinitis, *Ann. Allergy*, 55, 469–471, 1985.
54. Schultz, K.H., Hansen, B.M., and Noster, U., Allergy to the spores of *Pleurotus florida, Lancet*, 1, 625, 1974.
55. Sharon, N. and Lis, H., Lectins: cell-agglutinating and sugar-specific proteins, *Science*, 177, 949–959, 1972.

56. Shi, Y.L., James, A.E., Benzie, I.F.F., and Buswell, J.A., Mushroom-derived preparations in the prevention of H_2O_2-induced oxidative damage to cellular DNA, *Teratogenesis Carcinogenesis Mutagenesis*, 22, 103–111, 2002.
57. Sugano, N., Hibino, Y., Choji, Y., and Maeda, H., Anticarcinogenic actions of water-soluble and alcohol-insoluble fractions from culture medium of *Lentinus edodes* mycelia, *Cancer Lett*., 17, 109–114, 1983.
58. Suzuki, F., Koide, T., Tsunoda, A., and Ishida, N., Mushroom extract as an interferon inducer (I) Biological and physiological properties of spore extracts of *Lentinus edodes, Mushroom Sci*., 9(Part I), 509–520, 1973.
59. Suzuki, S. and Oshima, S., Influence of Shii-ta-ke (*Lentinus edodes*) on human serum cholesterol, *Mushroom Sci*., 9(Part I), 463–467, 1976.
60. Takehara, M., Kuida, K., and Mori, K., Antitumor effect of virus-like particles from *Lentinus edodes* (shiitake), *Arch. Virol*., 59, 269–274, 1979.
61. Takehara, M., Mori, K., Kuida, K., and Hanawa, M.A., Antitumor effect of virus-like particles from *Lentinus edodes* on Ehrlich ascites carcinoma in mice, *Arch. Virol*., 68, 297–301, 1981.
62. Tam, S.C., Yip, K.P., Fund, K.P., and Chang, S.T., Hypotensive and renal effects of an extract of the edible mushroom *Pleurotus sajor-caju, Life Sci*., 38, 1155–1161, 1986.
63. Tochikura, T.S., Nakashima, H., Ohashi, Y., and Yamamoto, N., Inhibition (*in vitro*) of replication and of the cytopathic effect of human immunodeficiency virus by an extract of the culture medium of *Lentinus edodes* mycelia, *Med. Microbiol. Immunol.* (Berlin), 177, 235–244, 1988.
64. Toth, B., Nagel, D., and Ross, A., Gastric tumorigenesis by a single dose of 4-(hydroxymethyl)benzenediazonium ion of *Agaricus bisporus, Br. J. Cancer*, 46, 417–422, 1982.
65. Tsuda, M., Purification and characterization of a lectin from the mushroom, *Flammulina velutipes, J. Biol. Chem*., 86, 1463–1468, 1979.
66. Tsukagoshi, S., Hashimoto, Y., Fujii, G., Kobayashi, H., Nomoto, K., and Orita, K., Krestin (PSK), *Cancer Treat. Rev.,* 11, 131–155, 1984.
67. Tsunoda, A. and Ishida, N., A mushroom extract as an interferon inducer, *Ann. N.Y. Acad. Sci*., 173, 719–726, 1969.
68. U.S. National Cancer Institute, *In vitro* anti-HIV drug screening results, developmental therapeutics program, Jan. 17, 1992.
69. Ushiyama, R., Nakai, Y., and Ikegami, M., Evidence for double-stranded RNA from polyhedral virus-like particles in *Lentinus edodes* (Berk.) Sing, *Virology*, 77, 880–883, 1971.
70. Wang, H.X., Ng, T.B., Liu, W.K., Ooi, V.E.C., and Chang, S.T., Isolation and characterization of two distinct lectins with antiproliferative activity from the cultured mycelium of the edible mushroom *Tricholoma mongolicum, Int. J. Peptide Protein Res.*, 46, 508–513, 1995.
71. Wang, H.X., Ng, T.B., Ooi, V.E.C., Liu, W.K., and Chang, S.T., Action of lectins from the mushroom *Tricholoma mongolicum* on macrophages, splenocytes and life-span in sarcoma-bearing mice, *Anticancer Res.*, 17, 415–420, 1997.
72. Wang, H.X., Ooi, V.E.C., Ng, T.B., Chiu, K.W., and Chang, S.T., Hypotensive and vasorelaxing activities of a lectin from edible mushroom *Tricholoma mongolicum*, *Pharmacol. Toxicol.*, 79, 318–323, 1996.
73. Wasser, S.P., Nevo, E., Sokolov, D., Reshetnikov, S.V., and Timor-Tismenetsky, M., Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations, *Int. J. Med. Mushrooms*, 2, 1–19, 2000.
74. Wasser, S.P. and Weis, A.L., Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (Review), *Int. J. Med. Mushrooms*, 1, 31–62, 1999.
75. Yang, B.K., Jeong, S.C., Park, J.B., Cho, S.P., Lee, S.D., Yun, J.W., Lim, W.J., and Song, C.H., Swimming endurance capacity of mice after administration of exo-polymer produced from submerged mycelial culture of *Ganoderma lucidum*, *J. Microbiol. Biotechnol.*, 11, 902–905, 2001.
76. Yang, B.K., Kim, D.H., Jeong, S.C., Das, S., Choi, Y.S., Shik, J.S., Lee, S.C., and Song, C.H., Hypoglycemic effect of a *Lentinus edodes* exo-polymer produced from a submerged mycelial culture. *Biosci. Biotechnol. Biochem.*, 66, 937–942, 2002.
77. Yang, Q.Y., A brief introduction to the research of PSP, in *1993 PSP International Symposium*, Yang, Q.Y. and Kwok, C.Y., Eds., Fudan University Press, Shanghai, China, intro. II, 1993.

78. Yoshida, O., Nakashima, K., Yoshida, T., Kaneko, Y., Yamamoto, I., Matsuzaki, K., Uryu, T., and Yamamoto, N., Sulfation of the immunomodulating polysaccharide lentinan: a novel strategy for antivirals to human immunodeficiency virus (HIV), *Biochem. Pharm.*, 37, 2887–2891, 1988.
79. Zadrazil, F., *Pleurotus*-Spores-Allergie, *Champignon*, 13, 9–10, 1973.
80. Zadrazil, F. and Kurtzman, R.H., Jr., The biology of *Pleurotus* cultivation in the tropics, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 277–298, 1982.
81. Zeisel, S.H., Regulation of "nutraceuticals," *Science,* 285, 1853–1855, 1999.
82. Zhou, S. and Gao, Y., The immunomodulating effects of *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Ling Zhi, Reishi mushroom) (Aphyllophoromycetideae), *Int. J. Med. Mushrooms*, 4, 1–11, 2002.
83. Zhuang, O. and Mizuno, T., Biological responses from *Grifola frondosa* (Dick.:Fr.) S.F. Gray-Maitake (Aphyllophoromycetideae), *Int. J. Med. Mushrooms*, 1, 317–324, 1999.

4 Overview of the Biology of Fungi

I. INTRODUCTION

Before beginning a consideration of special topics dealing with edible mushrooms and their cultivation, one should have a basic understanding of the biology of fungi in general. Those readers who already have such an understanding may skim through this chapter, pausing only long enough to become familiar with the terminology that we have elected to use. Readers who have never had formal instruction in a course in mycology (the study of the fungi) or read carefully in mycological textbooks covering classification (taxonomy), structure (morphology), function (physiology), inheritance (genetics), as well as the multitudinous and often complex patterns of reproduction exhibited by fungi, will find that reading this section makes for an easier comprehension and appreciation of all that follows.

II. THE FUNGI

A. Distinguishing Characteristics

What are the fungi? This question can be answered in many ways, some of which are more meaningful than others to individuals of various backgrounds and having different interests. They are the yeasts used in the making of beer and wine because of their fermentative activities, and the molds that are used for the commercial production of citric and other organic acids, and for the production of penicillin as well as the flavoring of many cheeses. They are causal agents of most of the diseases of plants and many diseases of humans. Several fungi are responsible for destruction of food in transit or storage and manufactured goods made of many different substances, but especially wood. Last, but not least, the fungi are important as a source of food.

It is not surprising that the fungi affect us in so many ways because they constitute a fairly large group of organisms. Some 69,000 species of fungi have been described, and estimates indicate that approximately 1.5 million species exist.

The most visible form, because of size, that fungi take, and the one of most concern to the readers of this book, is the mushroom — a fleshy, spore-bearing organ. The term *mushroom* is commonly applied to edible members of the gilled fungi, but we shall not restrict its use so closely. We define a mushroom as a macrofungus with a distinctive fruiting body of sufficient size to be seen by the naked eye and to be picked up by hand. In our definition mushrooms need not be Basidiomycetes, nor aerial, nor fleshy, nor edible. Although the greatest use of mushrooms has always been for their gastronomic and nutritional appeal, there has also been a long history of the use of certain mushrooms for their medicinal or tonic effects. In the present edition of this book, the increasing interest in medicinal mushrooms and mushroom products is examined and evaluated critically.

The fungi (sing., fungus) are eukaryotic organisms that have a cell wall that commonly contains the polysaccharide chitin along with other polysaccharides, lipids, and proteins. Some reproduce

sexually, and all reproduce asexually, in a variety of ways. Fungi lack chlorophyll and consequently cannot carry on photosynthesis. Therefore, nutritionally the fungi are described as saprophytic if they obtain nutrients from nonliving organic materials, or as parasitic if they obtain nutrients from living organisms. It should be mentioned that the fungi are different from other heterotrophic organisms in that they do not ingest their food, but obtain it by absorbing it into their cells by what is sometimes referred to as osmotrophic nutrition. This, then, is the nature of the fungi.

B. Habitats

Next, we consider where fungi are located. The answer to this is simple. They are all over! Fungal spores are present in the air. It is well known that fungi can be found in just about every conceivable terrestrial situation as well as in aquatic habitats — both fresh water and marine. This location in the air, on land, and in the sea tells us that fungi are truly ubiquitous. As a generalization, we can say that few, if any, substrates are free from fungi. Even if they are not breaking down the substrate for nutrients, they may take up a position there and make use of endogenous nutrients or take advantage of whatever nutrients are sent their way.

C. Role in Nature

If they are everywhere, and we might add, in large numbers, what is their role in nature? Fungi, along with bacteria, are the primary agents of decomposition in nature. This, of course, means that they are important in the cycling of a variety of elements such as carbon, nitrogen, and oxygen.

D. Classification

Although fungi share many characteristics with other living organisms, they are also unique in many respects. In recent years this uniqueness has received greater attention than it did in 1753 when Linnaeus placed the algae and fungi together in the division Thallophyta in the Kingdom Planta. This classification of the algae and fungi in the division Thallophyta lasted for some 200 years. Yet the photosynthetic algae and the nonphotosynthetic fungi obviously differ fundamentally in their nutrition and metabolism, and in modern times specialists became unhappy in the implied close relationship of these two groups of organisms.

There were those who recognized that all organisms did not fall easily into either of the two kingdoms, plant and animal, and a somewhat "catch-all" group, the Protista, has been used with some justification for the protozoans, simple algae, and the various types of slime molds. The members of the Protista that are cellular are mostly unicellular.

With the advent of the electron microscope and other modern techniques for studying organisms at the subcellular level, it became evident that there is a fundamental division of organisms based on the structure of the nucleus, the nuclear membrane, and various organelles. As a consequence of these differences, some scientists now divide the living world into two superkingdoms, the Prokaryonta and the Eukaryonta.[7]

The Eukaryonta have a true nucleus with membrane and membrane-bound organelles (e.g., mitochondria), all of which are lacking in the Prokaryonta. In the Prokaryonta is the Kingdom Monera, containing the bacteria, actinomycetes, and the blue-green algae. Currently, there is great interest in a kingdom called the Archaea, whose members seem to be closely related to the Prokaryonta, because members of the Archaea are thermophilic and are present in deep-sea, high-temperature water vents in oceans. In the Eukaryonta are placed four kingdoms — the protist, the animal, the plant, and the fungal. The latter bears the name of Kingdom Myceteae. Why should there be a separate kingdom for the fungi? What are the major characteristics of the fungi?

First of all, the fungi are eukaryotic. As such, they have certain characteristics common to both plants and animals. Unlike the members of the plant kingdom, the fungi are acholorophyllous. Lacking the ability to carry on photosynthesis, the fungi are thus dependent for their energy and

structural needs on organic materials, either from nonliving sources or from living hosts. As previously mentioned, in the former case we refer to the fungi as saprophytic, and in the latter, parasitic. Animals do not carry on photosynthesis either, but in their nutrition most fungi do not ingest food in the manner of animals, but more commonly secrete enzymes that break down complex insoluble or less-soluble food materials into smaller, soluble materials that can be absorbed into the vegetative fungal cells. This is sometimes referred to as absorptive nutrition, or it is said that nutritionally the fungi are osmotrophic.

The fungi have a cell wall, but the composition is different from the cell wall of plants in which cellulose and lignin are the main components. Glucans and chitin are the principal polysaccharides found in fungal cell walls, with cellulose found only in the walls of the class of fungi known as the Oomycetes.

Certainly, the presence of a cell wall in fungi is a plantlike characteristic, but the composition of the walls of plants and fungi are quite different, and when we consider their roles in nature (plants carrying out photosynthesis and fungi breaking down organic substrates and recycling carbon dioxide, thereby making it available again for photosynthesis by plants), it is apparent that the roles are opposite. Plants convert radiant energy to chemical energy. Fungi break down chemical compounds with the release of energy and the liberation of carbon dioxide.

Now that we have attempted to justify the placement of the fungi in a kingdom of their own, let us examine the structural features of the fungi.

III. VEGETATIVE STRUCTURE OF FUNGI

In terms of structure, it is the usual practice to divide the fungi into two groups — unicellular and filamentous. The more numerous are the filamentous fungi. The filament is a tubular structure called a hypha (pl., hyphae). These filaments grow only at the tips or at specialized regions where branches arise. Through branching, and in some species by means of anastomosis or fusion of hyphae, a network of these filaments is formed, which is called a mycelium.

A. Hyphae

The fact that hyphae grow at the tip has been known for some time from simple microscopic observation of the tip cell. Such observations reveal that the tip cell increases in length but those cells farther back that are delimited by septa do not. However, it has been demonstrated that materials essential for tip growth are not synthesized in the tip cell alone but are brought to the tip cell from cells farther back in the hypha.

In hyphal tip growth new cell wall formation and an increase in the cell membrane must take place. For this to occur, the cell wall at the tip must be slightly plastic. As the hypha grows, the cell wall at the tip is constantly undergoing some lysis as well as the formation of new cell wall. Autoradiographic studies with tritiated-labeled cell wall precursors have conclusively demonstrated that hyphal growth does occur at the tip. In addition, observations have been made from electron microscopic studies that permit hypotheses regarding the mechanism of apical growth. Clusters of vesicles (dictyosomes) are observed near the apex. These vesicles are probably derived from the Golgi apparatus, which, in turn, originated from the endoplasmic reticulum. It is suggested that these vesicles are driven to the apex by turgor pressure from cells farther back in the hypha that have become increasingly vacuolate. Other forces, for example, electrophoretic movement and contractile systems using microtubules or microfilaments, have also been hypothesized. Enzymes for wall lysis that break down the bonds of the wall components are presumably contained in these secretory vesicles. This means that the wall at the tip can expand by making use of the materials required for the formation of new cell walls and plasma membranes that are also contained in the vesicles. When the apex is reached, the vesicles coalesce and fuse with the plasma membrane. The wall now stretches due to pressure from within the hypha and the vesicles containing synthesizing

enzymes fuse with the plasma membrane. The new wall components form from precursors that have moved across the plasma membrane.

1. Coenocytic Hyphae

In some fungi the hyphae are coenocytic. This multinucleate condition stems from the fact that the coenocytic species lack cross walls, called septa (sing., septum), or else have septa only where the vegetative hypha is converted into a structure of different function, such as a stalk-bearing sporangia (i.e., a sporangiophore) or a stalk-bearing conidia (i.e., a conidiophore).

2. Septate Hyphae

Other species of filamentous fungi, those in the Ascomycetes, Basidiomycetes, and most of the Deuteromycetes, are septate. In septate fungi the cross walls divide the hyphae into what are commonly referred to as "cells," although we should remember that there are pores in the septa through which cytoplasmic connections occur, and organelles have been observed to move from one cell to the next in certain species.

A more careful study of an individual hypha will reveal that this cylindrical tube has walls that enclose a multinucleate protoplasm, and new cell wall material is laid down at the tips. Outstanding features of the fungal hypha are tip growth and the presence of a cell wall. The structure of the cell wall, its formation, its localized dissolution when hyphae fuse, and its chemical composition are important aspects of fungal biology, which can only be touched on in this overview.

B. Organelles

Next to the cell wall on the inside lies the outermost part of the cytoplasm, the cell membrane, and within this are found various organelles and inclusions. One of these, the nucleus, has a membrane and commonly one nucleolus. Vacuoles, another type of membrane-bounded organelle, may be conspicuous in the fungal hypha. This is especially the case in the older parts of the hypha, i.e., those regions at a distance from the tip where growth occurs. Mitochondria, another membrane-bounded organelle (the fungi are truly eukaryotic), are present. When the living hypha is examined by phase contrast microscopy, the mitochondria are frequently found to be clustered in regions where one would expect great metabolic activity to be taking place, such as in the region of tip growth, or where branches are originating, or where nuclear division is taking place. A diagrammatic representation of a fungal hypha to indicate location of these organelles is shown in Figure 4.1. This drawing represents a septate hypha such as is found in members of the Ascomycetes, Basidiomycetes, and most of the Deuteromycetes (Fungi Imperfecti). Septa are not of regular occurrence in the Zygomycetes or those filamentous members of other "Lower Fungi" such as Chytridiomycetes and Oomycetes. Septa do sometimes occur in these classes on aging or where a specialized structure is formed.

A few comments should be made about the fungal nucleus. Most studies of nuclear cytology of the fungi have been concerned with meiosis as seen in the ascus or basidium, and, until two or three decades ago, little attention was directed to the somatic nucleus. This was because the diploid nucleus is larger than the haploid somatic nucleus whose chromosomes frequently approach in size the limits of resolution of the light microscope. As a consequence of this basic difficulty imposed by the small size of the somatic nucleus, there are conflicting opinions in the literature about the mitotic process in fungal somatic cells. In some cases spindles have been reported; in other cases spindles are absent. In the single species, *Neurospora crassa*, an ascomycete, three methods for the division of the somatic nucleus have been reported — and it is conceivable that under certain conditions and locations more than one type of division may occur.

The electron microscope has been of tremendous help in resolving many problems of fungal cytology. As with other organisms the electron microscope has provided us with information about

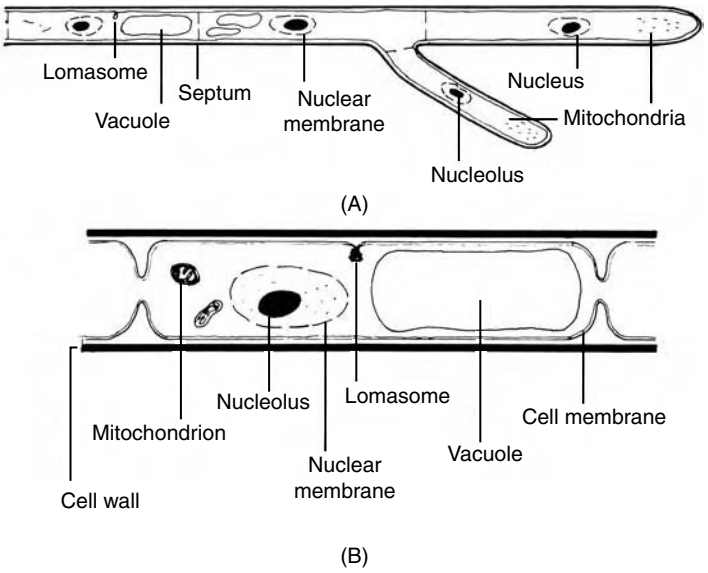


FIGURE 4.1 Diagrammatic representation of fungal hypha indicating location of various organelles. (A) Tip segment of hypha; (B) enlarged hyphal cell.

membranes and about wall structure. The fungal nuclear membrane is known to consist of two layers and to have pores at intervals.

C. Septal Structures

Several types of septa are found in the hyphae of fungi. In the Ascomycetes, and most of the Deuteromycetes, there is an ingrowth of the plasma membrane with new cell wall formation, and the end result is a septum that tapers toward a central pore (Figure 4.2).

In the Basidiomycetes a more complex septal structure is found. Here there is a swelling around the pore region, and this is referred to as a dolipore septum (Figure 4.3). Over these, on each side of the septum, are dome-shaped structures, the septal pore caps or parenthesomes, which are

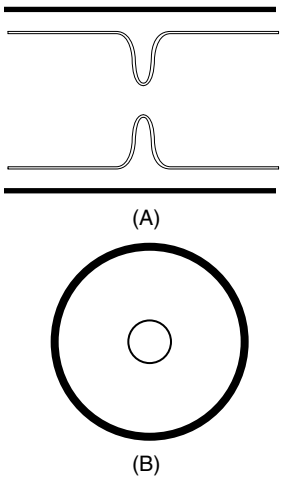


FIGURE 4.2 Diagrammatic representation of septum of Ascomycetes. (A) Longitudinal section showing septum; (B) transverse section through septum.

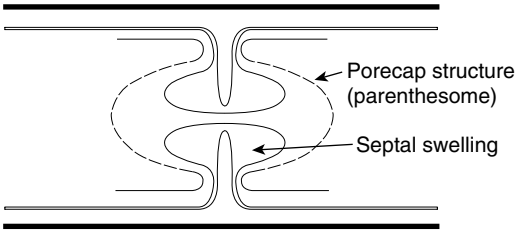


FIGURE 4.3 Diagrammatic representation of dolipore septum of Basidiomycetes (longitudinal sectional view).

perforate membranes continuous with the endoplasmic reticulum. The origin of this type of septum and considerations of mechanisms by which nuclei can migrate through such septa have occupied the attention of a number of mycologists. There are other types of septa in certain species. For example, a completely nonperforate septum is found in *Basidibolus ranarum*, and a septum with micropores is present in *Geotrichum*.

A body called the lomasome is evidently quite common in fungi. The lomasome is located between the plasma membrane and the cell wall (see Figure 4.1), appearing as spheres or variously shaped bodies along the border. They are apparently derived from the plasma membrane, but their function has not been established with certainty.

D. Secondary Mycelium of Basidiomycetes

Because the majority of edible fungi that are cultivated are Basidiomycetes, we now describe what is sometimes referred to as the secondary mycelium of the Basidiomycetes, because this is a very unique condition found in many, but not all, species of this class. The mycelium derived from a single spore is called the primary mycelium, while the mycelium formed following fusion of the two compatible primary mycelia is called the secondary mycelium.

The secondary mycelium is made up of hyphae, which bear clamp connections in which the dikaryotic condition is maintained. The dikaryon is a pair of unfused compatible nuclei, not just any two nuclei that would give a binucleate condition, but by definition two compatible nuclei. The hyphae of the secondary mycelium maintain this dikaryotic condition by the process of clamp connection formation, which is now described.

When a migrant nucleus from a compatible strain travels through a resident hypha and reaches a tip cell, the tip cell becomes dikaryotic — it has two unfused compatible nuclei. Near the position on the hypha where the two nuclei are located, a small hyphal outgrowth appears and then grows and bends back away from the tip. This growth is the so-called hook cell. Before the hook cell recurves onto and fuses with the main hypha, the two nuclei undergo a simultaneous (sometimes called conjugate) division, and one of the daughter nuclei moves into the hook cell. A daughter nucleus moves away from the tip to the region of the hypha near where the hook cell has approached the main hypha. Compatible nuclei are located in the tip region of the hypha. Now septa form across the main hypha where the hook cell has emerged and across the hook cell at that same location. Next, the hook cell fuses with the penultimate cell and the nucleus that was in the hook cell passes into the penultimate cell. (See Chapter 6, Figure 6.2.)

The structure that is formed by the hook cell is called a clamp connection. Clamp connection formation maintains the dikaryotic condition in the tip cell where growth is taking place. Since the nuclei are large relative to the diameter of the hypha, the clamp is thought to provide a by-pass, which permits the dikaryotic condition to be maintained.

Fusion of the nuclei does not normally occur until the fruiting body with its basidia is formed, and thus the dikaryotic condition (hyphae with clamp connections) is maintained in nature and can be perpetuated in culture for prolonged periods. It should be noted at this time that the dikaryon

is a special type of heterokaryon in which compatible nuclei are maintained in a 1:1 ratio by the process of clamp connection formation, and in genetic complementation the dikaryon functions in the manner of a diploid nucleus.

At room temperature most species of Basidiomycetes that have been studied form a new clamp connection about every 60 minutes, and the process of simultaneous nuclear division takes approximately 2 to 3 minutes. That is, when observed by phase contrast microscopy, the length of time from the disappearance of the dikaryon to the appearance of four daughter nuclei is about 2 to 3 minutes. (The nucleoli are the conspicuous nuclear structures in phase contrast microscopy, and these disappear during nuclear division.) Because it is normally the dikaryotic mycelium that gives rise to fruiting bodies, most studies of nutrition and most physiological investigations have made use of dikaryotic mycelia.

E. Cell Walls

The early work on fungal cell wall chemistry was based primarily on cytochemical studies, which attempted to identify the principal cell wall constituent. It was learned that certain taxonomic groups contained chitin, and others cellulose, and it was thought that both were not present in the wall of a single fungus. Later studies, using more modern techniques, demonstrated that there are species in which both chitin and cellulose are present in the same fungal wall.

With development of techniques that provide a greater resolution, information about details of fungal cell wall chemistry increased. The earlier studies made use of chemical digestion of the cell walls. With this technique it is impossible to be certain that only cell wall materials and all of the cell wall materials are present for the final analysis. With the use of mechanical methods for breaking open the cells for subsequent isolation of the cell walls has come greater accuracy in analysis of the cell wall.

When microscopic examination of stained preparations of cell wall fragments indicates that breakage of the cells is complete, it is necessary to separate the cell walls from the rest of the cellular material. This is done by washing and centrifugation.

Determination of the chemical nature of the cell wall polysaccharides cannot be made if the polysaccharide is reduced to its constituent monosaccharide units, for we would know nothing of how the units are linked together. For example, in the basidiomycete *Schizophyllum commune* it has been found that there are two different polysaccharides made up of glucose units, and hence called glucans. That there are two glucans in the cell wall of *S. commune* was revealed by differences in solubility in alkali. One of the glucans is soluble in cold alkali, and the other is not. Thus, the soluble glucan is called S-glucan, and the glucan that is resistant to cold alkali is called R-glucan. The determination of the structure of the glucans requires knowledge of how the components are linked to one another and their branching.

If a glucan is hydrolyzed severely (e.g., by treatment with 6 *N* HCl for 18 hours in sealed tubes at 110°C, or by treatment overnight with concentrated sulfuric acid followed by dilution to 1 *N* and boiling at 100°C), the result will be the recovery of the monosaccharide glucose without learning anything about linkages. A mild acid hydrolysis (e.g., 1 *N* HCl at 100°C for 1 to 6 hours) will break linkages and produce monosaccharides. The desired result in hydrolysis is to obtain disaccharides, as their linkages are known.

Our concern with polysaccharides might lead one to believe that polysaccharides were the only chemical compounds found in fungal cell walls. This is not the case, although glucose — usually as glucans, $\beta(1\rightarrow4)$ cellulose, $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ glycogen, $\beta(1\rightarrow3)$ and $\beta(1\rightarrow6)$ yeast glucan — constitutes from 80 to 90% of the cell wall material of many species, and glucosamine (in chitin) constitutes from 1 to 58% (range of values), usually 5 to 20%. Other substances found in the fungal cell walls may include lipids (extremes reported, 1 to 8%), protein (extremes reported, 3.5 to 13%), and ash (0.5 to 2.5%). Certain fungi may have components not mentioned above. For

example, mannose is found in the cell walls of yeasts. Xylose and fucose are found in some Basidiomycetes, and galactose and galactosamine may be found in the walls of some fungi.

The structural features of the cell wall require studies with the electron microscope. These studies are useful for learning about the development of the cell wall. In some fungi the electron microscopy studies have shown the cell wall to consist of two or three layers of unknown composition. In *S. commune*, Mayfield[25] has shown two layers.

The fibrillar nature of the cell wall is detected even after drastic chemical treatment. A comparative study of the dimensions of the fibrillar elements in the walls of *Phytophthora*, *Neurospora*, and *Schizophyllum* was made by Hunsley and Burnett.[20] By a shadowing technique, the chemically treated cell wall preparations of these three species were examined under the electron microscope, and the diameters of the microfibrillar elements were measured. Each species showed a significantly greater diameter of the microfibrils in the distal portion of the hyphae than in the apical portions, from which the authors conclude that "unit microfibrils are added during growth by a process of secondary intussusception, so increasing the size of the aggregates. This contrasts with the notion that thickening of hyphal walls occurs by the apposition of new microfibrillar lamellae as has been described in Phycomyces."

F. Unicellular Fungi

There are species of fungi that are unicellular throughout most of their life cycle. Here belong the yeasts and some members of the class Chytridiomycetes (e.g., the chytrids of the order Chytridiales). Many other fungi, perhaps most fungi, produce unicellular spores during a short reproductive phase, but these are not the fungi to which we are referring when we speak of unicellular fungi. The most familiar example of a unicellular fungus is the yeast, such as the well-known budding yeast, *Saccharomyces cerevisiae*.

IV. GROWTH

A. Growth Kinetics of Unicellular Fungi

Unicellular fungi such as *Saccharomyces cerevisiae* follow the growth kinetics well established for unicellular bacteria. In these organisms, when cell number is taken as the indicator of growth, there is an initial part of the growth curve in which there is apparently no growth (an increase in cell size or protein content may be occurring, however), and this is followed by a period of gradual increase in numbers until the logarithmic phase of growth is reached. Toxic products of metabolism or limitation in a nutrient (or nutrients) may bring about a deceleration of growth until a stationary phase of no increase and then a phase of autolysis and decline in numbers is reached.

Microbiologists are especially concerned with the phase of growth from which the cells that they use in their experiments are obtained because cells from different stages have different properties, and for reproducibility of results and the elimination of a lag phase, it is a common practice to take cells for an inoculum from the logarithmic phase of growth. There is no particular problem in doing this with unicellular fungi such as the yeasts, but what about the filamentous fungi in which growth occurs at hyphal tips?

B. Filamentous Fungi

Obviously, cell number is an impossible means for measuring growth of coenocytic fungi, and it is also an impossible means for septate species because cell size may vary, and there is no simple means for sampling the number of cells from a mycelium to gain an indication of the total number of cells.

1. Measurement of Growth

Thus, growth has been estimated in filamentous fungi by measurement of the linear growth of hyphae on an agar plate in which case growth occurs as a linear function of time. **Linear growth rates** can be compared under varying environmental conditions, and such measurements may provide a basis for determining the most suitable conditions for growth by more exacting techniques. Linear growth is not, however, an accurate measurement of growth since it disregards branching frequency and patterns and thus the density of growth, as well as the fact that on agar, a semisolid substrate, growth may occur in three dimensions. This latter feature can be negated, however, by having the growth occur on a permeable cellophane membrane placed on the surface of the agar.

The preferred method for determination of growth of a filamentous fungus is to grow it in liquid medium. If the medium is not aerated or agitated, the culture filtrate will become stratified as the result of removal of materials by the growing mycelium and also by the production of metabolic products. In such "stand cultures" there may be differences in pH, available nutrients, concentrations of oxygen and carbon dioxide, and "staling" products at various depths of medium within the container. Consequently, it is a common practice to aerate cultures, and this is generally done by means of a culture-shaking machine, of which there are various designs to provide either rotary or reciprocal agitation.

Of course, there are changes in the medium that occur as growth proceeds — changes in the nutrients provided, pH, and metabolic products — but at any one time all the mycelium is exposed to the same conditions. This last statement is not quite accurate, for growth in liquid on a culture-shaking machine induces the mycelium to grow in pellets. The hyphae at the interior of the pellet are exposed to conditions different from those at the surface and may be limited in the availability of oxygen, for example. The mycelium in liquid cultures is not limited spatially as is the case of growth on a solid or semisolid medium, and thus the colony becomes spherical. The morphology of pellets of different species may vary but they generally approximate a sphere. Theoretically, the rate of increase behaves as a squared function of time, and the total growth as a cubed function of the radius (or of time).

From the standpoint of experimental practice, it is necessary to consider the available methods for measuring the growth of mycelia in liquid culture. The biochemist may commonly measure growth by determining increases in protein. This involves sampling, digestion, and chemical assays that may be somewhat time-consuming; but a further drawback is that cases are known in which protein synthesis increases after an increase in weight has ceased. (The increase in mass is the generally accepted criterion for growth, but one should read carefully to see how growth is defined and measured for any particular experiment.) What, then, is the usual means for measuring growth of fungi grown in liquid medium? The answer is that the dry weight of the mycelium is obtained and compared with dry weights of mycelia of different ages grown under uniform conditions of inoculation and culture except for some experimental factor being tested.

Although commonly used, measurement of growth as dry weight of mycelia has its drawbacks. Washing to remove traces of medium may remove varying amounts of extracellular polysaccharide or polysaccharides loosely adhering to the outside portion of the cell wall. The dried out mycelium is commonly quite hygroscopic, so weighing of samples to obtain constant weight may prove difficult or give somewhat erratic results under varying conditions of humidity in the laboratory.

It has been known for over three and a half decades that there is a growth phase for mycelia cultured in liquid media that corresponds to the logarithmic growth phase in unicellular organisms.[17] When dry weights are determined and plotted against time on three different scales (linear, logarithmic, and cube root), it is found that the plot against the **cube root** scale gives a straight line for a longer period than either the linear or the logarithmic plot.

Recall that the mycelial colony in liquid grows as a sphere, and the volume of a sphere is $4/3\pi r^3$. Therefore, increases in volume occur as the cube of the radius. If the cube root of the mass

of a culture increases at a constant rate, conditions favoring unlimited growth are assumed to exist, as would be true for the straight-line portions of both the logarithmic and linear plots. Seldom do we find experimenters presenting such data (dry weights of mycelia grown in liquid media) as cube root plots, although it probably gives a more useful picture of the growth kinetics.

V. SPECIALIZED VEGETATIVE STRUCTURES

Our consideration up to this point has been of the vegetative mycelium of the filamentous fungi. Some specialized vegetative structures are **rhizomorphs** (ropelike strands of hyphae woven together and having the appearance of a root) and **sclerotia** (mats of closely woven together hyphae). These structures probably function primarily in assuring survival of the fungus in the vegetative stage under environmental conditions too severe for survival of the hyphae in a loosely knit mycelial system.

VI. SPECIALIZED REPRODUCTIVE STRUCTURES

A. Sexual

Specialized structures for reproduction in the fungi are numerous. Because separation into major taxa is based on sexuality, we consider the sexual structures first. The old class Phycomycetes is now divided into a number of classes, of which mention will be made of only three — the Chytridiomycetes, the Oomycetes, and the Zygomycetes. In the Chytridiomycetes, sexuality is by the fusion of motile gametes, produced in structures called **gametangia** (Figure 4.4A). The genus *Allomyces* belongs in this class. In the Oomycetes, motile spores are also present, but sexual reproduction is by oogamy in which there is a nonmotile structure, the **oogonium**, which contains egg cells (**oospheres**). Fertilization occurs when penetration tubes from the male gametangium (antheridium) that has become adherent to an oogonium break through the oogonial wall and discharge nuclei, which fuse with the oospheres and form the zygote (called here an oospore). There are a number of well-known Oomycetes — *Phytopthora, Plasmopara*, and *Achlya*, to mention a few. The first two genera are destructive plant parasites; they are the causal agents of the diseases known as late blight of potato and downy mildew of grape, respectively. *Achlya* (Figure 4.4B) is a water mold that has been used in experimental studies that have revealed the role of hormones in the sequential development of the male and female sex organs in this genus.

In the Zygomycetes, motile cells are absent, and sexuality occurs by **gametangial copulation**. Vegetative hyphae become specialized into progametangia, which on septation develop into the gametangia that fuse to form a zygote (here called a **zygospore**). In some species there is also evidence for hormonal control by volatile compounds of this sexual process. Well-known genera here are *Phycomyces* (Figure 4.4C), *Rhizopus, Mucor*, and *Absidia*.

Sexuality in the Ascomycetes involves the production of meiospores within a saclike structure that is called an **ascus** (Figure 4.4D). The haploid meiospores are thus called **ascospores**. For genetic studies, the best known of the Ascomycetes are members of the genera *Saccharomyces, Neurospora*, and *Sordaria*.

The Basidiomycetes produce their meiospores on a structure called a **basidium**. **Karyogamy** (nuclear fusion) and **meiosis** (nuclear division that results in four daughter nuclei, each with half the chromosome number of the original nucleus) take place within the basidium, and the haploid nuclei pass from the basidium out through short stalks (**sterigmata**) into the developing **basidiospores**, which are borne on the sterigmata (Figure 4.4E). Best known for genetic studies are the genera *Ustilago, Schizophyllum*, and *Coprinus*, and most of the edible fungi are also Basidiomycetes (e.g., *Agaricus, Lentinula, Volvariella, Flammulina*, and *Pleurotus*).

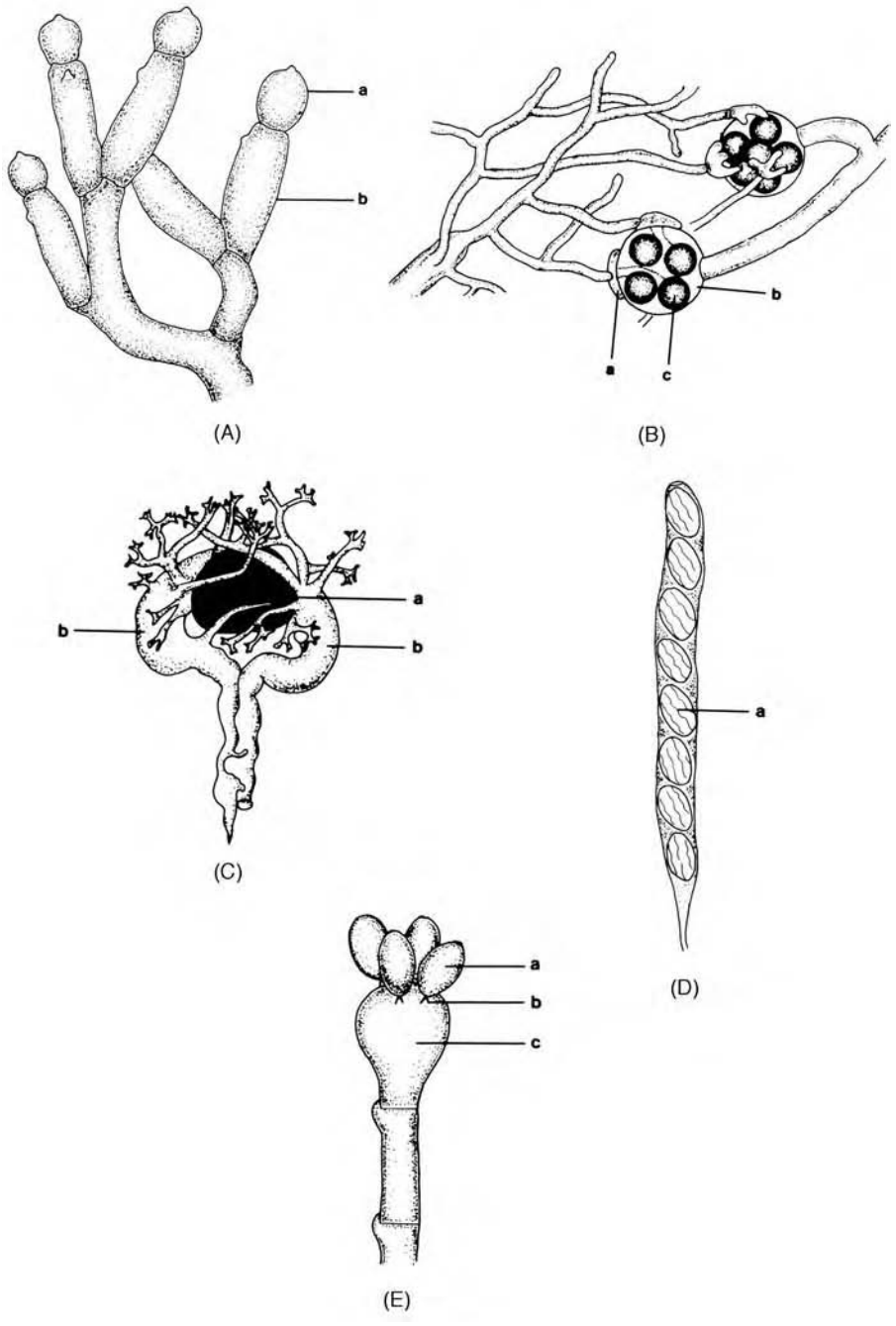


FIGURE 4.4 Sexual reproductive structures. (A) Gametangia of *Allomyces*; (a) male gametangium, (b) female gametangium. (B) Oogonium and antheridia of *Achlya*; (a) antheridium, (b) oogonium, (c) oosphere. (C) Zygospore of *Phycomyces*; (a) zygospore, (b) suspensor. (D) Ascus containing ascospores of *Neurospora*; (a) ascospore. (E) Basidium with basidiospores; (a) basidiospore, (b) sterigma, (c) basidium.

B. Nonsexual

Two things are outstanding about asexual reproduction in the fungi. One is the tremendous number of reproductive units that may be produced and disseminated within a very short time; the other is the diversity of structures that are involved in asexual reproduction. We make only some generalizations at this time.

Sporangiospores are those asexual spores that are produced within a structure (**sporangium**), which in terrestrial forms is produced on a stalk (**sporangiophore**) that lifts the spore mass above the substratum to assure widespread dissemination (Figure 4.5A). On the other hand, **conidiospores** or **conidia** (sing., **conidium**) are not produced within a structure but are formed exogenously in chains at the ends of specialized structures (**phialids**) terminating the conidiophores (Figure 4.5B). **Oidia** (sing., oidium) (Figure 4.5D) are formed in chains from the septation of individual hyphae. Oidia are also produced on stalks (oidiophores). **Chlamydospores** (Figure 4.5C) are thick-walled spores that may serve to enable the species producing them to survive extreme environmental conditions. In this respect, the chlamydospore is analogous to the spores formed in the bacterial genera *Bacillus* and *Clostridium*, in which spore formation does not provide for an increase in number of individuals, but for survival.

VII. REQUIREMENTS FOR GROWTH

A. Nutritional Requirements

As a framework for our consideration of fungal nutrition, a few generalizations about oxygen requirements and effects of hydrogen ion concentration may be useful. Although some fungi (certain yeasts, for example) will grow in the absence of free oxygen, i.e., anaerobically, most fungi are aerobic, and marked effects in form are produced by decreases in oxygen tension. Hydrogen ion concentration (pH) likewise has great effects on morphological development. In general, most fungi grow best on a slightly acidic medium, and a range from pH 4 to pH 8 is common. Of course, individual species may differ widely. The factors just mentioned, oxygen tension and pH, influence metabolic processes and consequently the ability of a fungal species to utilize certain substances as nutrients, which is the subject we now examine briefly.

1. Carbon

Carbon sources provide for both the structural and energy requirements of the fungal cell. The fungi are quite versatile in utilization of carbon compounds. There are fungal species that utilize various polysaccharides, monosaccharides, organic acids, amino acids, certain alcohols, polycyclic compounds, and natural products such as lignin and cellulose as carbon sources.

The principal substrates for fungi in nature are plant materials. The plant cell wall consists of the polysaccharides cellulose, lignin, and hemicellulose, which are the carbon compounds utilized by fungi. These polysaccharides are insoluble and consequently they must be broken down to soluble units if they are to be taken into the fungal cells and utilized. This is accomplished by the excretion of extracellular enzymes (e.g., cellulase, lignase) from the hyphae, which break down the polysaccharides to soluble monosaccharides.

The carbon source most commonly provided for growth of fungi in the laboratory is probably the 6-C monosaccharide glucose, which is well utilized by most fungi, although some species grow better on other carbon sources and some on a mixture of sources.

The concentration of the carbon source is an important consideration and should probably not exceed 2% when the requirements of the species being cultivated are not precisely known. There are, of course, exceptions to this generalization concerning concentration. Some species of yeasts, for example, require concentrations of glucose over ten times that recommended here to attain maximum growth.

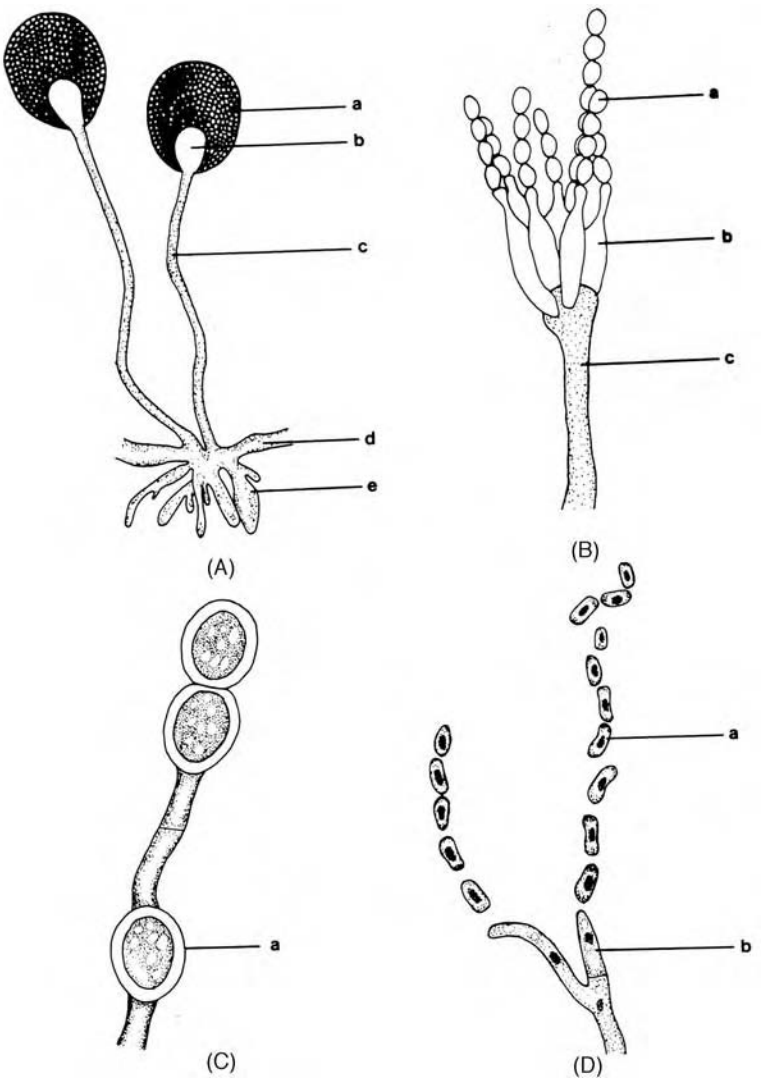


FIGURE 4.5 Asexual reproductive structures. (A) Sporangium of *Rhizopus*; (a) sporangiospore, (b) columella, (c) sporangiophore, (d) stolon, (e) rhizoids. (B) Penicillus structure of *Penicillium* showing conidia; (a) conidium, (b) phialid, (c) conidiophore. (C) Chlamydospore; (a) thick wall. (D) Oidia; (a) oidium, (b) oidiophore.

The role of carbon for the skeletal framework of all the organic compounds synthesized by the fungus is obvious. It should be pointed out that carbon compounds utilized by fungi in their nutrition also provide the energy that is required for the fungus to perform its life activities.

2. Nitrogen

Nitrogen is essential in the synthesis of proteins, purines, and pyrimidines. Chitin, a polysaccharide of common occurrence in the cell walls of many fungi, also contains nitrogen.

The fungi utilize a variety of sources to obtain the nitrogen for the synthesis of these essential compounds. These have been reported in the past to include **atmospheric nitrogen** (although no eukaryotic organisms including the filamentous fungi have been demonstrated to do this, using modern techniques). Other nitrogen sources utilized by fungal species include **nitrate**, the

ammonium ion, and **organic nitrogen**. Studies on nitrogen metabolism using microorganisms, including mutants of the ascomycete *Neurospora*, have led to the following generalization: A species utilizing nitrate can also utilize the ammonium ion and organic nitrogen, and a species utilizing the ammonium ion can also utilize organic nitrogen compounds. Robbins classified nitrogen utilization by fungi in the following way:

Class	N	NO_3	NH_4^+	Organic N
1	+	+	+	+
2	–	+	+	+
3	–	–	+	+
4	–	–	–	+

Evident from this table is that there are no fungi known that fall into Class 1 and all fungi can utilize organic nitrogen such as an amino acid.

3. Minerals

Although the importance of carbon and nitrogen for fungi is obvious, no less important are other elements, which may be required in lower concentrations. Most species of fungi utilize **sulfur** in the form of sulfate, but some, e.g., *Blastocladiella* and *Allomyces* (members of the division Mastigomycota, class Chytridiomycetes), require a reduced form of sulfur such as cysteine or methionine. Commonly, however, sulfur is provided as magnesium sulfate, and the requirement is around 10^{-4} *M*. Sulfur is necessary for certain sulfur-containing amino acids such as the aforementioned cysteine and methionine, the vitamins thiamine and biotin, and for certain products of metabolism such as penicillin and mercaptans.

Phosphorus is generally supplied in media for the growth of fungi as potassium phosphate in a concentration of around 10^{-3} *M*. Its presence in adenosine triphosphate (ATP), nucleic acids, and in the phospholipids of membranes indicates the basic importance of the element phosphorus in the living fungal cell.

The most abundant metallic element found in fungi is **potassium**. It is commonly supplied as potassium phosphate, and thus this salt provides two of the elements essential for fungal metabolism. A concentration of potassium of around 10^{-3} *M* satisfies the potassium requirement of fungi. Potassium has a role as a cofactor in some enzyme systems, is involved in carbohydrate metabolism, and is important in the maintenance of ionic balance. Loss of potassium from cells can be detrimental to living organisms including the fungi. In fact, the important antifungal antibiotics in the group known as polyene antibiotics have their antifungal activity because they create leakage of potassium from the fungal cells. The mechanism involved in these polyene antibiotics, such as amphotericin B, is that the polyenes become bound with ergosterol of the fungal membrane and release this sterol from its normal interaction with phospholipids. The ergosterol–amphotericin B complexes that are formed aggregate to form polar pores through which the potassium ions leak.

Magnesium is essential to all fungi. Many enzymes are activated by magnesium, and it is important in ATP metabolism. It is supplied at a concentration of about 10^{-3} *M*, usually as magnesium sulfate. Thus, just as we find that so many media for fungi include potassium phosphate, which supplies the essential elements potassium and phosphate, we also find that magnesium sulfate is a common constituent, supplying the essential elements magnesium and sulfur.

In addition to the aforementioned elements required by most fungi, there are a number of other elements, equally essential to various species, which are required in lower concentrations. These are the so-called **trace elements** (minor elements or microelements). Among these is **iron**, required at approximately 10^{-6} *M*. Iron is a constituent of catalase and of the cytochromes. Another trace element is **zinc**, which is required in amounts of around 10^{-8} *M*. Zinc is a constituent or activator

of many enzymes including alcohol dehydrogenase, which contains four atoms of zinc per molecule. Several fungi require **manganese**, which is supplied at about 10^{-7} *M*. Manganese plays a role in the activation of many enzymes, including those of the TCA (tricarboxylic acid) cycle and is involved in nucleic acid synthesis. **Copper** is an interesting trace element, which is essential for normal growth at a concentration of about 10^{-6} to 10^{-7} *M*, but higher concentrations result in toxicity (many copper-containing compounds act as fungicides). Another role for copper has been shown in *Aspergillus niger* (division Amastigomycota, class Deuteromycetes) in that pigmentation of the spores is dependent on the presence of copper, which is a constituent element in the enzyme tryosinase. A requirement, albeit a very small one (10^{-9} *M*), for **molybdenum** also exists for *A. niger*. Greater amounts of molybdenum are required if nitrate is used as the nitrogen source, as molybdenum is a constituent element in the flavoprotein nitrate reductase, which functions in the reduction of nitrate to the ammonium ion.

The determination of the requirement of elements in trace amounts for the growth of fungi is technically a difficult matter, because the element being tested may be present, in the small amounts required, as an impurity from the chemicals being used or in the inoculum. Consequently, there is uncertainty about the requirements for a number of other elements. **Calcium**, required by green plants, is apparently not required by most fungi, although some Ascomycetes require calcium for the production of perithecia, and it has been demonstrated to be required for fruiting body formation in the bird's nest fungus, *Cyathus stercoreus,*[24] a basidiomycete.

4. Vitamins

A vitamin is an organic molecule required in small amounts and is not used as a source of energy or structural material of protoplasm. It has a catalytic action and imparts specificity in its function as a coenzyme. As a coenzyme, such things as temperature and pH influence the vitamin requirement.

Most fungi have relatively simple nutritional requirements, but some do require one or more vitamins. The requirement indicates the inability of the fungus to synthesize the vitamin. This requirement may be complete or partial in that the vitamin is produced in insufficient amounts to produce optimal growth.

The vitamin most commonly required by fungi is **thiamine** (vitamin B_1), which is required in amounts of about 100 µg/l. Thiamine is a natural deficiency of several Basidiomycetes. Its role is as cocarboxylase, which functions as the coenzyme of carboxylase in the regulation of carbohydrate metabolism by the conversion of pyruvic acid into acetaldehyde and carbon dioxide. Thiamine consists of two moieties, a thiazole and a primidine. Fungal species are known that make one moiety but not the other, and their requirements for thiamine can be met by supplying only thiazole or pyrimidene to provide both moieties. For example, the requirement of *Schizophyllum commune* can be satisfied by provision of a pyrimidine to the medium. There are also species that require the intact thiamine molecule. It can be pointed out at this time that the requirements of a mushroom species for thiamine may differ for vegetative growth, fruiting body primordia formation, or fruiting body maturation.

The next most commonly required vitamin by fungi is probably **biotin** (known also as vitamin B_7 and as vitamin H). Biotin requirements are satisfied by amounts of about 5 µg/l. The role of biotin is as a coenzyme for carboxylations. For example, pyruvate carboxylase, for which biotin is coenzyme, acts as a donor or acceptor of carbon dioxide. Biotin is also involved as a cofactor in other carboxylation reactions occurring during the normal synthesis of fatty acids such as aspartic acid.

Other vitamins for which certain fungi have a natural requirement include **nicotinic acid** (B_3), **pantothenic acid** (B_5), and **para-amino-benzoic acid**.

The experimental determination of the requirement of a fungus for a vitamin requires great care because the vitamins are required in small amounts and may occur as impurities in the chemicals, especially those of natural origin, or in the inoculum.

B. Physical Requirements

Operating in conjunction with the nutritional requirements for growth are certain physical factors. Those that are considered here are **temperature**, **light**, **moisture**, **aeration**, and **gravity**. There are other physical factors affecting growth of fungi including hydrostatic pressure, viscosity, and mutagenic radiation, but, with the exception of mutagenic radiation, our knowledge of these is meager.

In the cases of these physical environmental factors, growth invariably occurs over a range of values. It is common to refer to three cardinal parameters in describing these factors — the **minimum** (the value below which growth will not occur), the **optimum** (the value at which the greatest amount of growth occurs), and the **maximum** (the highest value at which growth occurs). These values are affected by other factors such as nutrition, genetic strain, age of the mycelium, and the cultural conditions employed.

1. Temperature

Of all the physical factors affecting fungal growth, temperature is certainly one of the most important, and one that is most frequently studied. The temperature extremes (maximum and minimum) are of great importance in determining the survival and distribution of a fungal species in nature. The optimum temperature for growth, production of metabolic products, and sporulation are more frequently of interest to experimental investigators.

An increase in temperature generally increases enzymatic activity. In that part of the growth curve that is linear, the growth rates approximately double for each 10°C increase in temperature (i.e., the Q_{10} value is 2). High temperatures inactivate enzymes with a resulting effect on metabolism and, consequently, on growth. The failure of a fungus to grow at high temperature may be the result of inability to synthesize a needed vitamin, for example.

Fungi are classified according to their cardinal temperatures as follows:

- Psychrophiles (cold-loving) — with a minimum below 0°C, an optimum in the range of 17°C, and lack of growth above 20°C
- Mesophiles — with minimum above 0°C, maximum below 50°C, and optimum between 15 and 40°C (this is the largest group)
- Thermophiles — with minimum above 20°C, maximum at or greater than 50°C, and optimum around 35°C or higher

The composting process in which substrates, such as straw and manure, are wetted and acted on by naturally occurring bacteria, Actinomycetes and fungi, involves thermophilic microorganisms. These microorganisms break down the complex compounds of the substrate into simpler compounds, such as glucose, which can be taken in by the mushroom mycelium.

2. Light

Most fungi are exposed to alternating periods of daylight and darkness. Some, however, are situated in the darkness of soil or within the tissues of a host. The growth of most fungi is not sensitive to light, although strong light may inhibit or even kill (possibly a temperature effect). It has been reported that this inhibition by strong light may be overcome by the addition of natural materials, such as yeast extract, to the medium. A possible interpretation for this phenomenon is that the light may have destroyed certain vitamins, which are then replaced by those present in the natural material.

The most significant role that light plays for fungi is in the phototropic responses of reproductive structures (e.g., the sporangiophores of *Phycomyces* and *Pilobolus*) and in the formation of reproductive structures. The development of primordia of fruiting bodies of many Basidiomycetes is triggered by light. Light is also required for the development of other stages of fruiting body development. The positioning of the stipe and pileus, which is important if the basidiospores are

to fall free from the hymenial surface of the gills of the agaric or tubes of the polypore, has been shown in a number of mushrooms to be controlled by phototropic responses. Because light is actually inhibitory to the development of primordia and affects stipe elongation and pileus expansion of the button mushroom (*Agaricus bisporus*), this mushroom is grown in caves, tunnels, or mushroom houses in the dark.

Ultraviolet light in the region of 200 to 300 nm affects vegetative growth of fungi. This may be a lethal effect or it may induce mutations because these are the wavelengths absorbed by DNA (deoxyribonucleic acid). However, the effects of ultraviolet light can be reversed by exposure to visible light in the range of 360 to 420 nm by a process called **photoreactivation**.

3. Moisture

It is generally recognized that most fungi require high moisture levels. A relative humidity of 95 to 100% and a moisture content of the substrate between 50 and 75% support maximum growth of most Basidiomycetes; but, of course, there are fungi adapted to lower moisture concentrations. The extreme is the germination of powdery mildew spores, reported by Brodie[10] to occur at 0% relative humidity. Another exception is the dry rot fungus, *Serpula lacrymans*, whose mycelial strands can grow through substrates lacking moisture as a result of translocation of nutrients farther back in the hyphae and by the formation of "metabolic water."

4. Aeration

The components of the air that are of greater importance to most fungi are **oxygen** and **carbon dioxide**. Most fungi are obligate aerobes, but many will also grow in reduced levels of oxygen.

An effect of respiratory carbon dioxide on development has frequently been reported in a variety of organisms. The nature of the sporangium developed by the chytridiomycete *Blastocladiella*, whether an ordinary, thin-walled colorless (OC) sporangium or a dark, thick-walled resistant (RS) sporangium, has been shown by Cantino[12] to be determined by the carbon dioxide concentration.

The mushrooms of Basidiomycetes may be malformed in the presence of too much respiratory carbon dioxide — a fact that emphasizes the need for proper ventilation in mushroom growing houses. The effect of high concentrations of carbon dioxide on the development of fruiting bodies was first reported by Lambert[23] in 1933. The effect observed was an elongation of the stipe. The influence of carbon dioxide on fruiting body development of *A. bisporus* was extensively studied by Tschierpe,[32] who reported abnormal pileus formation as well as extensive stipe elongation. An extreme case, reported by Niederpruem,[26] is the absolute failure of primordium formation by the experimental basidiomycete *Schizophyllum commune* in the presence of 5% carbon dioxide. In *Flammulina velutipes*, the winter or velvet stem mushroom, *enokitake* in Japanese, a high concentration of respiratory CO_2 is obtained by special containers, which results in long stipes and small caps, a desired characteristic of this species.

The vegetative growth of *A. bisporus* has been shown by a number of workers to require low levels of carbon dioxide with an optimum concentration in the range of 0.1 to 0.5% reported by San Antonio and Thomas.[29]

5. Gravity

The influence of gravity on fungal growth is revealed most clearly in the development of fruiting bodies of Basidiomycetes. The role of the fruiting body is concerned with sexuality, and, if the value of sexual reproduction is to be obtained, the basidiospores must be disseminated.

The spores are discharged from the basidia, which are located on a hymenium that covers a gill, pore, or a tooth-shaped surface. After discharge from the basidium, the spore falls by gravity until it emerges from the region of the gills or pores; then air currents send it to its eventual destination. Thus, the orientation of the fruiting body and the gills or pores must be such that permits

FIGURE 4.6 *Lentinula edodes* growing on wood log in Thailand. Note the similar orientation of the stipes and caps — a geotropic effect. That the log was placed in a slanted position during the formation of the fruiting bodies is evident from the orientation of the fruiting bodies on the log. (Photograph courtesy of Mr. Sientong Nutalaya.)

a free fall, unobstructed by the gills or pores. This is accomplished by growth that adjusts the fruiting body to a vertical position, because the stipes of these mushrooms are negatively geotropic, and the hymenia maintain their vertical orientation by positive geotropism of the gills (Figure 4.6). Inadvertent displacement of the pileus from the horizontal, which would result in the spores not having space for free fall, can be overcome by development of the gills in a vertical orientation.

C. Transport and Translocation

Now that we have considered some of the nutritional requirements of fungi, we examine how the nutrients enter the fungal cell. Obviously, the supplied nutrients will be of no value to the vital activities of the fungus unless they enter the cell. This phenomenon of intake is sometimes referred to as "transport," which is the movement of materials across the cell wall and cell membrane. It is also apparent that metabolic products elaborated in one part of the fungus must be moved to other locations where needed for growth, for example. This is referred to as "translocation," and it is our present purpose to examine the processes of transport and translocation briefly.

1. Barriers to Transport

There are two barriers to transport. The first barrier is the **cell wall** and most compounds of molecular weight less than 4500 to 4700 can pass freely through the wall. If the cell wall has pores, even larger molecules may pass through it. For example, a mutant of the ascomycete *Neurospora* known

as "osmotic" permits the transport of compounds with molecular weight as great as 18,000. This was determined by studying the secretion of invertase, an extracellular enzyme. The second barrier encountered by materials entering the cell is the **cytoplasmic membrane**, which adds selectivity to transport as both size and charge determine movement across the membrane.

2. Passive and Active Transport

Transport is commonly referred to as either passive or active. **Passive transport** requires no energy and is a diffusion process that is essentially insensitive to temperature. The monosaccharides, such as glucose, which are derived from the activity of extracellular enzymes on substrate polysaccharides, may move into the hyphae by passive transport. **Active transport**, on the other hand, requires energy and is sensitive to temperature. Amino acids enter and exit hyphal cells by active transport involving enzymatically controlled processes, but there is not a specific enzyme for each amino acid. Cations such as potassium, ammonium, magnesium, and calcium move across membranes by active transport. Organic acids differ in that they are transported by both passive and active transport mechanisms.

3. Translocation

Translocation is the intercellular movement of substances within a hypha. In other words, it is the movement of substances from one part of a hypha to another. This important aspect of fungal physiology has been studied by Schutte,[30] by Thrower and Thrower,[31] and more recently by Jennings[21] and his associates. See Jennings[21] for detailed discussions and citations.

a. Methods of Study

Schutte[30] found that the fungi he studied could be divided into two groups — those that translocate, and those that do not. The group that translocates is the larger. His experimental design was to place a dish containing a nutrient agar medium within a dish containing non-nutrient agar with the levels the same (Figure 4.7). The nutrient agar was inoculated with the test fungus. If the fungus grew on the non-nutrient agar, it indicated that it could translocate. Similar experiments were conducted with fluorescein dyes and with radioactive compounds to test for the occurrence of translocation and, if it occurred, whether it was unidirectional or bidirectional. No bidirectional translocation was demonstrated by Schutte even though protoplasmic streaming can be shown to be bidirectional, and there is a correlation between translocation and protoplasmic streaming in

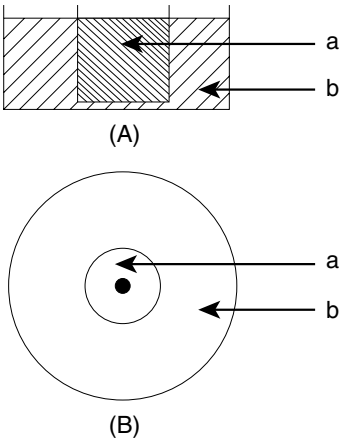


FIGURE 4.7 Dish method for studying translocation. (A) Side view, (B) top view; (a) nutrient agar, (b) non-nutrient agar.

such things as rate reduction at lowered temperatures and the cessation of the phenomenon during anaerobic conditions.

Translocation has also been demonstrated through the application of fluorescein dyes to fairy rings in which the dye was carried from the mycelium on the inside of the ring to the fruiting bodies. Schutte also showed that there were distinct translocation zones in the stipes and pilei through which translocation took place.

Thrower and Thrower[31] used the dish method of Schutte to study the ability of various fungi to translocate materials required for growth from a nutrient agar to a deficient (non-nutrient) agar as well as the ability of growing mycelium to move labeled carbon compounds. They found that even noncolonizing species were able to move labeled compounds when their mycelium grew out onto a complete medium. Thus, the technique of Schutte does not permit a conclusion regarding the non-occurrence of translocation in the case of a negative finding.

Jennings and co-workers[22] have experimented extensively with the timber decay organism *Serpula lacrymans,* which can grow for great distances over non-nutrient surfaces such as brick, concrete, or stone. As a consequence of this ability, *S. lacrymans* is the major timber decay organism in buildings in Northern Europe. Different lines of experimentation have resulted in the conclusion that the translocation of nutrients over a non-nutrient surface is brought about by a pressure-driven mass flow of solution. The process is as follows:

1. The mycelium of *S. lacrymans* breaks down cellulose in the wood to produce glucose.
2. The glucose enters the hyphae by active transport.
3. The glucose inside the hyphae is converted to trehalose (trehalose is the main carbohydrate that is translocated).
4. As a consequence of the accumulation of trehalose, the hyphae have a lower water potential than that outside the hyphae.
5. This higher water potential outside the hyphae than inside results in movement of water into the hyphae.
6. Hydrostatic pressure generated in the hyphae is responsible for the movement of solution through the mycelium (i.e., translocation).
7. The place of deposit, or sink, for the translocation materials is in the new protoplasm and wall material produced at the enlarging mycelial front.

b. Generalizations

A study by the Throwers[31] of species of fungi representing different taxa revealed that some fungi were capable of colonizing a deficient medium (referred to by Schutte as translocating fungi), some species were not, and others were indeterminate in this respect. They suggested that the ability to colonize is related to habitat rather than to taxonomic position. A last point in their studies was that movement of nutrients may occur without the intervention of cytoplasmic streaming, although the mechanism was unknown at that time.

Jennings et al.[22] have criticized the dish method of analysis for translocation studies on the grounds that the established mycelium may be made up of older, vacuolate hyphae, which might be without contents because of autolysis. Presumably, this could account for nontranslocation of the label. Other objections to the technique involved:

1. The failure to eliminate the possibility of diffusion as being responsible for movement of the label
2. The breakdown of the labeled compound by metabolism giving rise to radioactive carbon dioxide, which might be fixed in some manner by mycelium that had not actually received translocated material, thereby giving a false indication of translocation

These do not seem to us to be compelling arguments to negate the validity of the interpretation of results obtained in the dish method experiments. Jennings' viewpoint that rates of movement need to be known for an understanding of the mechanism of movement is certainly a valid one, and his studies of *S. lacrymans* provide rates from which he was able to conclude that pressure-driven bulk flow is the most likely mechanism for solute translocation, a view that is now generally accepted on the basis of experimental evidence.

VIII. METABOLISM

The term *metabolism* is commonly defined as the sum of all the chemical activities of a cell. Chemical changes in living cells supply energy for vital processes as well as provide for the assimilation of the structural materials of the cell. The breakdown of a substance to simpler chemical forms with the liberation of energy is known as **catabolism**, whereas the synthesis of the cell materials is called **anabolism**. We emphasize in this section the metabolism of carbon and deal also, to a lesser extent, with the metabolism of nitrogen and inorganic compounds.

A. Carbon

Previously we pointed out that the carbon requirements of fungi are not unique, that carbon is a basic constituent of proteins, lipids, nucleic acids, and the cell wall polysaccharides — all of which substances must be synthesized by anabolic processes within the fungus. The fungi, as they are nonphotosynthetic, do not obtain their carbon from CO_2 in any substantial amount, but rather from the catabolic breakdown of organic compounds. These organic compounds include monosaccharides, polysaccharides, organic acids, amino acids, alcohols, polycyclic compounds, and natural products such as cellulose and lignin. The insoluble compound cellulose is broken down into soluble components by enzymes secreted by the fungus, and then these soluble molecules are taken into the fungus by absorption. This mechanism used by the fungi is known as **absorptive nutrition** or **osmotrophism**.

Recall that the fungi prefer carbohydrates as a food source, with proteins, as a second source. In the laboratory, glucose is the most commonly used carbon source, and in a mixture of sugars it is commonly utilized first, although some species or strains grow better on other carbon sources and some on a mixture of carbon sources. The concentration of the carbon source is an important consideration in culturing fungi and should not exceed 2% in most cases; an exception is those species, such as certain yeasts, that are adapted to a habitat where there is a high sugar concentration, e.g., the nectaries of flowers in the case of yeasts.

The ability of any fungus to utilize a particular carbon source can be determined by respirometric methods. Indeed, the ability to break down a particular carbon substrate can be studied in cell-free mitochondrial preparations, but obtaining such preparations from fungi can be difficult, because it is necessary to burst open the cell wall without damaging the membrane system of the mitochondria.

1. Respiration

Respiration is a term that is used to refer to any cellular oxidation yielding energy to the cell. **Oxidation** is the loss of electrons from a substrate. Associated with this loss of electrons from one substrate is the gain of these electrons by another substrate. This second substrate is said to be reduced. Thus, the two reactions, **oxidation** and **reduction**, go along together — one substrate loses electrons and is oxidized, the other substrate gains electrons and is reduced. It should be mentioned that the removal of electrons from organic compounds is generally associated with a loss of hydrogen ions. The substrate oxidized is dehydrogenated, or it may be said to be a **hydrogen donor**, whereas the substrate reduced is said to be a **hydrogen acceptor**. The oxidation and reduction reactions occur together, and the terminology used to describe this is to say that they are

coupled. If we represent the substrate to be oxidized, i.e., the hydrogen donor, as AH_2, then the oxidation reaction can be represented as follows:

$$AH_2 \rightarrow A + 2H^+ + 2e^-$$

If we represent the substrate to be reduced, i.e., the hydrogen acceptor, as B, then the reduction reaction can be represented as follows:

$$B + 2H^+ + 2e^- \rightarrow BH_2$$

The overall result of these coupled reactions can be expressed:

$$AH_2 + B \rightarrow A + BH_2$$

but this represents the summation of a series of reactions in which electrons and the hydrogen ions are transferred from one substrate to another during oxidation reactions.

The comparative aspects of biochemistry deserve to be emphasized, and this has long been done. There is a basic similarity in the metabolism of organisms, which may differ widely phylogenetically, and thus it is not surprising to find a similarity in the metabolism of fungi with other organisms. On the other hand, while there is basic similarity, there are also some pathways that produce special products. In this overview, we examine only some very general aspects of fungal metabolism.

It has been pointed out by Burnett[11] that there are three main stages involved in the catabolism of carbohydrates that are related to the processes that yield energy. The first stage involves the conversion of the carbohydrates to hexose and its subsequent phosphorylation. This stage does not liberate energy and, in fact, generally requires ATP (a molecule with high energy bonds that serve as a storehouse of energy derived from respiration). The conversion of the carbohydrates to a hexose, such as glucose, is brought about by extracellular enzymes (enzymes secreted from the fungal cells), and the soluble glucose is taken into the fungal cells. Once inside the cell, the glucose is phosphorylated by the intracellular enzyme hexokinase into glucose-6-phosphate.

The second stage of catabolism of carbohydrates involves the breakdown of the hexose-6-phosphate to 3- or 2-C compounds. This energy-producing stage is known as **glycolysis**, and in the fungi three glycolytic pathways are known. The best known of these, and the one that is responsible for most of the glucose breakdown in fungi, is the Emden–Meyerhof–Parnas (EMP) pathway. There are numerous reactions controlled by as many as ten distinct enzymes that operate in the EMP pathway during the conversion of hexose to pyruvic acid. The energy value of the EMP pathway resides not so much in the ATP generated (4 ATP per mole of glucose) but in the pyruvic acid, which is then used to form acetyl-CoA. Acetyl-CoA combines with oxaloacetic acid to form citric acid and thus provides a means of entry for the products of glycolysis into the TCA (tricarboxylic acid) cycle, which is also known as the Krebs cycle or citric acid (CA) cycle.

The hexose monophosphate (HMP) pathways are also widespread in fungi, although the pentose phosphate (PP) is much more common than the other HMP pathway that is known as the Entner–Doudoroff (ED) pathway. Both of these involve the conversion of glucose-6-phosphate to 6-phosphogluconate by activities of the enzymes glucose-6-P dehydrogenase and 6-phosphogluconase; but the ED pathway proceeds ultimately to glyceraldehyde-3-phosphate and the synthesis of fatty acids and sugar alcohols along with other compounds.

The ED pathway is, as mentioned previously, of less common occurrence in the fungi than the PP pathway. In fact, its existence in fungi has been questioned because there is limited evidence for this, and then only in a couple of species in special phases of the life cycle. Both have similar stages initially in that the steps proceed from glucose-6-PO_4 to 6-phosphogluconate, but the ED

pathway then proceeds to 2-keto-3-deoxy-6-phosphogluconate and from there to glyceraldehyde-3-phosphate and pyruvic acid. The differences residing in the PP and ED sequences of these hexose monophosphate pathways are consequences of several enzymes present in one but not the other sequence. The only enzymes common to the PP and ED pathways in glycolysis are those of the initial step from glucose-6-phosphate to 6-phosphogluconate, in which the enzymes glucose-6-dehydrogenase and 6-phosphogluconase are involved.

The third stage related to energy-yielding processes in the catabolism of carbohydrates is the final breakdown to 1-C compounds, of which CO_2 is the principal compound. In this stage the 3-C compound pyruvic acid is completely broken down aerobically by the TCA cycle to CO_2 and water. (Recall that the TCA cycle is also referred to as the Krebs cycle and as the citric acid cycle.) The TCA cycle involves the formation of a number of di- and tricarboxylic acids. Pyruvic acid, in the presence of nicotinamide adenine dinucleotide (NAD) and coenzyme A (CoA), is converted to acetyl CoA and $NADH_2$ with CO_2 being liberated. CoA functions as a carrier for acetyl (CH_3CO^-) groups, and when acetyl CoA combines with the 4-C dicarboxylic acid known as oxaloacetic acid, the result is the formation of citric acid, a tricarboxylic acid. A sequence of enzymatically controlled reactions occurs, which leads back to the formation of oxaloacetic acid. Intermediates in this cycle may be removed for other reactions, such as amino acid formation, requiring the formation of oxaloacetic acid in other ways if the cycle is to continue. This oxaloacetic acid may come from carboxylation of pyruvic acid to form malic acid, with the subsequent conversion of malic acid to oxaloacetic acid; or it may come from phosphoenolpyruvate by carboxylation. If succinic acid is limited because it has been withdrawn for some reaction, then a shunt mechanism involving glyoxylic acid may operate. In this shunt isocitric acid, a 6-C tricarboxylic acid, in the presence of the enzyme isocitrase, is converted to glyoxylic acid ($CH_2OHCOOH$) and the 4-C dicarboxylic acid succinic acid. In the presence of acetyl CoA, glyoxylic acid will form the 4-C dicarboxylic acid malic acid.

We have seen that the TCA cycle by using acetyl, which is commonly derived from pyruvate, forms citric acid. The citric acid is ultimately oxidized stepwise to CO_2, but the intermediate compounds may serve for the synthesis of such things as amino acids, e.g., glutamic acid from α-ketoglutarate. This ignores, however, a most important role of the TCA cycle, which we now examine. This role has to do with energy production. In the TCA cycle, the hydrogen ions are transferred to NAD forming $NADH_2$ in the oxidations involving isocitric, α-ketoglutaric, and malic acids; and, in the oxidation of succinic acid, a flavoprotein known as flavin adenine dinucleotide (FAD) is involved in hydrogen transfer — the FAD becoming reduced to $FADH_2$. The electrons removed during these dehydrogenations are passed through a terminal respiratory chain to oxygen, which is reduced to form water. This electron transport chain in fungi involves a number of cytochromes and is probably very similar to the cytochrome system of other organisms. In general the sequence is from a flavoprotein to cytochrome *b* to ubiquinone (coenzyme Q) to cytochromes C_1 and C to cytochrome, and then to oxygen. The cytochromes of fungi have been identified by low-temperature absorption spectra and by their reactions to various inhibitors.

The passage of hydrogen ions and their electrons through the terminal respiratory chain (cytochrome chain) is associated with changes in energy, which is used in the synthesis of ATP.

$$\text{ADP} + \text{Pi (inorganic phosphate)} + \text{energy} \rightarrow \text{ATP}$$

Involved here is the coupling of oxidation and phosphorylation. For each pair of hydrogens, three molecules of ATP are generated. Approximately 7 kcal of energy are stored in 1 mol of ATP, and, since the conversion of pyruvic acid to acetyl CoA involves the formation of one $NADH_2$, 3 ATP are formed. The further complete oxidation of acetyl CoA through the TCA cycle brings about the release of eight hydrogen ions and electrons, and thus the formation of 12 moles of ATP. The total ATP formed from a mole of pyruvic acid is 15 mol, which amounts to approximately 105 kcal of energy (15 ATP × 7 kcal).

In this account, what we wish to emphasize is that the fungus enzymatically breaks down carbon substrates by respiration. The fungus uses: (1) the various intermediates for the synthesis of those compounds it needs for its life activities, and (2) some of the energy from the original carbon substrate is transferred to ATP, which serves as an energy "currency" for the organism's future energy requirements.

a. Methods of Study

The most commonly employed methods for studying respiration in fungi involve measurements of gas exchange or the determination of reduced forms of NAD and NADP at 340 nm by spectrophotometric methods.

In gas exchange, either oxygen uptake or carbon dioxide formation can be measured. In early studies, the Warburg–Barcroft constant volume manometer has probably been used for more determinations of fungal respiration than any other device, although it is not without its limitations. For example, the requirement for continuous adjustment to constant volume is a problem when continuous readings for a large number of samples are required at short intervals. Another objection is that the shaking of cell aggregates in suspension in the vessel may result in clumping and thus interfere with normal respiration. A simple respirometer that obviates some of the disadvantages of the Warburg–Barcroft apparatus has been described.[8] This volumeter has been used successfully for studies of respiration of filamentous fungi.

At 340 nm the spectrophotometer indicates by changes in optical density the absorption of the reduced forms of NAD and/or NADP when a substrate that has been added is oxidized. These changes reflect the enzymatic activity involved in substrate conversion. Similarly, the oxidation and reduction of cytochrome can be followed spectrophotometrically at 550 nm. By the use of specific inhibitors, the cytochrome system of the basidiomycete *Schizophyllum commune* was demonstrated by Niederpruem and Hackett.[27]

The preparation of the fungal material for subsequent respirometric studies has been performed in a variety of ways. For studies of respiration of whole cells, disks of mycelium growing on agar, or pellets of mycelium from liquid cultures grown in shake culture, or mycelium that has been briefly homogenized have been used. The presence of agar makes it impossible to obtain accurate measurements of the dry weight of the mycelium; this is important because references commonly used in respirometric studies are uptake of oxygen per unit of dry weight or unit of protein. The mycelial pellets from liquid shake cultures must be quite small, as diffusion of oxygen to the cells within the pellet may be limited by slight distances from surface to the interior of the pellet. Even brief homogenization will result in the fragmentation of the hyphae and thus the death of many cells.

As is evident, there are difficulties in preparing fungal cells for respirometric studies, and the investigations of Bonitati et al.[9] have indicated an approach to this problem that minimizes some of these difficulties. The main feature of the method involves the use of samples of moist mycelium thinly layered on tantalum grids that have been sterilized and preweighed. After measurement of respiration in a suitable apparatus, the dry weight of the respiring mycelium on the tantalum grid is determined. Such tantalum grids can be cleaned by boiling in 50% (v/v) nitric acid and rinsing in water, and they are then suitable for reuse.

Mitochondria from cell-free extracts also give valuable information regarding respiration, and many techniques have been used for the preparation of extracts from which the mitochondria can be separated by centrifugation. The simplest technique is to grind the mycelium in a mortar and pestle in a suspension medium with quartz sand or finely ground Pyrex™ glass. Drying of the mycelium with or without a preliminary freezing or drying in acetone renders the mycelium friable, and thus it is easier to extract the subcellular components. Freezing, followed by grinding with abrasives, has also been used successfully, as has mechanical disruption in a ball mill or in a variety of types of homogenizers. Sonic oscillation has also been used, but this frequently leads to rupturing of the membranes of the mitochondria causing the leakage of some of the mitochondrial enzymes into the supernatant fraction on centrifugation. Disruption of the hyphae by passage through a

Hughes press or other small-orifice-type press has been used successfully. There are other techniques, but those mentioned serve to indicate the general nature of the methods.

The suspension media for the extracts are commonly phosphate buffers, but they may also be salt mixtures or sucrose solutions. EDTA (ethylenediaminetetraacetic acid) is sometimes added to protect enzymes from inactivation by heavy metal ions.

There are many factors, other than species differences, that have an effect on respiration. The age and the developmental status of the fungal material have an effect, and a generalization can be made that respiration is highest during the period of most rapid growth. Membrane permeability may have a significant effect in respiration studies. For example, a substrate cannot be respired if it fails to pass through the membrane, and a low pH or conversion to an ester is required for certain organic acids to do this and become oxidized. Although there is this effect of acidity on exogenous substrates, endogenous respiration is generally independent of pH in the range of pH 5 to 8. Another generalization is that the rate of oxygen utilization is, as a rule, independent of oxygen pressure down to low levels, although there are some fungi that require relatively high oxygen pressures to achieve their maximum respiratory rates. In studies of fungal respiration phosphorus, potassium, and magnesium are usually provided for the respiring cells.

A matter of some concern in studies of fungal respiration is the metabolism of the fungal cells in the absence of external substrate, i.e., endogenous respiration. Endogenous respiration is generally high in fungi and can lead to some erroneous conclusions regarding the respiration of exogenous substrates if care is not taken to correct measurements for the endogenous respiration. One approach that has been used to minimize this effect is to starve the cells, thereby reducing their endogenous substrates; but a better method is to find a medium in which the cells do not accumulate reserve materials.

A determination that is frequently made is that of the **respiratory quotient** (R.Q.) — also sometimes referred to as the **respiratory coefficient**. The ratio of the volumes of carbon dioxide evolved and oxygen consumed is the respiratory quotient (R.Q. = CO_2/O_2). One of the values of this determination is that it gives an indication of the type of substrate being oxidized. For example, an R.Q. = 1 is obtained from the aerobic oxidation of a carbohydrate, and an R.Q. = 0.7 is indicative of the oxidation of a fat. Many studies have been made in which the R.Q. has been determined during development of a fungus from spore germination, through mycelial stage, to primordia formation, and finally to various stages of sporophore formation. As an illustration, we point to the study of Hou and Wu[19] of *Agaricus bisporus* in which they determined the endogenous respiration of fruiting body tissue to be of the order of 0.7, that of mycelium to be 0.93, and that of the primordia to be 0.9.

B. Nitrogen

It has previously been pointed out that nitrogen is essential for the synthesis of proteins, purines, pyrimidines, and some vitamins, as well as being a component of chitin, which is a polysaccharide found in the cell walls of most fungi. The sources of nitrogen for fungi are many, but not all are equally suitable for all species. Caution should be used in stating that a fungus is unable to grow when provided with a certain nitrogen source because the failure may be due to the lack of certain growth factors in the medium.

The fungal hyphae can take in or transport many types of nitrogen compounds. Recall that there are two barriers to transport — the cell wall and the cell membrane — and that compounds with molecular weight as high as 4500 to 4700 will pass through the walls. Thus, the wall is not an impediment to the common sources of nitrogen such as salts of nitrates or ammonium, or amino acids, but active transport is involved in the uptake of nitrate. In nitrogen metabolism it is known that some Basidiomycetes cannot utilize nitrate but are able to utilize the ammonium ion (NH_4^+). Such species probably lack the enzyme nitrogen reductase, which is responsible for the reduction of nitrate to NH_4^+, a step in the pathway of the conversion of nitrate to amino acids. Nitrate reductase

is a complex enzyme that contains molybdenum (Mo), FAD, and a heme moiety (cytochrome *b*). These enzyme moieties have been shown to participate in the transfer of electrons according to the following sequence:

$$\text{NADPH} \rightarrow \text{FAD} \rightarrow \text{cytochrome } b \rightarrow \text{molybdenum} \rightarrow \text{nitrate } (NO_3^-)$$

When the nitrate ion receives electrons, it is reduced to nitrite (NO_2^-). The reduction from nitrite proceeds through some intermediates — which are not known with certainty, such as nitroxyl (NOH), nitramide (NO_2NH_2), and hyponitrous acid ($H_2N_2O_2$) — to hydroxylamine and then to ammonia.

It has been pointed out by Griffin[18] that nitrite reductase is able to carry out six-electron transfer from nitrite to ammonia (nitrite → unknown → hydroxylamine → ammonia) in a single enzymatic step. The electron transport pathway of nitrite reductase begins with either NADH or NADPH and proceeds to FAD and thence to a heme-like prosthetic group containing iron known as siroheme, and finally to nitrite. According to this scheme, hydroxylamine reductase is not required; and, although there are reports of its activity, this is viewed as an artifact.

There is a relationship between TCA cycle intermediates and ammonia that results in the formation of amino acids. For example, α-ketoglutaric acid + NH_3 + $NADPH_2$ in the presence of the enzyme glutamic dehydrogenase gives rise to the amino acid glutamic acid + H_2O + NADP. Other amino acids can be formed by transamination reactions such as the following: glutamic acid + pyruvic acid in the presence of transaminase give rise to α-ketoglutaric acid + alanine. Fumaric acid also combines with ammonia to produce the amino acid aspartic acid, but this is less important than the production of glutamate from α-ketoglutarate. Much has been learned about amino acid synthesis from biochemical genetic studies of *Neurospora*.

C. Lipids

Lipids constitute a large class of heterogeneous compounds of diverse structure that are soluble in nonpolar solvents. Lipids generally contain glycerol and fatty acids. The fatty acids may be of variable chain length, and they may be saturated or unsaturated. Esterification of the glycerol with fatty acids may lead to the formation of mono-, di-, or triglycerides. There are also complex lipids, such as the phospholipids in which one hydroxyl group of glycerol is esterified by phosphoric acid, and the glycolipids in which glycerol is linked by a glycosidic bond to a sugar.

Lipases hydrolyze triglycerides, releasing glycerol and three fatty acids. Subsequent breakdown of glycerol (after phosphorylation) through glycolysis releases energy. The catabolism of the fatty acids is more complex, but the result is similar to the breakdown of glycerol in that the reserves stored in the fungus as fat become available to the fungus as energy and a variety of intermediates. Lipids are essential to the fungus as components of the cell membrane and the membranes of the various organelles and the endoplasmic reticulum. The most prominent of these membrane lipids are phospholipids, but sterols (the most common in fungi is ergosterol) are also present. In addition to serving as food reserves, lipids are present in the cell walls and on spore coats where it has been suggested that they serve a protective function as a water-repellant material. One other significant role for specific sterols in the genus *Achlya* of the class Oomycetes is as sexual hormones.

IX. REPRODUCTION

A. Introduction

Reproduction is the process by which an organism gives rise to new individuals. The fungi are extremely diverse in matters of reproduction, which may be accomplished through **sexuality** or by nonsexual means (commonly referred to as **asexuality**). **Sexual reproduction** is characterized by

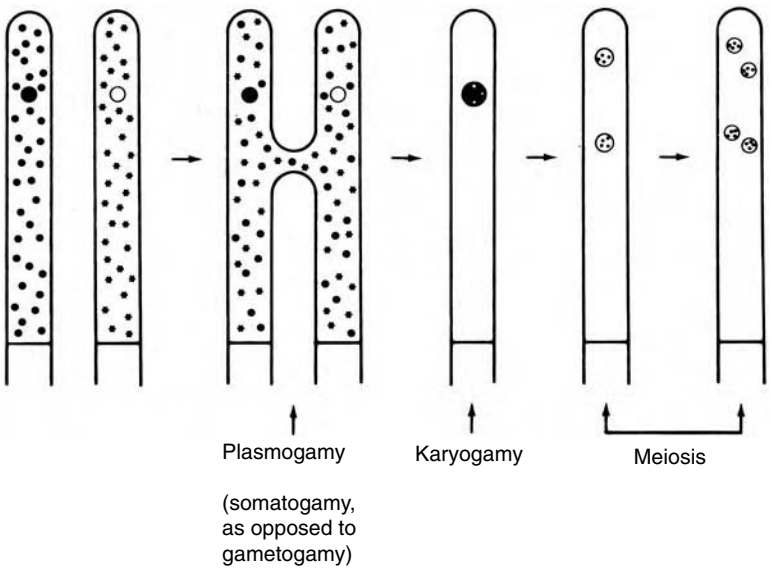


FIGURE 4.8 Sexual reproduction. It is convenient in fungi to recognize three stages in sexual reproduction: plasmogamy, karyogamy, and meiosis.

the union of two nuclei. It is the usual practice to describe sexuality as consisting of three cardinal events (Figure 4.8). These events are **plasmogamy**, which is the fusion of the protoplasts, bringing different nuclei into the same cytoplasm; **karyogamy**, or fusion of the unlike nuclei; and **meiosis**, which is a reductional division of nuclei that results in the formation of haploid nuclei. Nonsexual (**asexual**) reproduction does not involve the union of nuclei, or sex cells, or sex organs. In **nonsexual** reproduction, progeny are formed from a single parent, and thus there is no nuclear contribution from a second parent, so that any offspring resulting from asexual reproduction is genetically identical to the parent from which it arose.

It cannot be stressed too strongly that the advantage of sexual reproduction comes from the fact that the offspring that result vary from one another genetically. That is, sexual reproduction imparts **variation** to the species, with the consequence that some individuals will be more fit for a particular environmental situation than other individuals. On the other hand, in nonsexual reproduction the progeny formed are identical to the parent from which they arose, and, since they are commonly produced in very large numbers, an available habitat will soon become densely populated if the environmental conditions are favorable. Sexuality ensures the maintenance of the species under changing conditions, while asexuality provides for wide dissemination of the species so long as conditions are suitable for the existence of the genotype undergoing nonsexual reproduction.

In the fungi, reproductive organs may be formed. The reproductive organs may be sexual or nonsexual. If the entire thallus is converted into a reproductive structure or structures, as occurs in some of the Chytridiomycetes, the fungus is referred to as **holocarpic**. The more common situation, however, is for the reproductive organs to arise from only a portion of the thallus. Fungi of this type are referred to as **eucarpic**.

B. Sexual

Studies of sexuality in the fungi had their beginning with the investigations of A.F. Blakeslee,[6] who used single-spore isolates of members of the order Mucorales (class Zygomycetes) to demonstrate that only certain confrontations of single-spore mycelia resulted in the formation of zygospores (the sexual spores). Such species were referred to as **heterothallic**. In the heterothallic species

studied by Blakeslee, there were no morphological differences between the strains that gave rise to zygospores when mated, and he referred to them as + and − strains. In such heterothallic species, confrontations of + × − strains gave a sexual reaction, but confrontations + × + and − × − gave no such reaction. Species were found, however, in which the mycelium from a single spore would produce **zygospores** (sexual spores). These species were called **homothallic**.

In regard to life cycles, it could be said that there were two main sexual cycles, which may be distinguished as follows:

- Homothallic species. The sexual cycle is completed by a single thallus arising from a single spore.
- Heterothallic species. These require cross-mating between different homokaryotic thalli for completion of the sexual cycle.

1. Homothallism

Homothallic species are basically self-fertile, but there are different types of homothallism. In **primary homothallism**, a homokaryotic mycelium arises from a single spore, which contained a single postmeiotic nucleus. Among the cultivated edible fungi, only the straw mushroom (*Volvariella volvacea*) is frequently referred to as a primary homothallic species, but there are sufficient uncertainties, including variation displayed by single-spore cultures in successive generations,[14] so that the life cycle of this species will likely remain an enigma until careful studies using genetic markers have been performed.

In **secondary homothallism**, when two compatible meiotic nuclei enter a single basidiospore, these basidiospores produce a mycelium that is self-fertile. That is, a mating type system is operative. The common situation is for the basidia to bear only two basidiospores. *Agaricus bisporus* is now known to be a secondarily homothallic species with a unifactorial (bipolar) mating type system. If the meiotic nuclei enter the basidiospores randomly, not all the spores will be self-fertile because of the aforementioned unifactorial (bipolar) mating type system in which compatibility requires the presence of different alleles. Generally in *A. bisporus* two-spored basidia are produced. Not all of these basidiospores give rise to self-fertile mycelia, however. With meiosis there will be two nuclei bearing one mating type allele (e.g., *Ax*), while the other two nuclei will bear a compatible mating type allele (e.g., *Ay*). If we distinguish the four postmeiotic nuclei by labeling them *Ax1, Ax2, Ay1*, and *Ay2*, then spores of the following nuclear constitution are possible:

Ax1Ax2, Ax1Ay1, Ax1Ay2, Ax2Ay1, Ax2Ay2, and *Ay1Ay2*

Only those spores bearing both *Ax* and *Ay* alleles will give rise to fertile mycelia; those spores homozygous for *Ax* (e.g., *Ax1Ax2*) or *Ay* (e.g., *Ay1Ay2*) will give rise to sterile mycelia. Thus two thirds of the spores would be expected to be self-fertile, and one third to be self-sterile. In *A. bisporus* there are also a few three-spore and four-spore basidia formed, and these result in spores that produce sterile mycelia but may be cross-fertile according to the bipolar pattern of sexuality. In *A. bisporus*, clamp connections are not formed, rendering the fertile heterokaryotic mycelium morphologically indistinguishable from the sterile homokaryotic mycelium.

2. Heterothallism

In heterothallism there is a requirement for two compatible thalli to come together to bring about sexual reproduction and the completion of the life cycle. Each thallus is self-incompatible. In heterothallism an incompatibility system operates, just as we have seen to be the case in secondary homothallism. Two types of incompatibility systems have been well documented to occur in the Basidiomycetes. These are **bipolar incompatibility** and **tetrapolar incompatibility**. In bipolar

incompatibility there is a single genetic factor that controls the sexual cycle. Thus, bipolar incompatibility (bipolarity) is sometimes referred to as heterothallism with unifactorial control. In tetrapolar incompatibility (**tetrapolarity**) two unlinked genetic factors control the sexual cycle, and it may be referred to as heterothallism with bifactorial control.

In heterothallism the basic events are similar. On germination a single basidiospore will give rise to a homokaryotic mycelium whose nuclei are haploid. Confrontations between homokaryotic mycelia will lead to hyphal fusions. This is the previously described event called **plasmogamy**. When the mycelia are of compatible mating types, nuclei will migrate from one mycelium into the other, and when two compatible nuclei reach a tip cell, the dikaryotic condition (two unfused compatible nuclei in the same cell) that is established commonly becomes perpetuated by the growth of hyphae, which form clamp connections. The mycelium that is formed with clamp connections is known as secondary mycelium to distinguish it from the homokaryotic, unclamped mycelium that arises from a single spore, which is called primary mycelium. Nuclear migration from one mycelium to another after hyphae of compatible mycelia have fused is commonly, but not always, reciprocal; however, the nuclear condition of the dikaryotic mycelia formed on both sides of the mating are identical. Fruiting bodies will develop from dikaryotic mycelium under proper nutritional and environmental conditions and the presence of genes necessary for fruiting. In each basidium that forms on the hymenial surface, a pair of nuclei (the dikaryon) will fuse. This is the nuclear fusion event called **karyogamy**, and the consequence is the formation of a diploid nucleus. This diploid condition does not last long, for almost immediately the diploid nucleus undergoes **meiosis**, a two-stage divisional process that results in the formation of four haploid nuclei. These nuclei pass through stalks (**sterigmata**) that develop on the basidia into the four basidiospores that form on the sterigmata.

In bipolar incompatibility there is a single incompatibility factor that is known as the *A* factor, but multiple alleles of varying numbers in different species are present for this factor. Compatibility requires only that the mating type alleles of the confronting mycelia be different. Thus, in a confrontation between compatible homokaryotic mycelia (e.g., $Ax \times Ay$), dikaryotic mycelia ($Ax + Ay$) will be formed, which may lead to the development of fruiting bodies. In the basidia of these fruiting bodies, the dikaryotic nuclei ($Ax + Ay$) will undergo karyogamy, resulting in the formation of a diploid nucleus (*AxAy*), which soon undergoes meiosis forming four nuclei of the following mating types: *Ax, Ax, Ay, Ay.* One of these nuclei passes into each of the four spores on the basidium, so that from each basidium two spores are of *Ax* mating type, and two are *Ay.* Thus, when large numbers of basidiospores are produced, there is a 50% chance for a mating between two monosporous, homokarytotic mycelia to be compatible. Among the cultivated edible fungi, examples of bipolar heterothallism occur in *Pholiota nameko* and *Agaricus bitorquis*, but only in *P. nameko* is there a secondary mycelium with clamp connections, which makes that system more amenable to experimental studies and breeding work.

In tetrapolar incompatibility there are two incompatibility factors that are known as the *A* and *B* factors, with multiple alleles occurring at both factors. The *A* and *B* factors are unlinked, and, consequently, the spores from a single fruiting body are of four mating types (e.g., *AxBx, AxBy, AyBx,* and *AyBy*) occurring in a ratio of 1:1:1:1. Only those combinations that are heteroallelic for both mating type factors are completely compatible (e.g., $AxBx \times AyBy$, and $AxBy \times AyBx$). In *Schizophyllum commune* it has been clearly shown that there are two kinds of hemicompatible reactions ($A = B \neq$ and $A \neq B =$). In the common *A* (unlike *B*) matings (e.g., $AxBx \times AxBy$), an infertile heterokaryon is formed from the mycelia on both sides of the confrontation, since nuclear migration does occur. This is in contrast to the common *B* (unlike *A*) matings (e.g., $AxBx \times AyBx$), which are also infertile but in which the heterokaryon is formed only in the region of intermingling of the confronted mycelia, as nuclear migration does not occur. That is, nuclear migration requires a heteroallelic condition (either $A = B \neq$ or $A \neq B \neq$) at the *B* mating type factor. There are some microscopic differences in the hyphae involved in the hemicompatible reactions. In the common-*B* ($A \neq B =$) heterokaryon, the hook cell fails to fuse with the penultimate cell, resulting in the formation of pseudoclamps (false clamp connections). In such hyphae, the two nuclei forming the

heterokaryon divide simultaneously, sending two non-sister nuclei into the terminal cell, one nucleus into the penultimate cell, while the fourth nucleus remains in the unfused hook cell. In the common *A* ($A = B \neq$) heterokaryon the aerial mycelium is sparse, giving rise to the common name "flat" for this heterokaryon; the cells vary in the number of nuclei they contain per cell, there are no clamp connections, and partially formed septa are frequently encountered.

The compatible reaction, which may lead to the formation of a fruiting body, occurs when mycelia heteroallelic for both mating type factors are confronted (e.g., *AxBx* × *AyBy* and *AxBy* × *AyBx,* sometimes written as $A \neq B \neq$). In these confrontations true clamp connections are formed.

With equal numbers of spores of each mating type (*AxBx, AxBy, AyBx,* and *AyBy*) possible, confrontations of mycelia arising from these spores will give rise to equal frequency of the following reactions: compatible ($A \neq B \neq$), hemicompatible ($A = B \neq$), hemicompatible ($A \neq B =$), and incompatible ($A = B =$).

Among the cultivated edible fungi there are several examples of tetrapolar heterothallism, including *Lentinula edodes, Pleurotus ostreatus, P. sajor-caju, Flammulina velutipes, Auricularia polytricha*, and *Tremella fuciformis*. Although there are complexities in the genetic manipulation of the tetrapolar system, this is offset by a wealth of basic information derived from studies of *Schizophyllum, Coprinus, Flammulina*, and others which makes those species that have bifactorial incompatibility attractive to growers and breeders.

3. Hormonal (Pheromonal) Control

The term **hormone** has been used in the fungi in its classical sense as an organic compound produced in one part of the organism and transferred to another part where it elicits a special response. Currently, this term is used interchangeably with **pheromone**, which is defined as a chemical substance that acts at a distance.

The best-documented cases of hormonal regulation of sexuality in the fungi are those involving some of the aquatic fungi. The classical studies of J.R. Raper revealed the sequential development of sex organs of *Achlya* (class Oomycetes) under the control of several hormones, and two of these (antheridiol and oogoniol) have now been chemically identified. In *Allomyces* (class Chytridiomycetes) the female gametes have been shown to produce a hormone, sirenin, which attracts the male gametes to them. Sirenin was the first of the fungal sexual hormones to be chemically identified.

Hormonal activity has also been demonstrated in non-aquatic fungi. In the class Zygomycetes several heterothallic fungi have been shown to produce hormones that are responsible for the growth in air of zygophores (stalks that will bear zygospores) toward one another. In heterothallic species the + and − strains produce different hormone precursors. When these hormone precursors (prohormones) diffuse into the air and reach the strain of opposite mating type, they are converted to active hormones, and it is these active hormones that cause the zygophores to grow toward one another. These hormones that induce zygophore development are trisporic acids. The system of control is complex and much remains to be discovered about the induction of the morphogenetic changes.

In the higher fungi, the best-documented study of hormonal control in sexuality is in the ascomycete *Ascobolus*. It was shown by Bistis[3,4] that the following occur during sexuality of *A. stercorarius*, which has a bipolar mating type system with alternate mating type alleles, *A* and *a*:

1. The sexual organs (antheridia and ascogonia) are produced only when the *A* and *a* mycelia are in close proximity to one another.
2. Oidia, normally asexual reproductive units, may become activated in the presence of mycelium of opposite mating type, and an activated oidium may induce the growth of trichogynes toward the oidium when these two bodies are within a distance of 100 μm from one another.
3. If the position of the oidium is changed, there will be a new growth from the trichogyne at the point nearest to the oidium with growth occurring in the direction of the oidium.

4. Activated oidia placed on mycelium of the same mating type on which ascogonia with trichogynes were present will behave as though of compatible mating type, in that conjugation will occur between the trichogyne and the oidium (or antheridium); but the ascocarp ceases growth after a short initial period of development.

Bistis[5] has also demonstrated a role for sexual hormones in the ascomycete *Neurospora crassa*. Several decades ago a role for hormones in sexuality of this species had been suggested, but a definitive experimental demonstration for hormones in sexuality was not presented until the report of Bistis.[5]

In the filamentous Basidiomycetes there are no proven examples of an experimentally demonstrated role for sexual hormones other than those for mating type control, which will be considered later. There are cases in which observations of directed growth of hyphae might be interpreted as being the result of hormonal activities. It is not uncommon to observe hyphal tips growing toward one another. For example, in their study of hyphal fusions of *Schizophyllum commune* in which hyphae from confronting mycelia were studied microscopically, Ahmad and Miles[1] reported instances in which these hyphae curved and grew toward one another. In these studies it was also reported that there was a significantly higher frequency of hyphal fusions in confrontations involving heteroallelic *A* (i.e., $A \neq$) confrontations and that this effect was transmitted across a permeable membrane. Also in *S. commune*, Voorhees and Peterson[33] have demonstrated that there is a chemotropic attraction and fusion of hyphae to spores and that the attractant is water soluble. A mycelium is necessary for spores to produce the attractant, and the spores must be viable. When a spore and hypha fuse, the attractant is no longer produced.

In a number of studies of fruiting body development of Basidiomycetes, a role for hormones has been demonstrated although the chemical nature of the hormone may not be known. A role for cyclic adenosine monophosphate (cAMP) has been shown for both *Coprinus* and *Schizophyllum*, and Rusmin and Leonard[28] have reported that a low-molecular-weight compound caused fruiting in *S. commune*. This fruiting-inducing substance is referred to by them as FIS.

While a number of plant hormones (auxins, gibberellins, ethylene) have been found in Basidiomycetes, a hormonal role for these in the fungi has not been demonstrated. Some experimentation has indicated a role for a compound produced in the gills and causing stipe elongation, but as yet no chemical identification has been obtained. It is anticipated that soon we will have more information on hormones and their roles in the fungi because there are now more techniques available for identifying compounds active in low concentration.

The role of pheromones in mating type control is examined in Chapter 6, Section IV, which deals with the genetics of the mating type loci.

C. Nonsexual

In nonsexual reproduction, as in sexual reproduction, the individuals that are formed have the major characteristics that are typical of the species. Nonsexual reproduction is commonly referred to as asexual reproduction (reproduction without sex), and these terms are used interchangeably in this book. Other terms denoting reproduction in the absence of sexuality are **somatic** or **vegetative reproduction**. As previously noted, nonsexual reproduction does not involve the union of nuclei, or sex cells, or sex organs. That is, nonsexual (asexual, somatic, vegetative) reproduction occurs when progeny are formed from a single parent. There is no contribution from a second parent, and the offspring are genetically identical to the parent from which they arose. This is in marked contrast to sexual reproduction, where variation among the offspring is the rule.

The advantage of nonsexual reproduction to the fungus is that large numbers of progeny can be formed from an individual that is well adapted to a particular habitat, set of environmental conditions, nutritional availability, or even need, in the case of utilization by humans. Those offspring that reach a favorable substrate will grow and in turn reproduce under appropriate

environmental conditions. Many of the asexual reproductive units are easily disseminated and germinate rapidly so that the fungus can spread over a wide area within a short time. The obvious disadvantage of reproduction solely by nonsexual means is that the environmental conditions may change and not be satisfactory for the germination of the disseminated asexual reproductive unit, or for its subsequent growth and ability to become established in the new habitat. As long as the environmental conditions remain unchanged, there is a certain advantage to the species inherent in the genetic stability of asexuality. It is when the environmental conditions change in an unfavorable way that nonsexual reproduction is a disadvantage, because then only the infrequent mutations that permit growth under the changed conditions will allow the organism to survive.

There are a variety of methods by which fungi reproduce asexually. There are four general methods, some of which are found more frequently than others:

1. Fragmentation of a multicellular thallus. This is a common method used to subculture fungi in the laboratory.
2. Fission of somatic cells into daughter cells. This is found in a relatively few yeasts that are known as fission yeasts.
3. Budding of somatic cells or of spores. This is commonly found in yeasts with each bud formed giving rise to a new individual. The buds in some species arise from different places on the parent cell, but in other species they develop from the same place (one of the poles of the ellipsoidally shaped cell).
4. Production of spores. This is by far the most common and best-known method of asexual reproduction in fungi, and thus we treat it in more detail.

1. Types of Reproductive Units

Although we described previously (Chapter 4, Section VI.B) some specialized asexual reproductive structures, the various types of nonsexual reproductive units will now be examined more fully.

Classification of the fungi makes use of spore types. At the level of subdivision distinctions are made on the basis of presence or absence of sexual spores and, when present, the type of sexual spore. At lower levels the asexual spore and the structures in which they are located are of taxonomic use.

Spores vary in color, in size, in shape, and in the number of cells comprising them.. There is also variation in the arrangement of the cells supporting the spores and in the manner in which they are borne. Combinations of these various characteristics result in tremendous variation of spore types and structure, and some fungal species may produce more than one type of asexual spore.

a. Sporangiospores (Motile and Nonmotile)

Nonsexual spores may be borne within a structure. Such a structure is called a **sporangium** (pl., **sporangia**) which, by definition, means simply a structure containing spores. To distinguish these spores from other spores they are sometimes referred to as **sporangiospores**. Sporangia are saclike structures that in some species contain spores that are motile, and in other species nonmotile spores are contained within the sporangium. The motile spores are referred to as **zoospores**; the nonmotile spores are called **aplanospores**.

The motility of zoospores is due to the presence of **flagella** (sing., **flagellum**). In the fungi, zoospores possess either one or two flagella. There are also two types of flagella — whiplash and tinsel. The whiplash flagellum is divided into two parts, with a basal portion that is much longer than the terminal portion, which is usually very short and flexible. The tinsel flagellum is quite distinct in that it has a number of lateral hairlike projections, called **mastigonemes**, along its entire length. The number, type, and insertion of the flagella are used in classification. For example, in the Chytridiomycetes, of which *Allomyces* is a member, the zoospores have a single, posteriorly inserted, whiplash flagellum; in the Oomycetes, of which *Achlya* is a member, the zoospores have

two flagella which are laterally inserted, but the tinsel is anteriorly directed, and the whiplash posteriorly directed. Many details of flagellar structure have been learned from studies using the electron microscope, and it is interesting to note that transverse sections through the flagellum reveal a core (the axoneme) of two centrally located microtubules surrounded by nine pairs of microtubules near the periphery, an arrangement that is found in the flagella of all eukaryotic organisms.

Nonmotile sporangiospores are produced in sporangia that form at the tips of specialized stalklike hyphae known as sporangiophores. In some species the sporangiophores may have a very complex form. The terminal portion of the sporangiophore generally becomes swollen, forming a dome-shaped columella. Within the sporangial wall, cleavage of the cytoplasm into vesicles takes place. Details of the developmental events at the electron microscopic level are known from only a few studies, but it appears that these vesicles arise from the endoplasmic reticulum, and that the membranes of the vesicles fuse and are converted into tubules that branch and extend throughout the cytoplasm. Segments of protoplasm containing nuclei are cleaved out in this way, and these will become the sporangiospores. A wall is deposited outside the plasma membrane of the sporangiospore, this membrane having been derived from the membranes of the vesicles. Developmental details regarding the initial nuclear condition, uninucleate or multinucleate, of the developing sporangiospores are uncertain.

Sporangiophores may be simple or branched. Some fungi bear lateral sporangia in addition to the terminal sporangium. These lateral sporangia are smaller than the terminal sporangium and are termed sporangioles (sporangiola). A sporangiolum commonly contains only one or a few spores and lacks a columella.

b. Conidia

In contrast to sporangiospores, which are produced within a structure, **conidia** are borne externally, either terminally or laterally on hyphae or specialized hyphae called conidiophores. Conidiophores may be unbranched or branched in a multitude of ways, and they bear conidiogenous cells, which ultimately produce the conidia. Thallic development of conidia occurs when the hypha undergoes fragmentation or septation and the complete cell with its wall is delimited and may then swell. In blastic development there is a swelling process of only a portion of the cell, which gives rise to the conidium. This swollen portion becomes separated from the supporting cell by a septum. In some species conidia are produced from flask-shaped cells known as phialides. The phialides do not elongate, and the conidia are produced in succession from the narrow neck of the phialide, forming chains. Conidia may vary in shape, number of cells, size, and color.

c. Oidia

Oidia (sing., **oidium**) are thin-walled reproductive units that are produced from hyphae by a fragmentation process, or they may be produced from a specialized hypha, which fragments from the tip toward the base and is called an oidiophore (structure bearing oidia). Oidia behave like spores, but sometimes they also may function in sexuality, as has been demonstrated in the ascomycete *Ascobolus* and in the basidiomycete *Coprinus*.

d. Chlamydospores

Chlamydospores are thick-walled spores that are formed from vegetative cells and may be in chains or located in a terminal or intercalary position. Chlamydospores with their thick walls provide for survival under adverse environmental conditions.

e. Sclerotia

Survival under adverse environmental conditions is also provided by **sclerotia** (sing., **sclerotium**). The sclerotium is a hard, resistant body made up of fungal tissue, which after a period of dormancy germinates when conditions are favorable to give rise to a mycelium or other fungal structure. Dormancy may last for a long period of time. Sclerotia have been indicated as playing a vital role in the fruiting of the edible morel, *Morchella*.

f. Mycelia Fragments

In nature, nonsexual reproduction may be brought about when a mycelium becomes separated by any means into two or more parts. In the laboratory, mycelial fragmentation is one of the most commonly used means of propagation of fungi. The procedure is simply to take a sterile scalpel and cut out a small block of mycelium from a culture and transfer this aseptically to a nutritionally suitable sterile medium for incubation under appropriate conditions.

D. Spore Germination

Just as there are a variety of types of fungal spores, there are also different means of germination. It is not germane to the present treatment of spore germination to detail the many mechanisms of fungal spore germination, but we point out some general aspects of the topic.

Germination of spores may be affected by the physical feature of the spore; i.e., if the spore wall is thick as in chlamydospores or resistant sporangia, the spore may survive unfavorable conditions of temperature or desiccation, which would not be the case with thin-walled spores. Also, the contents of spores may vary. This includes water content, nutrients, and enzymatic capabilities.

1. Factors Affecting Germination

Whereas some spores will germinate immediately on being released from the parent structure if the environmental conditions are suitable, other species produce spores that remain dormant for a period of time. Dormancy is of two types — endogenous (constitutive) and exogenous.

Endogenous dormancy is imposed from within and may be due to the presence of low moisture content within the spore or the presence of inhibitors of germination. Thus, a wall that is relatively impermeable to water and a low water content of the spore will combine to keep the spore in the dormant stage, and this is a constitutive feature of the spores of certain species of fungi. The inhibitors of germination may be volatile or nonvolatile substances, and these must be removed for germination to take place. In addition, there are compounds that stimulate germination, and one of the ways in which these stimulators act is by overcoming the effects of self-inhibitors.

Among the edible mushrooms there are some species in which breeding is difficult because of poor or inconsistent germination of basidiospores. The outstanding case in which this is true is that of *Agaricus bisporus*, but it is also true with *Volvariella volvacea*; and in a number of other species, the scientist is plagued by inconsistent germination.

Because *A. bisporus* is the edible mushroom that is produced in greatest amounts and is the one for which the most advanced technology has been developed, much attention has been given to the study of spore germination in this species. Early observations indicated that isolated spores germinated very infrequently; but when many spores were close together, good germination occurred. A few spores germinated early and these seemed to stimulate the germination of other spores. These observations led to numerous experimental studies based on the premise that gaseous substances stimulated germination. This was supported by the finding that spore germination increased when the spores were in the same gaseous environment as the living mycelium of *A. bisporus*, or other fungi. Numerous volatile organic acids were then tested for a possible effect on spore germination, and isovalerate, produced by the mycelium, has been implicated as the stimulator of germination in a number of studies. The mode of action that has been suggested is that germination of the spores is suppressed by the accumulation of carbon dioxide in the spores and that isovalerate is a direct precursor of a carbon dioxide acceptor β-methylcrotonyl coenzyme A. Thus, isovalerate acts by removing carbon dioxide from the spore. Essentially what this does is to take away the carbon dioxide that normally is fixed to form oxaloacetate, production of which in this manner suppresses the activity of the enzyme succinic dehydrogenase of the tricarboxylic acid cycle (TCA or Krebs cycle). That is, in the absence of isovalerate, carbon dioxide is used to produce oxaloacetate, slowing the respiratory activities of the TCA cycle in the spores and keeping them in a dormant stage. Isovalerate, by removing the carbon dioxide, prevents the formation of

oxaloacetate from that carbon dioxide and thus activates the respiratory activity of the TCA cycle, which is required for germination.

A study of spore germination in *V. bombycina*,[15] a mushroom closely related to the straw mushroom *V. volvacea*, has suggested that germination of spores of *V. bombycina* operates in a manner similar to that of *A. bisporus;* namely, carbon dioxide acts as a self-inhibitor of germination, and a volatile substance that removes the carbon dioxide permits normal TCA cycle respiration to occur and triggers germination.

Exogenous dormancy is imposed from without; i.e., it is environmentally controlled. In some species of fungi, nutrients are required for germination, but in other species the spore contains sufficient nutrients for germination if water and suitable environmental conditions exist. The environmental factors important in spore germination are the same as those for mycelial growth and fruiting body formation: temperature, pH, aeration, and light. The optimal values for these three different developmental stages of fungi (spore, mycelium, and fruiting body) will differ, although commonly within the same range of values. The nutritional requirements for germination are difficult to generalize, because there are species, on the one hand, whose spores require nothing beyond water and an aerobic condition and, on the other hand, there are species that require inorganic salts and organic compounds such as glucose, or specific vitamins, or amino acids. Griffin[18] points out that in several fungi, carbon dioxide has been shown to be a requirement for spore germination and growth and, more importantly, emphasizes that carbon dioxide may be a universal requirement.

2. Measurement

Plant pathologists have long been concerned with studies of spore germination because of their interest in prevention of the spread of fungal diseases by spores. Thus, many of the techniques that have been developed for testing the effectiveness of various treatments to prevent spore germination have been developed by workers in the field of plant pathology, and certain standard procedures have been established. Of greatest interest from the standpoint of edible mushrooms, however, is the germination of basidiospores. Although the basidiospore commonly takes in water and swells as a first stage in germination, it is the emergence of the germination tube that is commonly accepted as the criterion of germination. Thus, microscopic examinations are made at intervals of time to determine the percentage of spores that have formed germ tubes, and these structures are called germlings. Besides the effects of nutritional and environmental factors on germination, the age of the fruiting body in reference to the time of discharge of the spores may also influence results, as will the density of spores in the germination chamber. Genetic factors also have an effect on spore germination.

X. RELATIONSHIP OF FUNGI WITH OTHER ORGANISMS — SYMBIOSIS

Whether in the water, soil, or other terrestrial habitats, the fungi carry on their existence in the presence of other living organisms. Only when we take the fungi into the laboratory and establish pure cultures are the fungi truly separated during their growth from other organisms. This living together, which is the normal situation in nature for all living things, is referred to as **symbiosis**. Symbiosis is commonly studied by taking a particular organism, or group of organisms, and examining any special relationships that species of a different taxon may have with it. For example, the relationship of insects with pollination of flowering plants or the role of bacteria of the genus *Rhizobium* in nitrogen fixation by leguminous plants are types of symbiotic relationships that have intrigued scientists. These happen to be examples in which both members of the association benefit, but that is not necessarily the case in symbiotic relationships. The word symbiosis simply means living together, and does not imply any advantage or disadvantage to either member of the partnership.

A. Parasitism

Because the fungi are heterotrophic organisms for carbon compounds, their relationship with other organisms commonly is related to this aspect of their nutrition. Thus, we find that some fungi fulfill their needs by obtaining their nutrients from living hosts; i.e., they parasitize these hosts. There are some species that are **obligate parasites** in that they cannot survive and grow away from the living host, and there are others that may obtain their nutrients both from living hosts and by saprophytic means. Such fungi are called **facultative parasites**.

Parasitism by the fungus is at the expense of the host, and while some hosts may be able to tolerate a certain amount of fungal growth, this kind of symbiotic relationship may bring about disease and even death of the host plant. The major diseases of plants are caused by fungi. An important aspect of the biology of fungi is their pathogenicity to plants. In Chapter 9 the diseases of mushrooms are examined.

B. Mutualism

Mutualism is a type of symbiosis in which both partners benefit. Sometimes only one partner benefits and the other is not affected either beneficially or adversely, in which case the term **commensalism** is sometimes used. One example of commensalism that is cited by Deacon[16] has to do with composting. Two fungi, *Chaetomium thermophile* and *Humicola insolens* var. *thermoidea*, are both thermophilic fungi and in abundance during the composting phases in which heat is generated. *Chaetomium thermophile* has strong cellulase activity, breaking down cellulose into cellobiose and glucose. *Humicola*, on the other hand, does not have strong cellulase activity, and it is thought that it utilizes the glucose made available by the cellulase activity of *Chaetomium* for its own growth needs. Thus, *Humicola* benefits from the presence of *Chaetomium*, which is not harmed in the process. There is some suggestion that this may be more a case of mutualism than commensalism because *Chaetomium* may also benefit by the removal of glucose. Glucose, an end product of the cellulase activity, slows the rate of action of the enzyme, so the removal of glucose would make for better cellulase activity by *Chaetomium*. This is, however, just another example of a situation in which systems in nature do not always fit perfectly into human-made categories. The symbiotic relationships existing between fungi and algae (i.e., lichens) and fungi and roots (i.e., mycorrhiza) fit into the term mutualism with less difficulty, however.

1. Lichens

A **lichen** is a distinct organism that is made up of two components: (1) the algal component is known as the phycobiont and generally consists of a green or blue-green alga; (2) the fungal component is known as the mycobiont and most commonly consists of an ascomycete although there are some in which the mycobiont is a basidiomycete. The number of lichen species is estimated to be between 13,500 and 14,000.[2,13] Although it has been possible to separate and grow the phycobiont and mycobiont members of a lichen in culture, and to bring them together again, only rarely and under low nutrient and poor moisture conditions has the reconstituted lichen resembled the one from which it originated. Study of the conditions — nutritional, physical and physiological — that are required for the development of a lichen has indicated the close, mutualistic association of the algal and fungal components.

2. Mycorrhiza

Once thought to be rare and something of a biological oddity, it is now known that the association of fungi with the roots of plants, which is known as a **mycorrhizal association**, is very usual and takes place with most taxa of plants. This association of fungi with roots is an example of mutualism and is of special importance with certain edible fungi. There are a number of important edible

fungi that are mycorrhizal fungi. Among these are the truffle (*Tuber* spp.), the matsutake (*Tricholoma matsutake*), and the boletes (*Boletus* spp.). While the mycelium of mycorrhizal fungi can be grown saprophytically, the formation of fruiting bodies is a product of the interaction of the fungus with the roots of a particular plant or group of plants; until the details of these interactions are discovered, the ability to control the fruiting of these mycorrhizal fungi will elude us. It is interesting that the edible species of mycorrhizal fungi include some of the most highly valued ones. These fungi belong to the type of mycorrhiza known as **ectotrophic**, in which the fungi form a sheath around the root with hyphae penetrating slightly into the root cortex. In ectotrophic mycorrhiza the hyphae grow between the cells of the root cortex, whereas in **endotrophic mycorrhiza** (also called **vesicular arbuscular mycorrhiza**) the hyphae form swollen or branched structures within the root cells. The endotrophic mycorrhizas do not form a sheath as is the case with ectotrophic mycorrhiza, and they are zygomycetes that are commonly associated with herbaceous plants. On the other hand, ectotrophic mycorrhizas are Ascomycetes or more frequently Basidiomycetes that are associated with forest trees (either broad-leaved deciduous trees or conifers).

C. Saprophytism

In saprophytism the fungus obtains its required nutrients from nonliving sources. In nature, the main sources of carbon for fungi are the lignocellulosic components of plant cell walls. Such compounds are not soluble in water and cannot be taken directly into the fungal hyphae. As pointed out in Section II.A of this chapter, the fungi have osmotrophic or absorptive nutrition, which means that they produce enzymes that are excreted from the hyphae into the surrounding environment where these extracellular enzymes can convert the insoluble lignocellulosic polysaccharides into soluble compounds such as glucose. These soluble compounds can move through the fungal cell wall and cell membrane into the cytoplasm where they become metabolized.

The growth of one species alters the conditions of the substrate by changing in quantity or quality the nutrients and moisture as well as the degree of acidity or alkalinity (i.e., the pH or hydrogen ion concentration). This alteration of the substrate condition may make the substrate suitable for growth of another species and a **succession of fungal species may occur**. A commonly observed succession can be seen in the growth of fungi on dung. Here, the first fungi to appear are members of the class Zygomycetes, e.g., *Pilobolus*, frequently followed by some species of the basidiomycete genus *Coprinus* and then by some imperfect fungi of the class Deuteromycetes.

XI. CHEMICAL COMPOSITION OF FUNGI

The fungi do not differ markedly from other organisms in the basic structure of such biologically important compounds as proteins, nucleic acids, lipids, and carbohydrates. That is, these compounds are similar in fungi to those found in plants and animals, but with a few differences worthy of note. These differences include the facts that there are some special fungal polysaccharides, that the concentration of DNA in fungi is somewhat lower than in other organisms, and that there are differences in the lipids of the membranes.

Within the fungal kingdom (the Myceteae) there are also differences in chemical composition, which make it difficult to make a generalization on the chemical composition of the fungi. With approximately 70,800 described species of fungi and estimates that 1.5 million species may actually exist in nature, it is to be expected that both quantitative and qualitative differences would occur. Differences in size, form, and habitat are present within the fungi, and variation in chemical composition could reasonably be anticipated to be associated with these differences. We would not expect the filamentous water mold *Saprolegnia* (class Oomycetes) to have the same chemical composition as the hard, almost woody fruiting body of *Ganoderma lucidum* (class Basidiomycetes) that grows on trees.

TABLE 4.1
Proximate Composition of Fungi

Class of Compounds	Dry Weight (%)
Carbohydrates	16–85
Lipids	0.2–87
Proteins	14–44
RNA	1–10
DNA	0.15–0.3
Ash	1–29

Source: Data from Griffin, D.H., *Fungal Physiology*, 2nd ed., Wiley-Liss, New York, 1994.

In addition to differences between widely separated species, there are also differences between strains of a species. Even the same strain can show chemical differences when different developmental stages are analyzed, as, for example, vegetative mycelium and spores. Analysis of the mycelium of the same strain at different ages may also result in variation in chemical analysis, because autolysis of mycelium occurs after the stationary growth phase. Growth conditions, such as the composition of the medium, or when grown in liquid medium, or whether the mycelium was grown on the surface of the liquid or submerged, can bring about changes in chemical composition.

A. Proximate Composition of Fungal Cells

In spite of the existence of many sources of variation in chemical analysis of fungi, it is still helpful to have information regarding the range of values and to attempt some generalizations. With this objective, a number of authors have published values for the proximate composition of fungi. Table 4.1 presents a compilation of such published values that was prepared by Griffin.[18] The extreme range of values for the various classes of compounds is the outstanding feature of this table and emphasizes the problems that are encountered in making statements about the chemical composition of fungi where different techniques of analysis may have been used, and especially diverse species of fungi. Examination of the table reveals a range of values extending from a onefold difference for DNA up to a 435-fold difference for lipids. Obviously, it is not possible to represent the chemical composition of fungi in general by specific values, and the significance of the table lies in the emphasis that it gives to the variation that can occur. Nevertheless, there are some generalizations about the chemical composition of fungi that are useful and that are given here.

Generalizations Concerning Fungal Chemical Composition

1. A common value for **water content** is 85 to 90%.
2. The **total carbon** is 40 to 50% of the dry weight. *Note:* This is little affected by cultural conditions or age, as most of the carbon is present in the cell wall.
3. The **nitrogen content** varies in published reports that were examined from 2.27 to 5.13%. *Note:* Only 60 to 70% of the nitrogen present in the fungal cell is protein. Thus, it is not correct to determine cellular protein by taking the value of nitrogen as determined by the Kjehldahl method and estimating protein as this nitrogen value × 6.25, which is the usual conversion factor that is used, making the assumption that protein is about 16% nitrogen. The relatively large amount of nonprotein nitrogen is present largely in the chitin of the cell walls, with some also in free amino acids and in nucleic acids. Thus, in many analyses for protein in fungi, certain compromises are made to obtain a more realistic estimate of the available protein in fungi used for food, and the conversion factor

of total N × 4.38 is used in many analyses for the determination of the protein content of mushrooms, for example.

4. Of the **mineral constituents**, phosphorus and potassium are the most abundant. The concentration of phosphorus in the mycelium will be low, however, when the fungus is grown on a medium with a low concentration of nitrogen because there will be a restriction in the synthesis of nucleic acids of which potassium is a significant component. Some elements may be accumulated by certain species of fungi even though they are not required. That is, the presence of an element in the fungal cell does not in itself indicate an important biological role for that element.
5. Many **fungal polysaccharides** are produced, and these are most commonly, but not exclusively, composed of glucose and galactose monomers.
6. **Lipids** are universally present, and their importance as components of the cell membrane and the membranes of cell organelles is well recognized. Lipid content is strongly dependent on cultural conditions, age, and species; therefore, it is of limited value to indicate a percentage composition of lipid for fungal cells on a dry weight basis.

B. Edible Mushrooms

With edible mushrooms, the proximate analysis is given to reflect compounds of significance in human nutrition. Thus, in Table 2.1 in Chapter 2, the proximate composition of 12 edible mushrooms is given. The six cultivated mushrooms that are consumed in greatest amounts are *Agaricus bisporus, Flammulina velutipes, Lentinula edodes, Pleurotus ostreatus, Volvariella volvacea*, and *Auricularia* spp. In that table, values of protein (estimated as total nitrogen × 4.38, according to the rationale presented in the previous section), fat, N-free carbohydrate, fiber, and ash are given. Other important nutritional characteristics for which mushrooms are commonly assayed include the amounts of various amino acids (Chapter 2, Table 2.3), vitamins, and minerals.

REFERENCES

1. Ahmad, S.S. and Miles, P.G., Hyphal fusions in *Schizophyllum commune*. Effects of environmental and chemical factors, *Mycologia*, 62, 1008–1017, 1970.
2. Alexopoulus, C.J., Mims, C.W., and Blackwell, M., *Introductory Mycology*, 4th ed., John Wiley, New York, 1996.
3. Bistis, G.N., Sexuality in *Ascobolus stercorarius*. I. Morphology of the ascogonium; plasmogamy; evidence for a sexual hormonal mechanism, *Am. J. Bot*., 43, 389–394, 1956.
4. Bistis, G.N., Sexuality in *Ascobolus stercorarius*. II. Preliminary experiments on various aspects of the sexual process, *Am. J. Bot*., 44, 436–443, 1957.
5. Bistis, G.N., Chemotropic interactions between trichogynes and conidia of opposite mating type in *Neurospora crassa, Mycologia*, 73(5), 959–975, 1981.
6. Blakeslee, A.F., Sexual reproduction in the Mucorineae, *Proc. Am. Acad. Sci. U.S.A.*, 40, 206–319, 1904.
7. Bold, H., Alexopoulos, C.J., and Delevoryas, T., *Morphology of Plants and Fungi*, 5th ed., Harper, New York, 1986.
8. Bonitati, J., Elliott, W.B., and Miles, P.G., Errors in volumetric respirometry: description of an improved microrespirometer, *Anal. Biochem.,* 43, 481–491, 1971.
9. Bonitati, J., Miles, P.G., and Elliott, W.B., New technique of sample preparation for respirometry of filamentous fungi, *Plant Physiol*., 45, 708–711, 1970.
10. Brodie, H.J., Further investigations on the mechanism of germination of the conidia of various species of powdery mildew at low humidity, *Can. J. Res*., 23, 198–211, 1945.
11. Burnett, J.H., *Fundamentals of Mycology*, 2nd ed., Edward Arnold, London, 1976.
12. Cantino, E.C., The relationship between biochemical and morphological differentiation in non-filamentous aquatic fungi, *Symp. Soc. Gen. Microbiol*., 11, 243, 1961.

13. Carlile, M.J. and Watkinson, S.C., *The Fungi*, Academic Press, San Diego, 1994.
14. Chang, S.T., Miles, P.G., and Wai, C.C., A study of monoporous isolates of *Volvariella volvacea, Mushroom Sci*., 11(2), 603–621, 1981.
15. Chiu, S.W. and Chang, S.T., The activation of spore germination in *Volvariella bombycina, Mushroom J. Trop.,* 7, 61–66, 1987.
16. Deacon, J.W., *Modern Mycology*, 3rd ed., Blackwell Scientific, Oxford, 1997.
17. Emerson, S., The growth phase in *Neurospora* corresponding to the logarithmic phase in unicellular organisms, *J. Bacteriol*., 60, 221–223, 1950.
18. Griffin, D.H., *Fungal Physiology*, 2nd ed., Wiley-Liss, New York, 1994.
19. Hou, H.H. and Wu, L.C., Respiratory changes in the cultivated mushroom, *Agaricus bisporus, Mushroom Sci*., 9(1), 37–49, 1972.
20. Hunsley, D. and Burnett, J.H., The ultrastructural architecture of the walls of some hyphal fungi, *J. Gen. Microbiol*., 62, 203–208, 1970.
21. Jennings, D.H., *The Physiology of Fungal Nutrition*, Cambridge University Press, Cambridge, U.K., 1995.
22. Jennings, D.H., Thorton, J.D., Galpin, M.F.J., and Coggins, C.R., Translocation in fungi, in *Symposia of the Society for Experimental Biology*, XXVII, Sleigh, M.A. and Jennings, D.H., Eds., Cambridge University Press, Cambridge, U.K., 139–156, 1974.
23. Lambert, E.B., Effect of excess CO_2 on growing mushrooms, *J. Agric. Res*., 47, 599–608, 1933.
24. Lu, S.H., Effect of calcium on fruiting of *Cyathus stercoreus, Mycologia*, 65, 329–334, 1973.
25. Mayfield, J.E., Septal involvement in nuclear migration in *Schizophyllum commune, Arch. Microbiol*., 95, 115–124, 1974.
26. Niederpruem, D.J., Role of carbon dioxide in the control of fruiting of *Schizophyllum commune, J. Bacteriol*., 85, 1300–1308, 1963.
27. Niederpruem, D.J. and Hackett, D.P., Cytochrome system in *Schizophyllum commune, Plant Physiol*., 36, 79–84, 1961.
28. Rusmin, S. and Leonard, T.J., Biochemical induction of fruiting bodies in *Schizophyllum commune*: bioassay and its application, *J. Gen. Microbiol*., 90, 217–227, 1975.
29. San Antonio, J.P. and Thomas, R.L., Carbon dioxide stimulation of hyphal growth of the cultivated mushroom, *Agaricus bisporus* (Lange) Sing., *Mushroom Sci*., 8, 623–629, 1972.
30. Schutte, K.H., Translocation in the fungi, *New Phytol.*, 55, 164–182, 1956.
31. Thrower, L.B. and Thrower, S.T., Movement of nutrients in fungi, 1. The Mycelium, *Aust. J. Bot*., 16, 71–80, 1968.
32. Tschierpe, H.J., Der Einfluss von Kohlendioxyd auf die Fruchtkörperbildung und die Fruchtkörperform des Kulturchampignons, *Mushroom Sci*., 4, 235–250, 1959.
33. Voorhees, D.A. and Peterson, J.L., Hypha-spore attractions in *Schizophyllum commune, Mycologia*, 78, 762–765, 1986.

5 Substrate and Mycelial Growth

I. INTRODUCTION

The mycelium of mushrooms, like all fungal cells, lacks chlorophyll and consequently is unable to utilize carbon dioxide, mineral ions, and water for photosynthesis as do green plants. Nutritionally, mushrooms are heterotrophs and obtain their nutrients by absorbing soluble inorganic and organic materials from substrates such as wood logs, manure composts, or other organic synthetic composts. Recall that in Chapter 4 we referred to this as absorptive or osmotrophic nutrition and mentioned the role of extracellular enzymes in breaking down insoluble compounds such as polysaccharides into smaller, soluble substances. Once organic compounds have entered the fungal cell, they can be converted to the various sugars, polysaccharides, proteins, lipids, purines, pyrimidines, vitamins, etc. required for the vital activities and structural needs of the fungus. The fungi are endowed with exceptional metabolic capabilities; many of them are able to grow and reproduce when supplied with a sugar as carbon source, a nitrogen source, various minerals, possibly a vitamin, and water. The carbon source provides the skeletal carbon for organic compounds and the energy for the anabolic processes. The nitrogen is essential for amino acids and, therefore, for proteins and enzymes. The minerals such as phosphorus, sulfur, magnesium, and potassium have a variety of functions, and the vitamins function as coenzymes.

Decades ago, Waksman and co-workers[43–49] conducted studies from which they concluded that most of the nutrients required for mycelial growth and mushroom development were obtained from lignin, cellulose, hemicellulose, and protein. These studies established the foundation for investigations of the preparation of composts from the standpoint of chemical and physical properties along with mixed culture fermentation for mushroom growth.

It is a long-established fact that elongation of a hypha is strictly apical and that laterals commonly arise immediately to one side of the apex. As described in Chapter 3, the apical growth can be demonstrated simply by measuring at different time intervals the distance from the hyphal tip to the first septum or branch and the distance between subsequent septa or branches. Only the apical segment will increase in length, and there is no increase in length between septa or branches once they have been formed.[29] The mycelium excretes enzymes into the substrate, and these enzymes degrade the insoluble compounds into soluble ones; then the mycelium absorbs these degraded and soluble molecules and uses them as nutrients for growth.

Research on the nutritional requirements for mushroom growth using different methods may be of both commercial and scientific value. An improved understanding of the biology of mushrooms has often led to improved commercial cultivation procedures and consequently increased yields of the mushrooms. The aims of research on the nutritional requirements of mushrooms have been outlined by Wood and Fermor[52] as follows:

- To increase understanding of the major nutritional requirements of the mushroom mycelium; in particular, to determine the carbon and nitrogen sources that can be utilized.
- To understand the effects of composting and other treatments of straw-based substrates in order to manipulate composting procedures for the improved utilization of the compost by mushroom mycelium.

- To assess the effectiveness and/or the value of supplementary materials added during composting or at spawn run.
- To facilitate the selection of strains of the mushroom having increased ability to degrade one or more of the major nutritional sources in the compost.

II. GENERAL NUTRITIONAL REQUIREMENTS FOR MUSHROOM GROWTH

Different types of mushrooms require different types of substrates. *Agaricus bisporus* grows on fermented compost, which is traditionally developed from wheat straw mixed with horse manure, and it requires a higher nitrogen content. Wheat straw contains about 0.62%, and horse manure contains about 1.5 to 1.8% nitrogen. The optimum C:N ratio for the mushroom is about 17.[38] *Volvariella volvacea*, the paddy straw mushroom, and *Stropharia rugoso-annulata* are grown on almost fresh or hardly composted plant residues, such as: rice straw, cotton waste, and banana leaves. Cellulosic plant materials contain different amounts of nitrogen: rice straw 0.58%, wheat straw 0.62%, barley 1.18%, cotton waste 0.65 to 1%, and banana leaves, 1.71%. It should be understood that *V. volvacea* is capable of growing on plant materials with a low nitrogen content. The optimum C:N ratio is about 75 to 80:1, but C:N ratios from 32 to 150:1 are almost as effective.[5] *Lentinula edodes* and *Pleurotus* spp. are fungi that can grow on wood. In addition to being distinguished by its high lignin content, wood can also be distinguished from other plant materials by its very low nitrogen content. Woody tissues contain 0.03 to 1.0% nitrogen as compared with 0.58 to 1.71% in herbaceous residues. The C:N ratio in most woody tissues is in the order to 350 to 500:1. Wood-inhabiting mushrooms are unique in that they can grow in such substrates. This suggests that these mushrooms can metabolize large amounts of carbohydrates, including lignin, in the presence of a very small amount of nitrogen.

The C:N ratio of the substrate is often used as a relative reference to characterize a compost. As the C:N ratio is considered to be the ratio of all the carbon to all the nitrogen in the substrate (compost), Gerrits[18] distinguished the "total" C:N ratio from the "available" C:N ratio. His argument is that the total amount of carbon includes all kinds of carbon from readily available to available with great difficulty. This same line of reasoning applied to nitrogen availability. It seems likely, however, that normally there will be a relationship between the "total" and "available" C:N ratio. If there is very little nitrogen (a wide C:N ratio), there will be little ammonia in the compost, and, if there is a lot of nitrogen (a narrow C:N ratio), there will be a high concentration of ammonia in the compost.

In the early history of mushroom research, many workers analyzed the compost to look for a relationship between different factors and mushroom growth and yield. This is difficult because compost is such a complicated substrate in both chemical and physical respects.

For obtaining precise information on the nutritional requirements for mushroom growth and development, agar cultures also are of limited value — particularly when inorganic nutrition is being studied. The reason for this is that agar contains a number of impurities in variable amounts. In precise work, therefore, liquid media of defined composition are necessary, with growth assessed by the dry weight of fungal material produced.[30] The most important and comprehensive early work on the nutrition of *Agaricus bisporus* in liquid culture was that of Treschow,[41] who critically reviewed the previous work on this subject and found that a wide variety of carbon compounds, amino acids, minerals, and trace elements can be utilized by the mushroom mycelium. Leatham[34] reported that he obtained fruiting of a dikaryotic stock of *L. edodes* on a chemically defined medium within 45 days after inoculation and that sometimes fruiting occurred as early as 27 days. By using liquid media to study the nutritional requirements of *V. volvacea*, the amino acids were found to be superior to the inorganic ammonium salts in supporting cellulose decomposition, with asparagine the best nitrogen source.[5,6,21,42] Nutritional requirements of *Pleurotus* spp. also have been extensively

studied in submerged culture.[25,31,32,37,40] From the above studies utilizing liquid culture techniques, certain basic general requirements for growth of mushrooms have been demonstrated. Apart from water, which of course is essential, the basic requirements include a usable source of carbon, an appropriate nitrogen compound, certain inorganic ions in appreciable quantities (namely, calcium, phosphate, potassium, sulfate, and magnesium), a number of trace elements (including iron, zinc, copper, manganese, and molybdenum), and sometimes special organic compounds (especially vitamins, again needed in minute amounts). However, an obvious disadvantage of using liquid media to determine the nutrient requirements of mushrooms is that, in the course of time, the composition of the medium is altered as the result of absorption of substances from the medium and the liberation of fungal metabolites into it. There is often a particular problem with pH, since the pH of the medium may drift rapidly.

III. PREPARATION OF SUBSTRATE

Mushroom substrate may be simply defined as a kind of lignocellulosic material that supports the growth, development, and fruiting of mushroom mycelium. The process of preparation of substrate is usually termed **composting**. The final product of composting is called the compost.

A. Composting

Generally, composting refers to the piling of substrates for a certain period of time and the changes due to the activities of various microorganisms, which result in a composted substrate that is chemically and physically different from the starting material. This is sometimes referred to as solid-state fermentation. Two types of composting are commonly described. One type involves the decomposition of heaps of organic wastes and the subsequent application of the residue to the soil. The aim of this type of composting is to reduce, in a sanitary manner, both the volume and the C:N ratio of the organic waste so that it is suitable for manuring soil to improve the growth of plant crops. When given directly to soil without composting, organic wastes with a high C:N ratio (such as straw) can give rise to a temporary nitrogen deficiency, which will then result in a reduction in yield of the plant crop.

The second type of composting is also a process of microbial fermentation, but in this case the substrate is used for the cultivation of edible mushrooms. Through composting, a mixture of rich organic materials is converted into a stable medium that is selective for the growth of a particular mushroom but is not suitable, or is less favorable, for the growth of competing microorganisms. The competitors exist in uncomposted materials, and often in partially composted materials, but they are far less active in well-composted mushroom substrates. Actually, this type of composting is derived mainly from the *Agaricus* mushroom–growing industry, in which a composting technique that renders wheat straw with horse manure specific for the growth of the *Agaricus* mushroom has been developed. It should be noted that the treatment of substrates for growing other mushrooms can be regarded as composting, but the procedures followed in composting and the nature of the product can be quite different. This is because the starting materials and the lengths of time accompanying the various changes in the substrate vary from mushroom to mushroom.

Our concern is to present the reader with the purposes of composting and the general changes that take place during this process. Consequently, we discuss this with special reference to *Agaricus*, because composting has been most extensively studied in this mushroom, and we do not attempt to include all the differences that occur in composting for other mushrooms.

Under natural circumstances when mushroom spawn is inoculated into raw substrate, the competing microorganisms may quickly gain dominance and prevent the mushroom mycelium from developing. The main purpose of composting, then, is to prepare a medium of such characteristics that the growth of mushroom mycelium is promoted to the practical exclusion of other organisms. During composting, certain chemical properties, physical qualities, and microbial flora

have to be developed in the substrate. All of these are equally important, and none is independent of the others. Broadly speaking, the proper chemical state is one in which the food materials best able to serve the nutritional needs of the mushrooms are accumulated. These foodstuffs must be in a form available to the mushroom. For example, it would be unsatisfactory if all the nitrogen were changed to nitrate instead of protein, as the mushrooms cannot use nitrates. Moreover, toxic substances that inhibit the growth of spawn must not be produced. The substrate must also have certain physical qualities. It must support aerobic conditions, hold waste without becoming waterlogged, and have a proper pH and good drainage. Biologically, the substrate must have a population of suitable microorganisms. It is literally teeming with millions of bacteria and fungi. The substrate in compost preparation for most mushrooms should not be sterilized.

Traditionally, horse manure and wheat straw are the basic ingredients of *Agaricus* mushroom compost; whereas wood logs and paddy straw are used for growing *Lentinula* and *Volvariella* mushrooms, respectively. Accordingly, any substrate that is composted of agricultural and/or industrial waste materials mixed with various organic supplements is called a "synthetic compost" when used for growing *Agaricus* or *Volvariella*, and a "synthetic log" when used for *Lentinula*. Numerous formulations employing every manner of lignocellulosic waste products and residues for the preparation of synthetic composts and logs have been devised (details are described in subsequent chapters). Synthetic compost in this sense is, in fact, the general substrate used for growing edible mushrooms, including *Agaricus* and *Lentinula*. Actually, the preparation of a compost suitable for mushroom growing is still very much dependent on the skill and experience of the individual grower. A well-prepared compost is essential for a successful crop because subsequent cultural operations cannot produce a good crop from a compost of poor quality. The comprehensive results of research on mushroom composts have been intensively reviewed.[15,22–24,28]

The process of mushroom composting, particularly with regard to *Agaricus*, takes place in two distinct phases. Although the principles of this complicated process are known and guidelines are available,[3,19,20,35,50] in practice modifications are necessary to meet various situations, e.g., the availability of raw materials, the facilities in the growing area, and, above all, the species of mushroom that is to be cultivated. There is no possibility that the technology of the *Agaricus* cultivation industry, even though it is considered to be the most advanced, can be uniformly followed or applied for the growing of other mushrooms in every situation. This is particularly the case for *V. volvacea*, because this mushroom lacks sufficient polyphenoloxidases to neutralize the poisonous phenol compounds that are liberated from the lignins during composting.

1. Phase I Composting (Compost Preparation)

The purpose of Phase I composting is to mix and wet the raw materials and to begin the composting process during which various microorganisms break down the straw (Figure 5.1). In straw or other plant residues, the main components are cellulose, hemicellulose, and lignin. The hemicelluloses are generally found in rather close association with cellulose in plant fibers. Both cellulose and hemicellulose are carbohydrates, which yield sugars after appropriate treatment. They are readily attacked by some bacteria and fungi and are easily decomposed under suitable circumstances. Lignin, on the other hand, is a resistant substance that more or less impregnates plant fiber and is not readily attacked by bacteria.

After composting, the easily decomposed carbohydrates, which serve as an excellent source of food for molds and bacteria, tend to diminish, and the substrate is no longer favorable for these potential competitors. Proteins increase as a result of the activity of the microorganisms that convert simple nitrogenous materials such as ammonia and nitrates to complex proteins. The heating of the heap is of special interest. Lambert and Davis[33] have identified four zones of activity in compost stacks, which were related to the distribution of temperature to the natural diffusion of air into the stacks and to microbial activity (Figure 5.2). Microbial activities in the anaerobic zone 4 and the outside cold zone 1 are considered to be unsatisfactory. Therefore, a good turner and turning

FIGURE 5.1 Different stages of composting for *Agaricus* mushrooms in France.

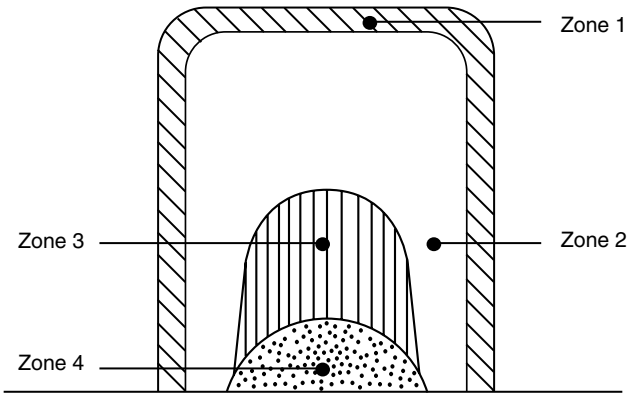


FIGURE 5.2 Diagrammatic representation of zonation in a compost heap. (Zone 1) temperature approximately 35°C, well-aerated, dry, under composed; (Zone 2) temperature 55 to 60°C, well-aerated, decomposed, white flecks of firefang fungus clearly visible; (Zone 3) temperature 70 to 80°C, aeration restricted; (Zone 4) temperature approximately 35°C, yellow, foul-smelling, anaerobic.

schedule should move the material between zones and give the entire compost the desirable aerobic zone conditions for a time. The composting stack can be made up in the open or, more commonly now, under the protection of open-sided compost sheds. For commercial purposes, yields from Phase I compost would not be high enough, because such compost is itself vulnerable and would also be subject to pathogens.

2. Phase II Composting (Compost Conditioning)

This is commonly known as pasteurization or peak-heating. The objectives of Phase II composting are twofold: (1) to eradicate insects and pests carried in with the Phase I composting substrate and to destroy spores of contaminating microorganisms and (2) to bring the substrate to a uniform temperature of about 50 to 55°C, which promotes decomposition of the substrates by thermophilic microorganisms. Through this final adjustment, a more selective medium favoring the growth of the mushroom is produced.

Live steam generated from a water boiler is generally employed for pasteurization, and it gives satisfactory results. It must be noted that it is the bed temperature that is critical, and the object is to manage the air temperature in any way necessary to keep the bed temperature at the desired level. This entire process must be adapted to meet the specific circumstances existing in different

TABLE 5.1
Major Groups of Microorganisms Isolated from Compost during Substrate Preparation

Bacteria	Actinomycetes	Fungi
Mesophiles		
Flavobacterium spp.	*Streptomyces* sp.	*Mucor* sp.
Pseudomonas spp.	*Nocardia* sp.	*Aspergillus* spp.
Serratia marcescens	*Micropolyspora* sp.	*Penicillium* spp.
		Phizopus stolonifer
Thermotolerants		
Pseudomonas spp.		*Aspergillus fumigatus*
Bacillus licheniformis		
Thermophiles		
Bacillus coagulans	*Thermoactinomyces* spp.	*Mucor pusillus*
Bacillus stearothermophilus	*Thermomonospora* spp.	*Humicola insolens*
Bacillus subtilis		*Rhizomucor pusillus*
		Talaromyces lanuginosa
		Humicola fuscoatra

Source: Data from Sparling, G.P. et al., *Soil Biol. Biochem*., 14, 609–611, 1982.

mushroom houses. Generally speaking, it takes approximately 2 hours after the introduction of live steam to have an air temperature of 60 to 62°C. This temperature should be maintained for 2 hours, and then a gentle stream of fresh air introduced to lower the temperature to 52°C. This temperature is maintained for the next 6 to 8 hours for *Volvariella volvacea,*[7] and for several days for *Agaricus bisporus.*[3,19] The steam supply is then shut off, and the compost is allowed to cool gradually to the desired temperature for spawning. How long it takes for this last step depends on the mushroom species and on the outdoor temperature.

B. Microorganisms Involved during Composting

A spectrum of different microorganisms has been isolated at various stages of composting.[7,13,26,28,51] In view of the complexity of the compost materials and the variations encountered even within the same composting stack, a considerable number of microbial species are encountered, but a list of some of the genera commonly isolated in these studies can be gained from examination of Table 5.1.

Temperature is one of the cardinal factors that determine the distribution of many microorganisms in the compost. Although these microorganisms of the compost can be generally classified as mesophiles, thermotolerants, and thermophiles, there is no clear dividing line between groups. If the temperature characteristics (in terms of minimum, optimum, and maximum) of a very large number of microorganisms were considered, these groups would definitely merge into one another. By far the majority of microorganisms are mesophiles, growing between 5 and 37°C.

Although a number of prokaryotic organisms are able to live and thrive at temperatures above 90°C, and for some of these 60 to 62°C is near the minimum temperature at which they will grow, the upper limit (maximum temperature) for eukaryotic organisms is near 60°C. However, the most widely used definition of a thermophilic fungus is that given by Cooney and Emerson,[9] who consider a thermophilic fungus one whose minimum temperature for growth is 20°C or above and whose maximum temperature for growth is 50°C or above. Such fungi have optima around 45 to 50°C. A thermophile can be simply defined as an organism that grows at temperatures above those considered to be the maximum limits for most forms of life.[33] However, there are a number of fungi that have maxima of 50°C or above, but minima below 20°C. They have optima at or around

40°C and grow slowly, or not at all, above 50°C. These are usually regarded as thermotolerants, rather than as true thermophiles.

Crisan[10] comprehensively reviewed the current hypotheses to explain thermophilism. Four of these hypotheses for explaining the ability of thermophiles to grow at high temperatures remain of major interest:

1. The **lipid solubilization hypothesis** proposes that thermal death occurs when the cell loses its integrity due to the solubilization at elevated temperatures of the protoplasmic lipids.
2. The **rapid resynthesis of essential metabolites hypothesis** suggests that thermophilic growth is not due to the presence of unusually thermostable metabolites such as enzymes but is the consequence of a particularly active metabolism, which, at elevated temperatures, replaces thermolabile metabolites at a rate equal to or greater than the rate at which they are being destroyed.
3. The **macromolecular thermostability hypothesis** proposes that thermophiles are able to produce some essential macromolecules, such as enzymes and other proteins, which exhibit an unusual degree of thermostability.
4. The **ultrastructural thermostability hypothesis** suggests that thermophiles contain some ultrastructural elements or organelles that exhibit a greater degree of thermostability than do similar components of mesophiles.

There is evidence that supports each of the above hypotheses; but it is difficult to evaluate the hypotheses, because the supporting evidence comes from studies of a variety of species and involves variation in cultural conditions and methods of analysis.

In mushroom composts, the initial activity of the microbial population is to absorb the soluble carbon and nitrogen compounds present in the stack materials. After these have been used up, growth of microorganisms takes place on the insoluble fractions, such as cellulose, hemicellulose, and protein.[27,47] It is of interest to note that little or no lignin is degraded during early stages of composting.[53] The later stages of the composting process support a great variety of Actinomycetes, which may be responsible for the observed lignin and cellulose degradation. At the same time, a layer of dark brown material usually accumulates on the outer layer of the straw surface. The material contains various intact and degraded microbial fractions and may be a nutritional source for the mushroom mycelium.[51] Further details of the preparation of compost and other procedures in mushroom cultivation can be found in Chang and Hayes.[8]

IV. BREAKDOWN OF SUBSTRATES BY EXTRACELLULAR ENZYMES OF MUSHROOM MYCELIUM

After proper composting, a suitable and selective medium is produced. This compost is a medium that supports good growth of mushroom mycelium but does not encourage growth of competitor or weed fungi. This is because during composting most of the soluble and common small nutritional compounds have been utilized for growth by various microorganisms. Therefore, at spawning, which is the process of planting mushroom mycelium (spawn) onto the bed materials, the compost primarily contains insoluble fractions of the substrate. These include lignin, cellulose, hemicellulose, protein, and microbial biomass. The mushroom mycelium of *Agaricus bisporus*, for example, can secrete various extracellular enzymes (Table 5.2) into the compost substrate,[12,17,47] as well as a large part of the microbial biomass.[14,51] These enzymes are constitutive; i.e., they are always produced or, more often, their production is induced by some substance, often a specific substrate, present in the medium.

Although the process of breakdown of lignin is not well understood, there is an apparent correlation between the ability to degrade lignin and the production of extracellular phenolases,

TABLE 5.2
Extracellular Enzymes of *Agaricus bisporus*

Enzyme	Nutrient in Growth Substrate (compost)	Product of Enzyme Activity for Assimilation by Fungus
Laccase	Lignin or phenols	?
Endocellulase Exocellulase β-Glucosidase	Cellulose and cellobiose	Cellobiose and glucose
Xylanase	Xylan	Xylose
Proteases	Protein (plant and microbial)	Amino acids
Phosphatase	Phosphate esters	Phosphate
β-*N*-Acetyl-muramidase (lysozyme)	Bacterial cell walls	?
β-*N*-Acetyl-glucosaminidase	Microbial cell walls	?
Laminarinase	β-(1→3)-Glucans	Glucose
DNAase RNAase	Microbial DNA Microbial RNA	Sugars and nucleotides
Lipase	Plant or microbial lipids	Fatty acids

Source: Data from Sparling, G.P. et al., *Soil Biol. Biochem.*, 14, 609–611, 1982.

such as laccase, peroxidase, and tyrosinase, which oxidize phenolic compounds, e.g., gallic and tannic acids. These oxidative reactions have long been considered involved in conversion of complex phenols to simple aromatic compounds that can be absorbed by mushroom mycelium and used for its growth. It should be noted that lignin is resistant to decomposition under anaerobic conditions.

Breakdown of cellulose, also a complex and insoluble carbohydrate, is brought about by the hydrolytic enzyme cellulase, which involves the cooperative functioning of at least three enzymes, namely, an exoglucanase, endoglucanase, and a β-glucosidase. The ability to degrade cellulose is restricted by the level of endoglucanase and not by β-glucosidase.[4] The products of cellulase action are simple and soluble carbohydrates; the end product is glucose. These soluble carbon compounds can be absorbed by the fungal mycelium and used for growth and as the energy source.

The extracellular enzymes produced by mushroom mycelium have been considered to be involved in the degradation of microbial biomass that was built up during composting.[2,12,51] The total microbial biomass in a horse manure and wheat straw compost immediately before spawning has been estimated to approximately 2% of the compost by weight, assuming that microbial biomass contains 50% carbon. The ratio of fungal and actinomycete to bacterial mass in this compost was estimated to be 2.8:1. However, the relative ratio of fungal to actinomycete mycelia in the compost is unknown.

The microbial biomass has been calculated to provide less than 10% of the total mushroom biomass.[39] This indicates that *A. bisporus* probably obtains the bulk of its carbon nutrition from straw. However, the biomass does contain a high level of nitrogen, and, since microorganisms also accumulate minerals during composting, the microbial biomass could act as a good source of both nitrogen and minerals for mushroom growth.

V. GENETIC IMPROVEMENT OF MUSHROOM CULTURE IN REGARD TO SUBSTRATE UTILIZATION BY INCREASED PRODUCTION OF EXTRACELLULAR ENZYMES

Franklin[16] reported that A.J. McCarthy of the University of Manchester Institute of Science and Technology had isolated a range of Actinomycetes with the ability to break down lignin from wheat

lignocellulose. Some isolates were capable of fast solubilization of as much as 40% of the lignin substrate. The Actinomycetes appear to offer additional advantages. Their initial attack is faster and their degradative system is more robust. Moreover, according to the present state of genetic knowledge, recombinant DNA technology as a method of improvement is feasible with Actinomycetes.

The isolation of hypercellulolytic mutants of *Trichoderma reesei* has greatly improved the potential for conversion of lignocellulosic materials to produce monomer sugars. Cellulase produced through mixed cultures of *T. reesei* and *Aspergillus phoenicis*[11] and of *T. harzianum* and *A. ustus*[36] also showed increased β-glucosidase activity and greatly improved hydrolytic potential.

Arst[1] reported that scientists of the University of Essex had devised and successfully used a new cloning strategy for the filamentous fungus, *Aspergillus nidulans*. The method screens for chromosomal aberrations sufficiently large that changes in restriction patterns of genomic DNA can be easily detected in Southern blots probed with cloned DNA that overlaps the aberration. Their method has already enabled them to clone several genes of interest.

The primary factor that determines the ultimate secretion capacity for the extracellular enzymes of an organism is its genetic constitution. Extracellular enzymes, produced by microorganisms of monocultures or mixed cultures, when in compost can increase the productivity of mushrooms, because their presence makes the lignocellulose substrate more suitable for mushroom growth. The biotechnological improvement of current commercial strains of mushrooms for increasing yield of extracellular enzymes is also important and is a potentially useful strategy; however, the present state of biological knowledge and genetic techniques in mushrooms does not yet permit the exact procedure of cloning and transformation of the genes important in the production of these enzymes. The improvement of mushroom culture by increasing production of extracellular enzymes through conventional breeding procedures is highly probable, however, and would be very useful, because such strains could increase substrate bioconversion.

REFERENCES

1. Arst, H.N., Jr., A new cloning strategy for filamentous fungi, *Microbiol. Sci*., 167, 1985.
2. Atkey, P.T. and Wood, D.A., An electron microscope study of wheat straw composted as a substrate for the cultivation of the edible mushroom (*Agaricus bisporus*), *J. Appl. Bacteriol.,* 55, 293–304, 1983.
3. Carapiet, G., Phase II or cookout, *Mushroom Sci*., 11(Part I), 311–318, 1981.
4. Chandhary, K., Mittal, S.L., and Tauro, P., Control of cellulose hydrolysis by fungi, *Biotechnol. Lett*., 7, 455–456, 1985.
5. Chang-Ho, Y. and Ho, T.M., Effect of nitrogen amendment on the growth of *Volvariella volvacea, Mushroom Sci*., 10, 619–628, 1979.
6. Chang-Ho, Y. and Yee, N.T., Comparative study of the physiology of *Volvariella volvacea* and *Coprinus cinereus, Trans. Br. Mycol. Soc.,* 68, 167–172, 1977.
7. Chang, S.T., Cultivation of *Volvariella volvacea* from cotton waste composts, *Mushroom Sci*., 10, 609–618, 1979.
8. Chang, S.T. and Hayes, W.A., Eds., *The Biology and Cultivation of Edible Mushrooms,* Academic Press, New York, 1978.
9. Cooney, D.G. and Emerson, R., *Thermophilic Fungi — An Account of Their Biology, Activities and Classification*, Freeman, San Francisco, 1964.
10. Crisan, E.V., Current concepts of thermophilism and the thermophilic fungi, *Mycologia,* 65–67, 1171–1198, 1973.
11. Duff, S.J.B., Cooper, D.G., and Fuller, M., Cellulase and β-glucosidase production by mixed culture of *Trichoderma reesei* Rut C30 and *Aspergillus phoenicis, Biotechnol. Lett*., 7, 185–190, 1985.
12. Eddy, B.P. and Jacobs, L., Mushroom compost as a source of food for *Agaricus bisporus, Mushroom J.,* 38, 56–67, 1976.
13. Fergus, C.L., Thermophilic and thermotolerant molds and actinomycetes of mushroom compost during peak-heating, *Mycologia*, 56, 267–284, 1964.

14. Fermor, T.R. and Grant, W.D., Degradation of fungal and actinomycete mycelia by *Agaricus bisporus, J. Gen. Microbiol.,* 131, 1729–1734, 1985.
15. Flegg, P.B., Mushroom composts and composting: A review of the literature, *Rep. Glasshouse Crops Res. Inst.,* 1960.
16. Franklin, F.C.H., The impact of biotechnology on agriculture, *Biotechnol. News*, 5, 7, 1985.
17. Gerrits, J.P.G., Organic compost constituents and water utilized by cultivated mushroom during spawn running and cropping, *Mushroom Sci*., 7, 111–126, 1968.
18. Gerrits, J.P.G., The supplementation of horse manure compost and synthetic compost with chicken manure and other nitrogen sources, *Mushroom Sci*., 9(Part II), 77–98, 1974.
19. Gerrits, J.P.G., Factors in bulk pasteurization and spawn-running, *Mushroom Sci*., 11(Part I), 351–365, 1981.
20. Gerrits, J.P.G., Bels-Koning, H.C., and Muller, F.M., Changes in compost constituents during composting, pasteurization and cropping, *Mushroom Sci*., 6, 225–243, 1967.
21. Ghosh, A.K. and Sengupta, S., Studies on biochemistry of higher fungi. I. Submerged growth of *Volvariella volvacea* in synthetic medium, *J. Food Sci. Technol*., 14, 6–9, 1977.
22. Gray, K.R., Sherman, K., and Biddlestone, A.J., A review of composting. Part 1, *Process Biochem,* June, 32, 1971.
23. Gray, K.R., Sherman, K., and Biddlestone, A.J., A review of composting. Part 2: The practical process, *Process Biochem*, October, 22, 1971.
24. Gray, K.R., Biddlestone, A.J., and Clark, R., Review of composting. Part 3: Processes and products, *Process Biochem*., October, 11, 1973.
25. Hashimoto, K. and Takahashi, Z., Studies on growth of *Pleurotus flabellatus, Mushroom Sci*., 9, 585–593, 1974.
26. Hayes, W.A., Microbiological changes in composting wheat straw/horse manure mixture, *Mushroom Sci*., 7, 173–186, 1968.
27. Hayes, W.A., Mushroom nutrition and the role of microorganisms in composting, in *Composting*, Hayes, W.A., Ed., W.S. Maney & Sons, Leeds, U.K., 1977.
28. Hayes, W.A., *Composting*, W.S. Maney & Sons, Leeds, U.K., 1977.
29. Hudson, H.J., *Fungal Biology,* Edward Arnold, London, 1986.
30. Ingold, C.T., *The Biology of Mucor and Its Allies*, Edward Arnold, London, 1978.
31. Khanna, P. and Garcha, H.S., Physiological studies on *Pleurotus* spp. I. Nitrogen utilization, *Mushroom Newsl. Trop.*, 5(3), 16–19, 1985.
32. Khanna, P. and Garcha, H.S., Physiological studies on *Pleurotus* spp. II. Carbon utilization, *Mushroom Newsl. Trop.*, 6(1), 9–14, 1985.
33. Lambert, E.B. and Davis, A.C., Distribution of oxygen and carbon dioxide in mushroom compost heaps as affecting microbial thermogenesis, acidity and moisture therein, *J. Agric. Res*., 48, 251–253, 1934.
34. Leatham, G.F., A chemically defined medium for the fruiting of *Lentinus edodes, Mycologia*, 75, 905–908, 1983.
35. Lomax, K.M., Collins, N.E., Jr., and Denneny, C.M., Simulating mushroom compost heat production, *Biocycle,* April, 38–40, 1984.
36. Macris, B.J., Paspaliari, M., and Kekos, D., Production and cross-synergistic action of cellulolytic enzymes from certain fungal mutants grown on cotton and straw, *Biotechnol. Lett.,* 7, 369–372, 1985.
37. Mueller, J.C., Gawley, J.R., Lanz, H., and Hayes, W.A., Mineral and heavy metal content of *Pleurotus sajor-caju* grown on cellulosic residues from a bleached kraft pulp mill, *Mushroom Newsl. Trop.*, 5(3), 9–16, 1985.
38. Smith, J.F. and Hayes, W.A., Use of autoclaved substrates in nutritional investigations on the cultivated mushroom, *Mushroom Sci*., 8, 355–362, 1972.
39. Sparling, G.P., Fermor, T.R., and Wood, D.A., Measurement of the microbial biomass in composted wheat straw, and the possible contribution of the biomass to the nutrition of *Agaricus bisporus, Soil Biol. Biochem*., 14, 609–611, 1982.
40. Srivastava, H.C. and Bano, Z., Nutritional requirements of *Pleurotus flabellatus, Appl. Microbiol*., 19, 166–169, 1970.
41. Treschow, C., Nutrition of the cultivated mushroom, *Dansk Bot. Ark.,* 11, 1–180, 1944.

42. Tzeng, D.S., Studies on Nutritional Requirements and the Improvement of Techniques in Cultivation of Straw Mushroom, *Volvariella volvacea*, M.S. thesis, National Chung Hsing University, Taichung, Taiwan, 1974 [in Chinese with English summary].
43. Waksman, S.A. and Allen, M., Comparative rate of decomposition of composted manure and spent mushroom soil, *Soil Sci*., 34, 189–195, 1932.
44. Waksman, S.A. and Cordon, T.C., Thermophilic decomposition of plant residues in composts by pure and mixed cultures of microorganisms, *Soil Sci*., 47, 217–225, 1939.
45. Waksman, S.A., Cordon, T.C., and Hulpoi, N., Influence of temperature upon the microbiological population and decomposition processes in composts of stable manure, *Soil Sci*., 47, 83–113, 1939.
46. Waksman, S.A. and McGrath, J., Preliminary study of chemical processes involved in the decomposition of manure by *Agaricus campestris, Am. J. Bot*., 18, 573–581, 1931.
47. Waksman, S.A. and Nissen, W., On the nutrition of the cultivated mushroom, *Agaricus campestris* and the chemical changes brought about by this organism in the manure compost*, Am. J. Bot*., 19, 514–537, 1932.
48. Waksman, S.A. and Reneger, C., Artificial manure for mushroom production, *Mycologia*, 26, 38–45, 1934.
49. Waksman, S.A., Umbreit, W.W., and Cordon, T.C., Thermophilic actinomycetes and fungi in soils and composts, *Soil Sci*., 47, 37–61, 1939.
50. Wang, H.C. and Shou, C.H., The development of phase II composting for mushroom cultivation in China, *Mushroom Sci*., 11(Part I), 319–326, 1981.
51. Wood, D.A., Microbial processes in mushroom cultivation, a large scale solid substrate fermentation, *J. Chem. Tech. Biotechnol.*, 34B, 232–240, 1984.
52. Wood, D.A. and Fermor, T.R., Nutrition of *Agaricus bisporus* in compost, *Mushroom J*., 114, 194–197, 1982.
53. Wood, D.A. and Leatham, G.F., Lignocellulose degradation during the cycle of *Agaricus bisporus, FEMS Microbial. Lett*., 20, 421–424, 1983.

6 Sexuality and the Genetics of Basidiomycetes

I. DISCOVERY OF SEXUALITY BY KNIEP AND BENSAUDE

Although Blakeslee in 1904 demonstrated sexuality in the Mucorales (class Zygomycetes) through matings of mycelia established from single spores,[3] it was not until 1918–1920 that the mechanism of sexuality in the Basidiomycetes was discovered. During World War I, Hans Kniep[16] in Germany studied mating reactions of single-spore cultures of *Schizophyllum commune*, and in France Mathilde Bensaude[2] of Portugal based the research for her doctoral dissertation on a study of the mating reactions of single-spore cultures of *Coprinus fimetarius*. Bensaude showed that the mycelium arising from single spores was made up of hyphae with simple septa (Figure 6.1B) and that the hyphae of the fruiting bodies and mycelium, which gave rise to fruiting bodies, bore clamp connections (Figure 6.1A). Furthermore, she demonstrated that when mycelia that had originated from single spores were confronted in various combinations, only certain combinations gave rise to clamped mycelium, while in other combinations the hyphae had simple septa.

A. Tetrapolarity

Blakeslee's studies in the Mucorales showed that in those Zygomycetes that are heterothallic the single-spore cultures are either + or − as far as mating is concerned. However, things are more complicated in the Basidiomycetes. The genetic basis of this pattern of sexuality, known as **tetrapolarity**, was first most completely described by Kniep, who pointed out the involvement of two mating type factors (*A* and *B*), with heterozygosity at both loci required for the formation of the clamped (dikaryotic) mycelium. Thus, the spores arising from the basidia following meiosis are of four types, in terms of mating type.

Diagrammatically we can illustrate the situation as follows:

AxBx × *AyBy*	(Monokaryons)
↓	
AxBx + *AyBy*	(Dikaryon, clamped hyphae)
↓	
Fruiting body with basidia	
↓	Karyogamy in basidia
AxAyBxBy	2*n*
↓	Meiosis
Spores	(*n*)
AxBx	
AxBy	Compatible reactions
AyBx	(give rise to dikaryons)
AyBy	

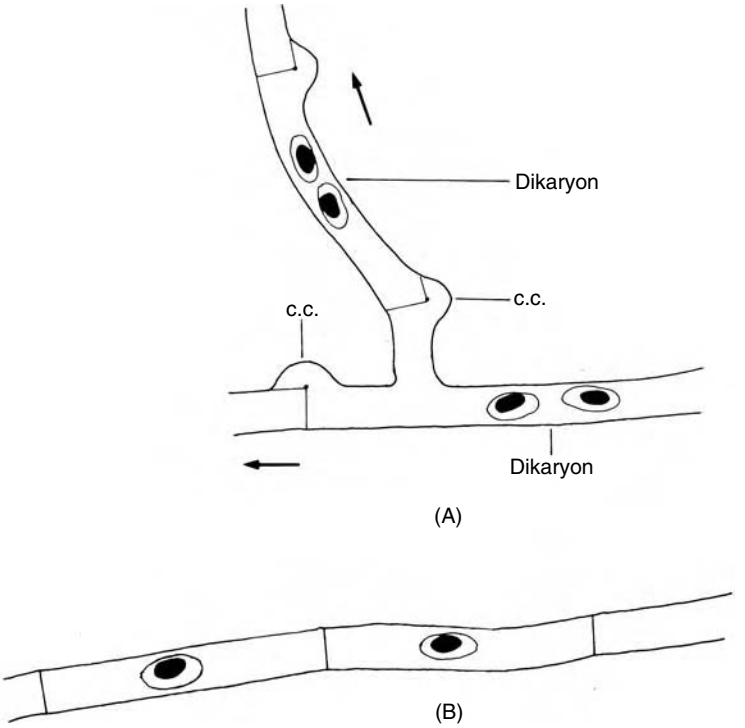


FIGURE 6.1 Hyphae showing clamp connections (A) and simple septa (B). (A) Dikaryotic hypha with clamp connections (c.c.); note pairs of nuclei (= dikaryons). (B) Homokaryotic hypha with simple septa (i.e., lacking clamp connections); note septa and single nucleus per cell.

The dikaryotic mycelium is formed when monosporous mycelium (mycelium arising from a single spore) of one mating type, e.g., *AxBx*, is confronted with monosporous mycelium of a compatible mating type, e.g., *AyBy*. Because there is a single type of nucleus in a monosporous mycelium, the term **monokaryon** is also used. Fusion of hyphae of the two compatible monokaryotic strains is followed by the migration of nuclei from one strain through the established hyphae of the other strain. When the migrant nucleus reaches a tip cell of a hypha that the nucleus is invading, that cell then contains two compatible nuclei. The cell in this special binucleate condition, in which two compatible nuclei coexist within it, is called a **dikaryon**.

B. Clamp Connection Formation

The dikaryotic condition is maintained during growth of the hypha by a process known as clamp connection formation — a process that assures that each developing cell of the hypha becomes dikaryotic.

The process of clamp connection formation had been described in the 19th century by the German mycologist Brefeld,[4] and Bensaude[2] in her dissertation showed the nuclear situation. With the invention of the phase contrast microscope by Zernicke in 1932, it became possible to study this process in the living, growing hyphae, and in the 1950s it was shown by a number of investigators that in many Basidiomycetes a situation such as is diagrammed in Figure 6.2 is quite typical.

Near the position on the hypha where the two nuclei are located, a small hyphal outgrowth appears and then grows and bends back away from the tip. This growth is the so-called hook cell. Before the hook cell recurves onto and fuses with the main hypha, the two nuclei undergo a simultaneous (sometimes called conjugate) division, and one of the daughter nuclei moves into the hook cell. A daughter nucleus of the other compatible nucleus moves away from the tip to the

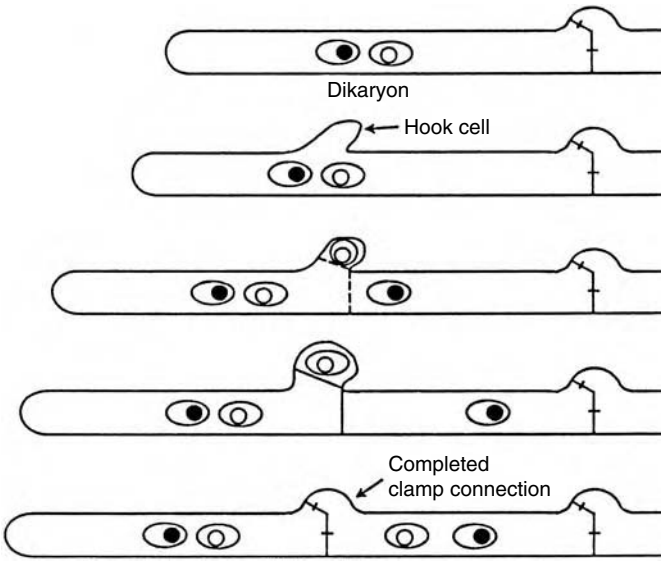


FIGURE 6.2 Diagrammatic representation of clamp connection formation. (A) Conjugate nuclear division occurs. (B) Beginning of septal formation. (C) Septal formation completed and fusion of hook cell with penultimate cell. (D) Passage of nucleus from hook cell into penultimate cell. (E) Completion of clamp connection.

region of the hypha near where the hook cell has approached the main hypha. Compatible nuclei are located in the tip region of the hypha. Now, septa form across the main hypha where the hook cell has emerged and across the hook cell at that same location. Next, the hook cell fuses with the penultimate cell, and the nucleus that was in the hook cell passes into the penultimate cell. The structure that is formed by the hook cell is called a clamp connection or simply a clamp.

Because the nuclei are large relative to the diameter of the hypha, the clamp is thought to provide a bypass, which permits the dikaryotic condition to be maintained. As fusion of nuclei does not normally occur until the fruiting body with its basidia is formed, the dikaryotic condition (hyphae with clamp connection) is maintained in nature and can be perpetuated in culture for prolonged periods.

It should be noted at this time that the dikaryon is a special type of heterokaryon in which compatible nuclei are maintained in 1:1 ratio by the process of clamp connection formation, and in genetic complementation the dikaryon functions in the manner of a diploid nucleus.

At room temperature most species of Basidiomycetes that have been studied form a new clamp connection about every 60 minutes, and the process of simultaneous nuclear division takes approximately 2 to 3 minutes. That is, when observed by phase contrast microscopy, the length of time from the disappearance of the dikaryon to the appearance of four daughter nuclei is about 2 to 3 minutes. As it is normally the dikaryotic mycelium that gives rise to fruiting bodies, most studies of nutrition and most physiological investigations have used dikaryotic mycelia.

Fruiting bodies (mushrooms) are normally formed by dikaryotic mycelia. Basidia are commonly formed in a hymenial layer (e.g., the gills of members of the order Agaricales), and it is in the basidia that karyogamy (nuclear fusion) takes place to form a diploid nucleus (Figure 6.3). This diploid nucleus almost immediately undergoes meiosis (process of nuclear division consisting of two division events in which the diploid number of chromosomes is reduced to the haploid number). The four haploid nuclei produced as a consequence of meiosis move through the stalks (sterigmata), which support the basidiospores on the basidia. One haploid nucleus enters each basidiospore. Each basidiospore has one *A* mating type allele and one *B* mating type allele. A random collection will result in basidiospores of four mating types in equal frequency, as shown shortly.

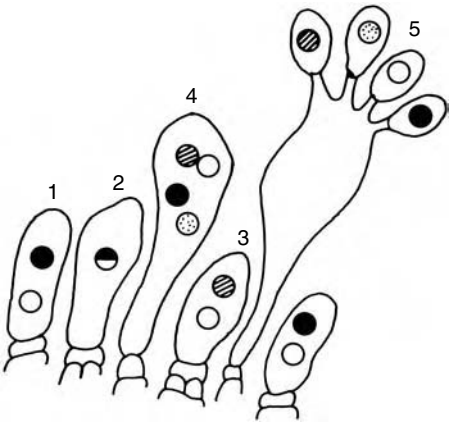


FIGURE 6.3 Nuclear events in basidium: (1) dikaryon (two unfused compatible nuclei in basidium); (2) basidium after nuclear fusion (nucleus is diploid); (3) basidium after meiosis I; (4) basidium after meiosis II (four haploid nuclei); (5) mature basidiospores (here indicated as a tetratype tetrad, but both parental and nonparental ditype tetrads will occur).

II. OTHER EARLY FINDINGS IN SEXUALITY IN BASIDIOMYCETES

In the intervening years between the original studies of Bensaude and Kniep and the early 1950s, much additional information was learned about sexuality in the Basidiomycetes:

1. The finding that each of the mating type loci contained a series of multiple alleles. This was later investigated more thoroughly by Raper and co-workers,[33] who have shown that each of the mating type "loci" consists of a chromosomal segment with subunits of the factors within which crossing-over takes place.
2. In some heterothallic species there is only one mating type locus (such species are referred to as "bipolar"), but multiple alleles exist here also.
3. In addition, there are homothallic species — those in which mycelium arising from a single spore can complete the life cycle.

A. Results of Tetrad Analysis

It was Kniep[17] in 1922 who provided an answer to an observation, previously made, of complete interfertility between progeny obtained from fruiting bodies of different origin. This was part of a study in which he was seeking to understand the mechanism involved in the occurrence of more than two mating types. For this, he wanted to analyze tetrads derived from individual basidia.

Finding it difficult to obtain tetrads from *Schizophyllum commune*, Kniep[17] discovered *Aleurodiscus polygonis* to be a more suitable species for tetrad analysis because the four spores from single basidia are discharged in groups, and it is possible to obtain monosporous mycelia from the germlings. (Almost three decades later Papazian[27] in 1951 did succeed in obtaining tetrads from *S. commune* using a micromanipulator, but it was still a difficult and time-consuming operation.) Kniep's studies have been described fully by Raper,[32] and the description that follows is based on that excellent account. Kniep obtained the four spores from each of 35 tetrads from a single fruiting body. The four members of each tetrad were mated in all possible combinations.

	1	2	3	4
1	–	–	+	+
2		–	+	+
3			–	–
4				–

Note: +, compatible reaction; –, no reaction.

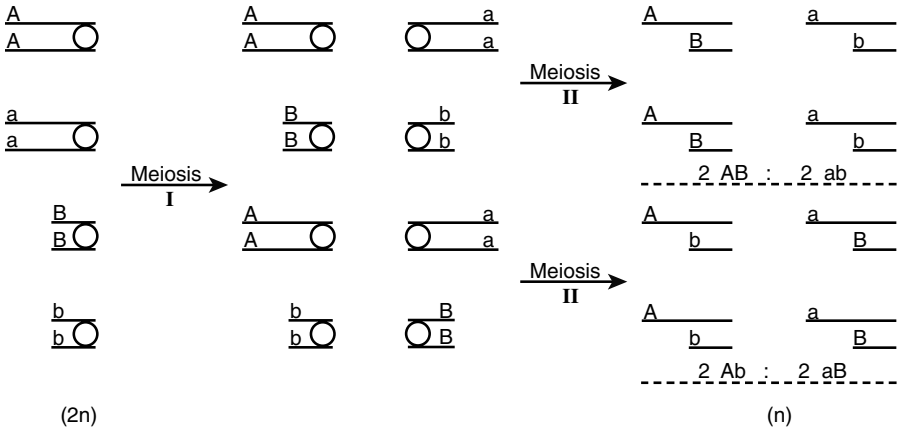


FIGURE 6.4 Diagram of Kniep's[17] tetrad analysis of *Aleurodiscus polygonis* in which each tetrad gave rise to only two mating types, which were interfertile. The tetrads were of two different types in approximately equal ratio, but the mating type classes of one type of tetrad were not fertile with the mating type classes of the other type of tetrad, indicating the segregation at the first division of meiosis of two factor pairs located on different chromosomes.

Each tetrad gave rise to only two mating types. Approximately half of the tetrads consisted of the same two mating type classes, which were interfertile. The other half of the tetrads also consisted of two interfertile mating type classes, but these were not fertile in matings with the two classes of mating types of the first type of tetrad. These results could be interpreted as indicating **segregation at the first division of meiosis of two factor pairs located on different chromosomes**. (This is presented diagrammatically in Figure 6.4.)

Later, Kniep[19] found the tetratype tetrad in *Aleurodiscus polygonis*. The tetrad resulted from segregation at the second meiotic division (presented diagrammatically in Figure 6.5).

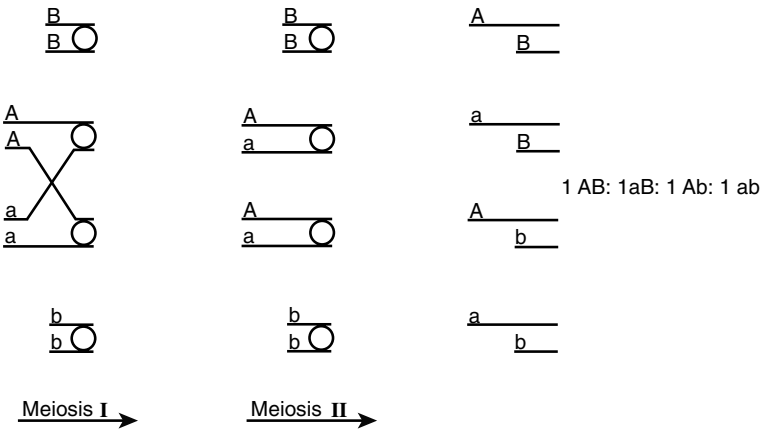


FIGURE 6.5 Diagram of Kniep's[19] tetrad analysis of *Aleurodiscus polygonis* in which the tetrad gave rise to four mating types. This tetratype tetrad indicated segregation at the second division of meiosis.

Random spore analysis also indicated the independent segregation of the two pairs of factors. From two different fruiting bodies the following results were obtained:

Mating Type	Fruiting Body *A*	Fruiting Body *B*
I	50	22
II	54	25
III	52	22
IV	54	17
Total	210	86

That the progeny of these two fruiting bodies were completely interfertile indicates the presence of multiple allelomorphs (multiple alleles) at each of two loci.

		AB	***ab***	***Ab***	***aB***
Fruiting body I	*AB*	–	+	–	–
	ab	+	–	–	–
	Ab	–	–	–	+
	aB	–	–	+	–

Note: + dikaryon, clamps; – no dikaryons, no clamps.

		A′B′	***a′b′***	***A′b′***	***a′B′***
Fruiting body II	*A′B′*	–	+	–	–
	a′b′	+	–	–	–
	A′b′	–	–	–	+
	a′B′	–	–	+	–

Note: $AB \times A'B' = + \rightarrow AB$, AB', $A'B$, and $A'B'$.

When these progeny from the "hybrid fruiting body" were mated back against testers from fruiting bodies I and II, the following results were predicted and obtained:

		AB	***AB′***	***A′B***	***A′B′***
Fruiting body I	*AB*	–	–	–	+
	ab	+	+	+	+
	Ab	–	–	+	+
	aB	–	+	–	+
Fruiting body II	*A′B′*	+	–	–	–
	a′b′	+	+	+	+
	A′b′	+	+	–	–
	a′B′	+	–	+	–

The formation of the dikaryon with clamp connections occurs only when there is a heterozygous condition of both the *A* and *B* factors.

One should be wary about saying "always" and "in every case" when describing biological phenomena. If enough cases are examined, some will be exceptional. The dictum "cherish your exceptions" is worth remembering. Thus, it is not surprising that Kniep[18] encountered some exceptions

in the mating reactions of *S. commune*. An isolate turned up occasionally that was compatible not with just one, but with two, of the four mating types. For example, using the simplified terminology introduced by the late Professor John R. Raper:

A1B1 × ***A2B2*** →	***A1B1***	***A1B2***	***A2B1***	***A2B2***
A1B1	–	–	–	+
A1B2	–	–	+	–
A2B1	–	+	–	–
A2B2	+	–	–	–
*A*B1*	–	+	–	+
*A1B**	–	–	+	+

Kniep suggested that the asterisked mating type factors were mutations even though, when they appeared, it was in frequencies higher (~2%) than would be expected for spontaneous mutations; and these mutations occurred only in cultures newly obtained from spore germlings. Established mycelia appeared to be stable in regard to the mating type factors. We return in Section IV to this problem and its later solution.

B. Geographical Races

The term "geographical races" was used by Brunswik,[6] because the stocks of *Coprinus* collected from widely separated locations had different mating type factors, but the term is misleading because completely cross-fertile stocks have been reported from opposite sides of a single dung ball; and Miles et al.[22] studied mating types of *Schizophyllum commune* in a small area and found different mating type factors in isolates from fruiting bodies on the stump of a single tree. This is not an uncommon finding.

C. Bipolarity

In 1923–24, several workers (Vandendries,[47,48] Brunswik,[6,7] Newton[24]) discovered a number of heterothallic species that produced only two mating types in each fruiting body. This was similar to the pattern of sexuality found in the Mucorales and called "bipolar sexuality." **Bipolarity** then is the situation in which there is a factor pair at a single locus. In the Basidiomycetes that are bipolar, however, there is a system of multiple alleles at the single locus, which is different from the Mucorales in which alternate alleles are present.

D. Illegitimate Matings

Another type of exception to appear was the case of fertile matings between incompatible strains. These were reported in *Coprinus* by Brunswik[7] in 1924, and also by Vandendries[47] in 1923. Such matings were described as "illegitimate," a term that we shall see used again at a later time. The illegitimate matings were found by Brunswik to occur only in the event of common *B* matings, and in these cases a variation of the clamp connection was formed. These were called **pseudo-schnallen** (= false clamp or pseudo-clamps) (Figure 6.6).

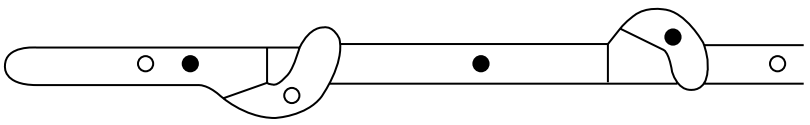


FIGURE 6.6 False clamp connections.

False clamp connections differ from true clamp connections in that in the case of the false clamp connection the hook cell does not fuse with the subterminal cell of the hypha. Cytological and genetic studies by Oort[25] and Quintanilha[30] confirmed the routine presence of false clamps in such common *B* matings and further revealed the usual nuclear situation, which was for the terminal cell to be binucleate, the hook cell to contain a single nucleus, and the penultimate cell to be uninucleate.

E. Buller Phenomenon (= Di-Mon Mating)

The famous Canadian mycologist A.H.R. Buller during his career made many significant contributions to sexuality in the Basidiomycetes. One that has interested and continues to intrigue us was the discovery that homokaryotic mycelia could be dikaryotized by dikaryons; i.e., unclamped homokaryotic mycelia could be induced to form clamp connections if such mycelia were confronted by the clamped mycelia of a dikaryon.[8] The name **Buller Phenomenon** was given to this kind of reaction by Quintanilha[31] in honor of its discovery by Buller. Later, Papazian[26] referred to this as a **di-mon mating**, a more descriptive term for the confrontation of dikaryotic and monokaryotic mycelia.

Broadly speaking, di-mon reactions are of two types:

1. **Legitimate:** When the mating type of the monokaryon is compatible with that of both nuclei of the dikaryon, it is referred to as compatible, e.g., $(AxBx + AyBy) \times AzBz$. If it is compatible with only one nucleus of the dikaryon, it is referred to as hemicompatible, e.g., $(AxBx + AyBy) \times AyBy$.
2. **Illegitimate:** When a mating reaction occurs between a homokaryon and a dikaryon, and the nucleus of the homokaryon is not compatible with either of the components of the dikaryon, e.g., $(AxBx + AyBy) \times AxBy$.

A number of suggestions have been offered as explanation for the di-mon reaction. For example, it was suggested that following hyphal fusion, both nuclei of the dikaryon enter the monokaryon and migrate through the mycelium to the hyphal tips,[36] or mutation of one of the mating type factors of the dikaryotic nuclei might make the nucleus compatible with that of the homokaryon, or fusion of the nuclei of the dikaryon followed by reduction to give the specific *A* and *B* factors required by the homokayron,[8] or recombination of the *A* and *B* factors in the vegetative dikaryon without nuclear fusion and meiosis, which was suggested by Quintanilha.[29] In this last case in a trinucleate cell (cell in which nuclei of the homokaryon and of the dikaryon are present), Quintanilha envisaged that during metaphase of conjugate division of the dikaryon there could be an exchange of chromosomes with the nucleus of the homokaryon. This latter hypothesis of Quintanilha involving internuclear genetic exchange was confirmed experimentally, but the mechanisms involved were not elucidated.

III. REACTIONS OTHER THAN THOSE FORMING DIKARYONS

We have already seen that certain matings gave reactions other than that of compatible strains forming the dikaryons with clamp connections. Recall the illegitimate reaction reported by Brunswik[7] in which matings involving a common *B* situation produced false clamp connections. Vandendries and Brodie[49] and Brodie[5] described in *Lenzites betulina* in nature and *in vivo* the regular occurrence of what they called the "barrage sexual," which occurred when the two mated strains had a common *B* factor. The barrage was a line of sparse growth in which there was an apparent aversion between the hyphae of the two strains that had been mated.

In 1950, Papazian[26] published the results of his doctoral research on *Schizophyllum commune* — a study replete with many significant contributions. The most significant in our present

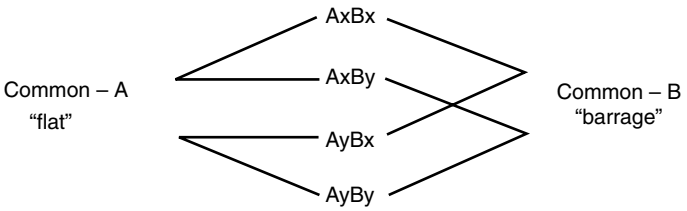


FIGURE 6.7 Matings giving rise to heterokaryons in tetrapolar species. *Note:* Descriptive terminology from *Schizophyllum commune*. (Data from Papazian, H., *Bot. Gaz.*, 112, 143–163, 1950.)

considerations was the demonstration of the occurrence of predictable reactions when single-spore mycelia are mated in manners other than in the combinations that bring about the dikaryotic reaction. These resulted in the formation of heterokaryons as shown in Figure 6.7. Fulton[13] reported comparable findings in *Cyathus stercoreus*.

It was by making use of these reactions that Raper was able to work out the genetic structure of the mating type loci, for these permitted the determination of alleles as identical, and not just different; e.g., the mating results $Ax \times Ay = +$, and $Ax \times Ax = -$, tell us only that Ax is different from Ay, but if $Ax \times Az = F$, this tells us that Ax and Az are identical and different from Ay.

IV. GENETICS OF THE MATING TYPE LOCI AND SEXUAL MORPHOGENESIS IN *SCHIZOPHYLLUM COMMUNE*

A. *Schizophyllum commune*

In this section we examine in some detail the genetics of the mating type loci and sexual morphogenesis in the experimental organism *S. commune* (Figure 6.8). Although *S. commune* is not an edible mushroom, it is now known that *S. commune* does produce a polysaccharide, **schizophyllan**, which has β(1→3) linked chains with branches of a single glucose unit attached by β(1→6) linkages at every third unit along the chain, on the average. Schizophyllan is now being used extensively for the treatment of cervical cancer. We have chosen to describe studies with this organism for a number of reasons.

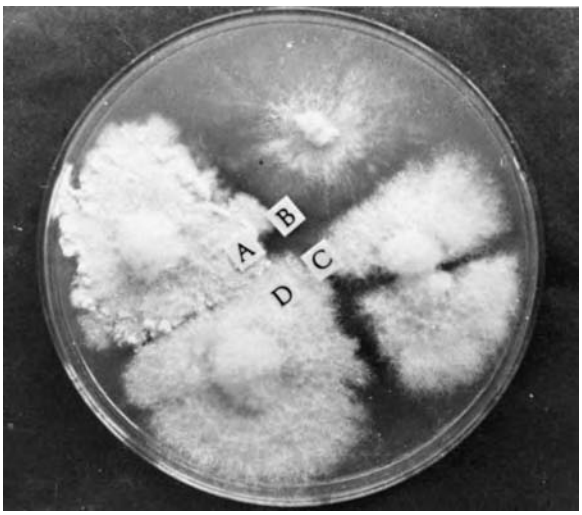


FIGURE 6.8 Four mating reactions of *Schizophyllum commune:* (A) $A \neq B \neq$; (B) A=B≠; (C) $A \neq B$=; (D) A=B=.

1. Much of the important information on the topics at hand was first gleaned from studies of *S. commune*.
2. The studies with *S. commune* provide a framework for studies with edible fungi that are heterothallic.
3. Studies of heterothallic edible species generally show a close parallel in regard to mating type loci and sexual morphogenesis to the basic findings in *S. commune*. For example, the results obtained by Takemaru[40–43] with *Flammulina (Collybia) velutipes* in regard to the genetic structure of the mating type locus, and the studies of Tokimoto et al.[44] on the number of mating type alleles in *Lentinula edodes*, and of Eugenio and Anderson[10] in *Pleurotus ostreatus* are similar.
4. The authors have both carried out research involving genetics and morphogenesis with *S. commune* and are thus familiar with the experimental techniques and literature of *Schizophyllum*.

It was no accident that the discovery of sexuality in the Basidiomycetes by Kniep[16] and the discovery of common *A* and common *B* heterokarysis by Papazian[27] involved research with the organism *S. commune* Fr. This small, wood-rotting fungus (see Figure 7.3) has a short life cycle, the spores germinate easily in high percentage, and the dikaryotic mycelium fruits in culture without any elaborate treatment.

It was also no accident that *S. commune* was used by Papazian and by Raper and his associates for studies of the genetics of the mating type loci. In addition to the previously mentioned attributes, *S. commune* could be grown on a simple, chemically defined medium, and auxotrophic mutants were available for use in selective techniques if this should be desired.

Earlier, in considering the presence of multiple alleles at the mating type loci, the lack of support for the idea of mutated factors as advanced by Kniep was mentioned. Furthermore, Kniep had mentioned "favored classes of new factors" and frequent "back mutations" to the original parental factors. This suggested to Papazian[26] the possibility that intrafactor recombination might be responsible for the origin of the different mating type alleles.

B. The *A* Locus of *Schizophyllum commune*

The number of alleles at the mating type loci was known to be numerous. Whitehouse[50] in 1949 estimated the number to be in the order of 100 for both the *A* and *B* loci. Subsequently, Raper et al.[34] studied the mating types of *S. commune* from fruiting bodies obtained from various parts of the world and identified 96 distinct *A* factors and 56 distinct *B* factors from 114 homokaryotic strains. From these data, estimates of the number of mating type factors in the total natural population are 339 *A* and 64 *B* factors. The two mating type factors, *A* and *B*, were also known to be unlinked from the studies of Kniep on the basis of equal frequency of parental and recombinant mating types from compatible matings.

Papazian, by both tetrad analysis and random spore matings involving two known *A* alleles, recovered factors that were compatible with both parental *A* factors. From these results he concluded that the *A* incompatibility factor is controlled by at least two closely linked genes. Evidence was not obtained that would permit a distinction to be made between a few loci with multiple alleles or many loci with paired alleles. This work by Papazian was done in Raper's laboratory, located at the University of Chicago. Another student, Vakili,[46] enlarged the sample studied by Papazian and recovered about 3% nonparental *A* factors. Vakili made the interesting discovery that differences in recombination frequency occurred at 23°C (3%) and 33°C (8.5%).

This study was continued when Raper moved to Harvard University. Vakili had extended the number of crosses, and now it was further enlarged to include different pairings from various locations, and progenies were collected from these and testcrosses were made to identify recombinant mating types. Unfortunately, another natural phenomenon in the biology of *S. commune*

occurred that greatly diminished the number of matings from which definitive results could be obtained. This upset the experimental plan and lessened considerably the value of the results.

The phenomenon referred to was the spontaneous occurrence of the morphological mutant, *thin*, which carried with it, under the conditions that were then being used, a unilateral mating behavior. (In unilateral matings one strain behaves as a donor of nuclei, but does not accept nuclei or become dikaryotized and form clamped hyphae, even in a compatible confrontation.) This meant that in unilateral × unilateral confrontations no mating reactions occurred.

If recombinants were unilateral, it could not be determined if the recombinants were identical or different, which was normally done on the basis of a "flat" or dikaryotic (clamped) reaction when the two recombinants were confronted. (Of course, different *B* factors were necessary for this test.)

The *thin* mutant was especially troublesome because it tended to outgrow wild-type mycelium. Later Schwalb and Miles[39] discovered that unilaterality in *thin* could be circumvented by a different mating procedure and growth on a different medium, but at that time, with the techniques being employed, *thin* behaved only as a unilateral mater. Thus, the chief concern was to avoid the *thin* mutation.

Because many of the test strains mutated to *thin*, the experimental plan was changed from mating strains in all possible combinations to mating each strain with one specific strain that was stable in regard to mutation at the *thin* locus. Fortunately, such a strain was available.

Strain #699 (MT A41 B41) had been isolated as a single-spore culture from a fruiting body collected at Fresh Pond, Massachusetts. It had been kept in culture for some time without mutating to *thin*. To take advantage of the relative stability of strain #699 in regard to mutation to *thin*, the other homokaryons from different locations were each mated with strain #699.

Spores were collected at 23 and 33°C from the fruiting bodies formed from these matings, and the progeny were screened for nonparental *A* factors by appropriate matings. For example, from a mating of *A*41 × *A*42, progeny were obtained that would be tested by mating with tester strains bearing factors *A*41 and *A*42 as follows:

Progeny	*A*41	*A*42
*A*41	F	+
*A*42	+	F
*A**	+	+

Note: *A** = nonparental *A* factor.

Such nonparental *A* factors (*A**, above) were then cross-mated in all intrastock and interstock combinations. They were also mated with the previously distinguished *A* factors from nature to determine identity with one of those *A* factors.

Strain #699 of *S. commune* was crossed with 11 other strains from different localities, and progeny collected for screening for nonparental *A* factors. As previously indicated these nonparental *A* factors were then mated in all the various intrastock and interstock combinations as well as with the known 96 *A* factors previously identified when isolated from nature. In each of nine crosses in which recombinants occurred, these recombinants could be assigned to two self-sterile, cross-fertile classes. The members of these two classes were of about the same number. In all, 15 different classes of nonparental *A* factors occurred. Interestingly, of these 15 classes, 5 were identical to factors from nature.

In view of these results and previous information that had been gained about the mating type factors, a model was developed for the genetic structure of the *A* factor. The proposed model was that the *A* factor consists of two linked loci with multiple alleles at each locus. These loci were designated *A*α and *A*β, and #699 was designated *A*41α1-β1.

*A*51, when mated with *A*41, gave two new factors and was designated *A*51α2-β2. *A*49 recombined with *A*41, but one class of recombinants from this cross was the same as one of the recombinant classes from the cross of *A*41α1-β1 × *A*51α2-β2. Therefore, *A*49 was designated *A*α2β3. (*A*49α1-β1 × *A*51α2-β2 gives recombinants α1β2 and α2β1; *A*49 × *A*41α1-β1 gives recombinants α1β3 and α2β1. Consequently, *A*49 is α2-β3.)

It was possible to test the model by recovering parentals from matings involving recombinants. For example, *A*α1-β5 × *A*α3-β1 gave recombinants *A*α1-β1 and *A*α3-β5. *A*41 is α1-β1 and *A*42 is α3-β5. Thus, two *A* factors are fully compatible when there is heterozygosity at either or both of the subunits α and β. The specificity of *A* factor is determined jointly by the *A*α and *A*β loci.

Further tests were made to check the correctness of the two-locus *A* factor, with special attention to such things as the number of α and β alleles, and to recover intra-*A* recombinants (unsuccessful).

C. The Two-Locus Mating Type Factor Occurs Elsewhere

It should be pointed out that the *B* factor also was shown to consist of two component loci, designated as *B*α and *B*β. Nor is this system unique to *S. commune*. It has been found in species of *Coprinus*, in *Collybia velutipes* (now *Flammulina velutipes*), in *Lentinula edodes*, and in other tetrapolar Basidiomycetes. Although multiple alleles occur in bipolar species, they are fewer in number, and there is no evidence for the single mating type factor, *A*, having more than a single locus.

D. Findings from Molecular Genetic Studies

The advent of modern molecular genetic techniques applied to studies of mating type control of *S. commune* has led to greater resolution of the structure and function of mating types. Important in this respect was the cloning of $B\alpha_1$ and $B\beta_1$ and the determination that both contain three putative pheromone genes and a single receptor gene.[45] The six pheromone genes were sequenced. It has been pointed out by Fowler el al.[12] that compatible combinations of pheromones and receptors are produced by individuals of different *B* mating types and initiate a fertilization pathway that is required for sexual development. They demonstrated that a large cluster of genes at the $B_{\beta 2}$ mating type locus encodes a single pheromone acceptor and eight different pheromones. It was also shown that mutations within these genes produced small changes in both the pheromone and acceptor molecules that could alter their specificity in reactions. A model has been proposed, for which there is some experimental evidence suggesting how mutations and duplication could lead to the observed mating type variants found in nature.

The *A*α mating-type genes of *S. commune* have been investigated by Ulrich's group at the University of Vermont.

E. Sexual Morphogenesis

Dikaryosis is the normal situation in systems containing different *A* factors and different *B* factors (i.e., *A*≠*B*≠). Thus the features that are present in the dikaryon but absent in the homokaryon can be assumed to be under the control of either or both of the mating type factors.

The clamp connection is the visible difference between homokaryotic and dikaryotic hyphae. Further information is available from examination of the hyphae in common *A* and common *B* heterokaryons, as these heterokaryons display some features of the dikaryon but lack others. Recall that in common *B* matings false clamps (pseudoschnallen) characteristically occur, and in false clamps the hook cell fails to fuse with the penultimate cell (see Figure 6.6). The assumption is thus that a difference in *B* alleles is required for fusion of the hook cell with the main hypha.

It is also known that in common *B* matings, nuclear migration does not take place. Therefore, it can be stated that migration requires paired unlike *B* factors.

J.R. Raper and C.A. Raper[35] made extensive studies of morphogenesis in *S. commune*. In their studies they made use of a number of modifier mutations that affected the morphogenetic sequence of dikaryosis, and these have been most helpful in bringing about an understanding of the various stages in this process. These various stages and the factors that control them are indicated in the following table:

	Controlling Factors	
	Different *A* Factors	Different *B* Factors
Nuclear migration		+
Nuclear pairing	+	
Conjugate division	+	
Hook–cell formation	+	
Hook–cell septation	+	
Hook–cell fusion		+

At this time we wish to remind the reader of the important role of sexuality in the life cycle of the higher fungi. It is, of course, through sexuality that effective recombination of genetic characters is brought about, and with this the formation of individuals that are better adapted to particular environmental situations (along with individuals less well adapted, of course).

In the fungi certain changes must be made in the vegetative hyphae to accommodate sexuality. In the description of sexuality in the Basidiomycetes that we have just examined, it should be noted that nuclear migration and nuclear pairing are two such events; and for these, hyphal fusion is a required preliminary step. Genetic studies in the Basidiomycetes are unique, and, consequently, of interest in the matter of genetic structure and operation of the mating type factors in sexual morphogenesis. This is examined next.

To comprehend adequately genetic events in the Basidiomycetes, it is desirable that we have some familiarity with the techniques that are used in genetic experimentation with fungi.

V. GENETICS OF FUNGI

Of course, for any study of inheritance it is necessary to have two individuals that differ in some character that can be either visibly distinguished or detected by (1) growth or lack of growth, or (2) the production or lack of production of some product of metabolism, which may or may not be essential for its life activities. Those characters that commonly occur in nature are referred to as **wild type** (sometimes the term **normal** is used), whereas a sudden, heritable change that can be distinguished from wild type is known as a **mutation** (or the term **mutant** may be used). Mutations constitute the material for genetic studies, so we devote the rest of this chapter to mutations: how to induce them, how to isolate them, and how to characterize them.

A. Induction of Mutants

Mutations occur naturally, but at low frequency. Even prior to the studies of Mendel, investigators of inheritance recognized the sudden, heritable changes of a character, which they referred to as a "sport." Plant or animal breeders might even select for a sport if it seemed to be a character of potential value. With the rediscovery of Mendel's laws in the 20th century, and attempts to determine their applicability to various groups of organisms, and to add to knowledge of fundamental genetic principles, mutations were accepted for the fact that they were different from wild type without concern for practical value.

The event that accelerated interest in the mutation process was the discovery by Muller in 1924 that ionizing radiation from X rays increased the frequency of mutation. The rate of mutation with mutagenic treatment was compared with the spontaneous rate of mutation.

1. Spontaneous Mutation Rates

In the filamentous fungi, it is difficult to measure the spontaneous mutation rate. It is even more difficult in those filamentous Basidiomycetes that do not produce uninucleate asexual spores. Most of the studies of spontaneous mutation rate that have been made with the fungi have been with the ascomycete *Neurospora crassa* and involved biochemical mutations called **auxotrophs**. An auxotrophic mutant has lost the ability to carry out some biochemical step, and, consequently, it will not grow on a chemically defined, minimal, medium, which supports the growth of wild-type strains. The addition of a specific substance or substances to the minimal medium will permit growth of the auxotrophic mutant. A strain that grows on the minimal medium without supplementation is called a **prototroph**.

With uncertainties in the filamentous fungi as to the number of nuclei, the number of genes per nucleus, and where in the development of a conidium a mutation may have occurred, only rough estimates can be made of the spontaneous mutation rate. The experimental procedures that have been used include the determination of the number of spontaneously occurring auxotrophs from a population of microconidia (these are uninucleate) of *N. crassa*, or the number of a particular kind of auxotrophic mutant in a large population of conidia; or, better yet, the spontaneous rate of reversion from auxotrophy to prototrophy (such a reversion is called a reverse or back mutation). This last procedure has the advantage that the minimal medium can be used to select for the reverse mutation, as the auxotrophic strain will not grow on the minimal medium.

Rough estimates of forward mutation (i.e., from wild type to mutant) are in the region of one per million per nucleus per gene. Estimates of spontaneous reverse mutation rates vary somewhat with the allele, but, again with *N. crassa*, a value of 1 to 10 per 100 million macroconidia (macroconidia are multinucleate) is found in the literature.

2. Mutagenic Treatment: X Rays

Because spontaneous mutants are rare, techniques are used to increase the frequency of mutation. As previously mentioned, the studies of Muller on the fruit fly *Drosophila* demonstrated that ionizing radiation increases the rate of mutation. Radiation is also a useful mutagenic treatment for fungi. In general, there is a direct correspondence between X-ray dosage and the frequency of mutations obtained among the nuclei that survive the radiation.

Experimentally, we can determine the dosage that will result in a certain percentage of survival of spores or hyphal fragments and relate this to the approximate percentage of mutants among the survivors. As screening for biochemical mutants in filamentous fungi involves much time and labor, it is desirable to use a dosage that will give the highest percentage of mutants among the survivors.

We can obtain a rough estimate of the dosage range that should be employed from reports in the literature for other species of fungi (e.g., 15 to 40 kiloroentgens for *N. crassa*). For efficiency in obtaining mutants with a species for which data are not available, however, it may be worthwhile in the long run to perform an experiment in which survival and mutation frequency are determined.

3. Mutagenic Treatment: Ultraviolet

For most investigators seeking to obtain mutants of fungi, it is simpler to use ultraviolet (UV) light than X rays, because a simple, inexpensive, germicidal UV lamp is available. The germicidal effect is related to the absorption maximum of nucleic acid at 260 nm (1 nanometer = 10 angstroms, symbolized Å), which is included in the wavelengths delivered by the germicidal lamp. Actually, most germicidal lamps deliver a broader range of wavelengths, but experimentation has demonstrated that 260 nm is the effective wavelength. This wavelength has also been shown to increase mutation frequency.

Both the germicidal (lethal) effect and the mutagenic effect are believed to result from the formation of covalent bonds between adjacent pyrimidine nucleotides, generally thymine but sometimes cytosine. These linked pyrimidine nucleotides are referred to as dimers, either thymine

dimers or cytosine dimers, depending on the specific nucleotides that have bonded together. The consequence of dimer formation is an inhibition of normal DNA synthesis.

It has also been demonstrated that there is a mechanism that can repair the UV-induced damage. The mechanism requires exposure to wavelengths in the approximate range of 360 to 480 nm. Light in this range has the effect of activating an enzyme that splits the dimer, thus permitting the return to normal DNA synthesis and lack of mutagenicity. This light repair is called **photoreactivation**. In experimental work in which UV is being used to obtain mutants, it is important that cells not be exposed to the wavelengths inducing photoreactivation. This can be accomplished by illuminating the work area with yellow light, which does not transmit wavelengths causing photoreactivation.

Mutations are caused by UV through dimer formation, which is followed by replication of DNA. The dimers are responsible for gaps in the strands of DNA that are synthesized. This method of mutagenesis is different from the mode of action of ionizing radiation, e.g., X rays, which cause alteration in the bases of the DNA and breaks in the DNA strands.

4. Mutagenic Treatment: Chemical

There are many chemical substances that may act as mutagens. Fincham and Day[11] present a clear account of chemical mutagens in particular reference to the fungi. They point out that there are two classes of chemical mutagens, those that act directly on preexisting DNA (e.g., nitrous acid, hydroxylamine, and alkylating agents) and those like the base analogues that cause errors in further synthesis of DNA.

The base analogues, as the name implies, have a structure that is very similar to one of the bases of DNA, and consequently they can become incorporated into DNA at the time of replication in place of the normal base to which it bears a similarity. For example, 2-aminopurine (AP) is an analogue of adenine, and 5-bromodeoxyuridine (BDU) is an analogue of thymine. The result of the incorporation of the analogue is a mutation.

Nitrous acid is one of the compounds that act directly on DNA to produce a change in one of the bases. It does this by reacting with primary amino groups to give hydroxyl groups. For example, adenine becomes hypoxanthine, guanine becomes xanthine, and cytosine becomes uracil. Subsequently, base pair changes of the transition type (purine for purine, and pyrimidine for pyrimidine) are produced, which are responsible for the mutations.

Alkylating agents that are effective mutagens contribute a methyl or ethyl group to the 7-nitrogen atom of the base guanine of DNA, producing either 7-methyl- or 7-ethylguanine. This causes mutation for the following reasons:

- The 7-ethylguanine is readily removed by hydrolysis from the DNA.
- A gap is left in the DNA strand by the removal of 7-ethylguanine.
- Any base may fill the position on the complementary strand that was left by the gap.

A second method for mutation involves base-pairing errors of the 7-ethyl- or 7-methylguanine at replication. Effective alkylating agents in regard to mutagenesis of fungi are ethyl methane sulfonate (EMS) and *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (nitrosoguanidine, or NG).

Extreme care must be taken in working with the chemical mutagens; some of them are carcinogenic (cancer-causing agents). Care must also be taken in working with X rays and UV. Glasses should be worn when working with UV light to protect the eyes.

B. Isolation of Mutants

1. Total Isolation

Isolation of a few specific types of mutant individuals from a large population can be very laborious in the absence of any techniques for selecting the types being sought. If a large random sample of

conidia, spores, or mycelial fragments that have been treated with a mutagenic agent are individually isolated after they have started to germinate or grow, and then all of these isolates are scored as mutant or wild type, it will be found that only a very low percentage is mutant (~3 to 4%). This total isolation procedure is very costly in time and labor, but there are some cases in which it must be followed because enrichment methods that are suitable for certain species may not operate in others. We now examine some of the techniques that have been helpful in lessening the time and labor involved in isolating mutants.

a. Restriction of Linear Growth

With filamentous fungi the inoculation of numerous spores or hyphal fragments on the agar surface of a petri plate may soon lead to confluent growth of the mycelium, because the mycelia do not grow as discrete colonies. When this happens, it is not possible to make isolations from the original, individual sources of inoculum. One approach to this problem is to include a growth-restricting agent in the medium. The sugar L-sorbose is not utilized by *Neurospora*, but when it is included in the medium prior to autoclaving, it results in a colonial type growth that facilitates isolation of individual colonies. There are a number of other substances that will retard hyphal growth, resulting in a more discrete colony form. Among these are some of the bile salts, such as sodium taurocholate and sodium deoxycholate, and the photodymic dye rose bengal.

There are also mutants of *N. crassa* that produce a colonial growth, and in *Schizophyllum commune* the *fir* mutant has a restricted growth morphology, but when these are used in mutagenic studies, it is necessary to undergo an additional sexual cycle to separate the new mutant from the growth-restricting mutant.

b. Isolation of Auxotrophs

Auxotrophic mutants are of great utility in genetic studies, because strains bearing auxotrophic markers can be selected by use of differential media. Isolation of auxotrophs can be accomplished in several ways, but the general procedure requires that all isolates first be grown on a complete medium (i.e., a medium that supports the growth of auxotrophic mutants), and then transferred to a minimal medium. On the minimal medium the auxotrophic mutants will not grow, but the prototrophs will grow.

2. Filtration Enrichment Method

As pointed out earlier, a total isolation procedure is inefficient, so means for enrichment for the number of auxotrophic mutants among the isolates have been developed. One such is known as the **filtration enrichment method**, which has been successfully used with the Ascomycetes *Ophiostoma* and *Neurospora*. The essential objective of the process is to permit the mutagenically treated conidia to germinate in a liquid minimal medium and then to remove the hyphae of the germinated conidia by sterile filtration. This process may be repeated more than once in order to remove conidia with prototrophic nuclei that have been slow to germinate.

The conidia that have passed through the filter will consist of nonviable conidia and a higher percentage of conidia with mutant nuclei than was present in the original conidial suspension. Of course, after filtration the conidial suspension must be plated on a complete medium to obtain growth of the viable conidia. Both auxotrophic and prototrophic colonies will develop, so these must be distinguished by subculturing to plates of minimal medium.

3. Starvation Selection Method

It has been found that strains with two biochemical deficiencies (i.e., two auxotrophic mutations) survive for a longer time in minimal medium than do the strains with a single deficiency. From this information, a method for obtaining an increased percentage of auxotrophs from mutagenically treated cells was developed. This technique is known as the **starvation selection method** and involves the following:

- The mutagenic treatment of the conidia of an auxotrophic mutant.
- The plating of the conidia onto minimal medium, which is then overlaid with more minimal medium.
- During a period of incubation the conidia bearing the original single auxotrophic mutant die.
- A layer of complete medium is poured onto the plate and, after incubation, subcultures are made from the colonies arising onto selective media, which will determine the presence of an additional auxotrophic mutation.

4. Rescue Method

The principle of the **rescue method** is to add nutrients in some manner to a minimal medium upon which prototrophic cells have already formed colonies after a period of incubation. Colonies that develop after the addition of the supplementary nutrients are likely to be auxotrophs. This technique can be utilized for the selection of specific auxotrophs by use of specific supplementary nutrients.

5. Selective Elimination of Prototrophs by Use of Chemical Method

A method that finds less use because of the limited number of antifungal antibiotics that affect only growing cells is the **selective elimination of prototrophs by antibiotics**. In this method strains sensitive to the chemical agent (e.g., the antifungal antibiotic nystatin) are treated with a mutagenic agent and incubated in a minimal medium in the presence of the antibiotic. Only the prototrophic cells are killed because the antibiotic is effective only against growing cells (penicillin acts in this way with bacteria). The auxotrophs are not affected by the antibiotic, as they do not grow in a minimal medium. The antibiotic is removed by centrifugation, and the cells, spores, or conidia that were used are plated onto a complete medium. The colonies that grow are then tested for prototrophy or auxotrophy.

None of the above techniques will enable the isolation of only auxotrophic mutants. There are too many variables involved. Especially important in this connection is the variation in germination time of spores that may be the result of the mutagenic treatment, because most of the techniques make use of a differential in prototrophic growth in minimal medium.

C. Characterization of Mutants

We mention four types of mutants: auxotrophic, morphological, developmental, and fruiting. Different means are used to characterize the various types of mutant. For example, the auxotrophic mutants are characterized on the basis of their ability or inability to grow on minimal media to which various known supplements have been added. The other types of mutants are characterized by morphological features visible to the naked eye or with the use of a microscope. In some cases color may be a distinguishing feature, e.g., color of conidia or spores. There are also cases in which the production of a metabolic product may be determined.

1. Auxotrophic Mutants

The basic screening procedures for characterizing auxotrophic mutants were described by Beadle and Tatum for *Neurospora crassa.* The isolates that were determined to be auxotrophic mutants on the basis of growth on a complete medium and lack of or very limited growth on minimal medium were tested for growth on minimal media with various known supplements. If the auxotrophic mutant failed to grow on minimal plus a mixture of amino acids, or minimal plus purines and pyrimidines, but grew on minimal plus vitamins, then this latter medium contained the substance (a vitamin) that the auxotrophic mutant had lost the ability to make. The next step involved adding the vitamins separately to minimal medium to see which vitamin or vitamins would permit growth to occur.

The Lederbergs developed a technique for use with bacteria (it is also useful with yeasts), which permitted the rapid distinction of auxotrophs. The technique is known as **replica plating**. A piece of velvet cloth is sterilized and placed over a circular mold of proper size so that the mold with the velvet covering on the outside will fit inside the bottom of a petri dish. The velvet is pressed gently against the colonies growing on the surface of the agar in the petri dish, and then it is removed and pressed lightly against the agar surface of petri dishes containing minimal medium or minimal medium with various supplements. The many short, stiff threads of the velvet material serve as tiny inoculating needles and transfer cells from the original plate of complete medium, on which the colonies were growing, to plates containing desired selective media. Differential growth on complete, minimal, and supplemented minimal media will enable selection of auxotrophic mutants and determination of the nature of their deficiencies. This technique has its widest application with bacteria, but it can also be used with yeasts and fungi that have a yeastlike growth phase.

A modification of the replica plating technique has been developed for those filamentous fungi that produce an abundant number of conidia at an early age. Instead of a velvet cloth, which would tend to pick up too many conidia and scatter them all over, a template is made of steel pins, such as insect pins, that are stuck at close intervals through a piece of thin plastic. The sharp tips of the pins are pushed into an agar plate to make them sticky, and then they are pressed onto the surface of the agar plate containing the colonies. The sticky tips of the pins pick up conidia, which can then be transferred to other plates by pressing the template against the agar surfaces of the minimal and variously supplemented minimal media. Other modifications of the replica plating technique have been tried with varying degrees of success.

The procedure for determination of the actual requirement of an auxotrophic mutant has been described previously. Because much time and labor is involved in an approach that involves total isolation, it is apparent that the replica plating technique can be very useful. As the majority of auxotrophs fall into mutants with a requirement for a relatively few number of supplements (various amino acids, vitamins, purines, and pyrimidines), the screening process can be simplified by having a number of possible growth requirements in different arrangements in several media. The procedure outlined by Holliday[14] is to have a series of media in which the substances are arranged in a systematic manner so that each substance occurs in only one or two of the media.

For example, n different substances per medium when arranged in n different media will permit the testing of $n(n + 1)2$ different substances. To use a simple illustration, three substances per medium arranged in three different media will permit the testing of six different substances, A, B, C, D, E, and F.

Medium	Substances Added to Minimal Medium	If Auxotroph Grows on	Substance Required
1	A, B, C	1 only	C
2	D, B, E	2 only	D
3	F, A, E	3 only	F
		1 and 2	B
		1 and 3	A
		2 and 3	E

2. Morphological Mutants

The mycelial morphological mutants have been studied to the greatest extent in *Neurospora crassa, Schizophyllum commune*, and *Coprinus* spp. They can sometimes be recognized with the naked eye, and, if this is not sufficient, a simple microscopic examination reveals distinguishing features. Their usefulness as genetic markers is lessened, because isolates bearing two or more morphological mutant genes are difficult to score satisfactorily since they are commonly reduced in size and do not necessarily reveal the major features of the genes involved. Furthermore, slower growth may

lead to a selection against these multiple morphological mutants when germlings are being isolated, and there may even be diminished viability. Several studies have been made to relate the morphological mutant with biochemical events controlled by the gene governing the morphology. These mutants are potentially of great value in determining what it is that the gene does on an enzymatic and biochemical level to induce the mutant morphological change from wild type.

It is also interesting to note that there are classes of morphological mutants in the ascomycete *N. crassa* that are similar to those that occur in the basidiomycete *S. commune*. A very unique morphological mutant of *S. commune* called *puff*,[20] because of puffs of growth at intervals along main hyphae, has been demonstrated to have its counterpart in a single gene mutant of the edible mushroom *Lentinula edodes*.[23]

3. Developmental Mutants

Mutants in which some aspect of the sexual development of the species has been altered by mutation are known in a number of fungi, and they are of particular interest to us in connection with the development of fruiting bodies of Basidiomycetes. One class of practical importance is composed of those that lead to a failure to produce spores, or in which spore production is greatly restricted (see Chapter 8). The practical significance of such sporeless mutants lies in the fact that workers in mushroom houses may suffer from pulmonary and allergenic problems if they inhale spores in great numbers (see Chapter 3). Consequently, species that produce spores before the fruiting body is ready for harvest, such as species of *Pleurotus*, may cause this problem.

4. Fruiting Mutants

Mutants affecting the development of fruiting bodies have just been mentioned, but there are also strains that vary in time and amount of fruiting. These are important in determination of the genetic control of fruiting, which operates in addition to the mating type control of sexuality. This topic is examined in more detail in Chapter 8.

D. Utilization of Methods of Molecular Biology in Genetic Studies of Fungi

The understanding of biological systems at the level of the molecule has been one of the great advances of biological science in recent decades. The term **molecular biology** is applied to such studies and the techniques of molecular biology have been applied to problems of mushroom biology in general and especially to fungal genetics. These techniques are now commonly used in most research laboratories and need not be presented in detail, but we do want to emphasize the kinds of applications of molecular biology that are germane to the subject at hand.

1. Taxonomic Studies — Distinguishing Species and Strains

The discipline of **taxonomy** (the science that deals with the identification, naming and classification of the diverse types of fungi) has long relied on morphology, cytology, and cytogenetics for the resolution of taxonomic problems. However, distinctions that are based on morphological features, and the difficulty with many species in achieving mating reactions, may be inadequate to achieve the degree of resolution that is required — especially when we consider that 1.5 million species of fungi may exist.

In genetic studies both alleles of a heterozygote must be recognized. **Allozyme analysis** permits this. Enzymes that differ in electrophoretic mobility because of allelic differences in a single gene are called allozymes. It is possible to correlate genotypic differences between individuals with electrophoretic banding patterns. Royse and May[37] have reviewed the methods used for allozyme electrophoresis. More specifically, allozyme variation as determined by electrophoretic mobility

has been used to determine the genetic diversity of natural populations of several edible mushrooms. Multilocus enzyme electrophoresis has been used in studies of many edible mushrooms (e.g., *Agaricus bisporus*, *Lentinula edodes*, *Volvariella volvacea*, *Pleurotus* spp., and others). Such studies have resulted in genetic maps of allozyme linkage groups.[37]

A valuable and widely used technique in DNA research is known as the polymerase chain reaction (PCR), which duplicates specific pieces of DNA. An essential ingredient in this reaction is the Taq polymerase enzyme, which was derived by Brock from the thermophilic bacterium *Thermus aquaticus*. The polymerase chain reaction is an *in vitro* enzymatic amplification of a specific DNA sequence that was present in a minute amount. A modification of PCR called the arbitrarily primed-polymerase chain reaction (AP-PCR) has proved useful in distinguishing strains. There is an advantage in the AP-PCR method over allozyme electrophoresis in that AP-PCR makes use of markers based on DNA probes and gives direct assessment of the genetic diversity of different strains. Chiu et al.[9] have exploited the AP-PCR technique in strain identification of *L. edodes* and have described the experimental procedures for this technique. Briefly, the amplified DNA fragments are revealed by electrophoresis or characterized by direct DNA sequencing. In their studies, Chiu et al.[9] found that polymorphism among different strains in the same species was not revealed by mobility in gel electrophoresis but could be demonstrated by DNA sequencing or restriction digestion. With AP-PCR, a nonspecific primer, the DNA profiles reveal polymorphisms. Differences in polymorphisms indicate dissimilarity of strains.

2. Demonstration of Genetic Variation in Natural Populations

Survival of a species in nature under changing environmental conditions requires genetic variation. Such genetic variation is also necessary for a controlled breeding program for a mushroom species under cultivation. Thus, it is desirable to be able to determine how much variation is present in natural populations and also to preserve that variation. Such preservation requires that extensive collections be made of wild populations that are subject to loss through destruction of habitat and climate changes that might have adverse effects on features of the life cycle that control viability of the vegetative mycelium and fruiting. Because commercial cultivars have been used for long periods of time and have limited genetic variability, future breeding for improved cultivars needs the genetic variation of wild collected strains as discussed by Anderson.[1]

In the previous section, it was mentioned that allozyme variation analyzed by electrophoretic mobility is useful in determination of the genetic diversity of natural populations. Allozymes are good genetic markers and can be used to determine the genetic diversity of various mushroom cultivars as well as strains collected in nature. As useful as allozyme markers are, direct examination of DNA variation has become of increasingly great importance.

One widely used DNA technique is known as **restriction fragment length polymorphisms** (RFLPs). The RFLPs are based on cleavage patterns produced by bacterial endonucleases. These bacterial endonucleases recognize specific short sequences in the foreign DNA that has entered the bacterial cell, and they make a cut at or near the site of a specific sequence.

Anderson[1] has pointed out that these restriction enzymes are actually part of the defense systems of bacteria by fragmenting foreign DNA that has entered the bacterial cell. The prokaryotic bacteria are relatively "leaky" to invading DNA as compared to the situation in eukaryotes such as the fungi. Experimentally, the DNA fragments that have been cut off by the endonucleases can be separated by agarose gel electrophoresis. Analysis of the fragments can then be accomplished by using radioactive DNA molecules that contain the nucleotide sequences of interest. Commonly employed, however, to detect the specific restriction fragments among the multitude of fragments that appear in the genome is the Southern blot procedure, which has been concisely described by Anderson[1] as follows. The genomic DNA is cut with a restriction enzyme and the fragments produced by a specific cleavage are separated on the basis of size by agarose gel electrophoresis. Alkali treatment is used to make the DNA in the gel single stranded. The single-stranded DNA is

then transferred to a hybridization membrane. A small segment of homologous DNA is used as a probe, which has been made radioactive. The DNA of the probe is then allowed to hybridize with the DNA on the hybridization membrane. Fragments of genomic DNA hybridizing to this radioactive probe can be detected by autoradiography.

Royse and May[37] stress the value of combining allozyme and DNA data. Kerrigan et al.[15] combined allozymes and RFLPs in their study of wild *Agaricus bisporus* populations from different habitats in California along with cultivated heterokaryons (cultivars) probably of European origin. Their detailed analysis suggested that the California isolates could be placed in either the cultivar group (which had escaped from cultivation) or in a group associated with a particular habitat (the cypress groves of California). A less distinct group of isolates, intermediate to the two main ancestral groups, is thought to represent hybrids between these two groups. It has been emphasized by Royse et al.[38] that this is the type of information that is essential for "our understanding of genetic behavior of isolates of a cultivated species in the wild."

3. Demonstration of Genetic Variation in Germplasm Collections

To obtain a true picture of genomic variation within a species, it is important that genomic analyses be made of commercial cultivars as well as strains collected from diverse localities in nature. Dissimilarity of commercial cultivars may not be great even though they have different designation because, for years, strains of cultivated mushrooms were exchanged, sometimes by consent, and sometimes without permission. For either of these reasons the result would be less variation than might be supposed if the strains were truly unique. It cannot be emphasized too strongly that acquisition and retention of germplasm variability is of utmost importance, for the germplasm provides the genetic material for breeding programs. Differences in germplasm can be detected by allozyme analyses and DNA-based markers. The detection of RFLPs constitutes a DNA technique that has been described previously and it is useful in demonstrating genomic variation in germplasm collections. Another DNA-based marker, useful in distinction of germplasm variation, is known as RAPD (random amplified polymorphic DNA). RAPD is modified from the PCR. RAPD produces fragments similar to those produced by RFLP by a random amplification of DNA with a short primer of arbitrary sequence. Royse et al.[38] point out that although the RAPD fragments behave as simple Mendelian loci, they are dominant and alternative alleles are not expressed. Consequently, it is not possible to distinguish heterozygous and homozygous individuals in diploid fungi. RAPD analysis has been described succinctly by Anderson.[1] The advantages of RAPD analysis lie in its speed and simplicity (requires few materials, amplification products can be visualized without Southern hybridization or radioactive materials). He has also pointed out some of the drawbacks of the RAPD analysis. These drawbacks involve sensitivity to the conditions of analysis including contaminants in the target DNA preparation, concentration of reaction components, source of enzyme, and particularly the cycling temperature.

4. Linkage Studies

The traditional methods of determining linkage involve making sexual crosses followed by statistical analysis of the recombination frequency of genetic markers. Such analysis will determine if the recombination frequency is matter of chance or due to markers being linked. These traditional methods may be of limited value with edible mushrooms because of difficulty (1) in crossing, (2) in obtaining fruiting in a timely manner to obtain the progeny needed for analysis, or (3) in the germination of the sexual spores. Fortunately, allozymes and the DNA markers (RFLPs and RAPDs) are now available for linkage studies. The length of each chromosome can be estimated in megabases (mb). This has been demonstrated for *Coprinus cinereus* by Pukkila.[28] The cytological determination of fungal chromosome number and size for establishment of karyotypes is difficult because of the small size of fungal chromosomes. Thus, determination of karyotype by

electrophoretic methods such as pulsed field gel electrophoresis (PFGE) has been used to establish the chromosome number and their estimated sizes for a number of mushroom species as presented in a table in Miles and Chang.[21]

5. Confirmation of Crosses

The mushroom geneticist who has mated two different strains must have evidence that the cross has occurred. This is confirmed by the formation of a heterokaryon (a hyphal system in which different nuclei are present). In some of the mushroom species, the observation of clamp connections after mating is evidence that a dikaryon has been formed. (The dikaryon is a special type of heterokaryon in which the compatible nuclei are present in the same cell in a 1:1 ratio.) The establishment of a heterokaryon on a minimal medium when two differing auxotrophic mutants have been mated is another useful technique for confirmation of a cross. Similarly, with the rise of drug-resistant markers, the heterokaryon will grow on a medium containing the chemicals, but both homokaryotic strains fail to grow. However, with the advent of molecular techniques, it is relatively simple to use allozyme markers. The determination that a strain has allozyme markers from two different monokaryotic strains that were mated constitutes proof that the two strains were successfully crossed. Royse and May[37] mention that the use of allozymes to confirm a cross between two single spore–derived lines is used commercially by some spawn makers in the United States in their breeding programs.

6. Patent Labeling

Mushroom producers that have devoted much time, energy, and money to the development of a cultivar do not want their cultivar to be used without authorization by another commercial grower. Because it is a relatively simple matter to obtain tissue cultures from fruiting bodies purchased in the market, the originator of the cultivar needs to have exclusive rights to the cultivar. This is done by obtaining a patent. To obtain a patent it must be shown that the cultivar can be characterized by specific genes or gene sequences. To be useful for patenting purposes, the markers must be codominant; that is, the markers must be expressed in the heterozygote. For this purpose allozyme markers and various DNA markers, such as those obtained by RFLPs, RAPDs, and AP-PCRs can be used. Consequently, molecular methods are making it easier to protect patent rights.

REFERENCES

1. Anderson, J.B., Molecular tools in breeding *Agaricus bisporus*, in *Genetics and Breeding of Edible Mushrooms*, Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach, Philadelphia, 225–247, 1993.
2. Bensaude, M., *Recherches sur le cycle evolutif et la sexualite chez les Basidiomycetes*, Ph.D. thesis, University of Paris, published Nemours, France, 1918.
3. Blakeslee, A.F., Sexual reproduction in the Mucorineae, *Proc. Am. Acad. Sci*., 40, 206–319, 1904.
4. Brefeld, O., *Untersuchungen über Schimmelpilze*, 3rd ed., Verlag von Arthur Felix, Leipzig, 1887.
5. Brodie, H.J., The barrage phenomenon in *Lenzites betulina, Genetica*, 18, 61–73, 1936.
6. Brunswik, H., Über die Sexualitätsverhältnisse bei den Basidiomyzeten, *Verh. Zool. Bot. Ges. Wein*, 73, 153–154, 1923.
7. Brunswik, H., Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung, *Coprinus, Bot. Abh. K. Goebel*, 5, 1–152, 1924.
8. Buller, A.H.R., *Researches on Fungi,* Vol. 4, Longmans, Green, London, 1931.
9. Chiu, S.C., Kwan, H.S., and Cheng, S.C., Application of arbitrarily primed polymerase chain reaction in molecular studies of mushroom species with emphasis in *Lentinula edodes*, in *Genetics and Breeding of Edible Mushrooms*, Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach, Philadelphia, 265–284, 1993.

10. Eugenio, C.P. and Anderson, N.A., The genetics and cultivation of *Pleurotus ostreatus, Mycologia,* 60, 627–634, 1968.
11. Fincham, J.R.S. and Day, P.R., *Fungal Genetics*, 3rd ed., Blackwell Scientific, Oxford, 1971.
12. Fowler, R.J., Mitton, M.F., Vaillancourt, L.J., and Raper, C., Changes in mate recognition through alterations of pheromones and receptors in the multisexual mushroom fungus *Schizophyllum commune*, *Genetics,* 158, 1491–1503, 2001.
13. Fulton, I.W., Unilateral nuclear migration and the interactions of haploid mycelia in the fungus *Cyathus stercoreus, Proc. Natl. Acad. Sci. U.S.A.,* 36, 306–312, 1950.
14. Holliday, R., A new method for the identification of biochemical mutants of micro-organisms, *Nature* (London), 178, 987, 1960.
15. Kerrigan, R.W., Horgen, P.A., and Anderson, J.B., The California population of *Agaricus bisporus* comprises at least two ancestral elements, *Syst. Bot*., 18, 123–136, 1993.
16. Kniep, H., Über morphologische und physiologische Geschlechst Differenzierung (Untersuchungen an Basidiomyzeten), *Verh. Phys. Med. Ges. Wurzburg*, 46, 1–18, 1920.
17. Kniep, H., Über Geschlechtsbestimmung und Reduktionsteilung, *Verh. Phys.-Med. Ges. Wurzburg,* 47, 1–28, 1922.
18. Kniep, H., Über erbiche Änderungen von Geschlechtsfaktoren bei Pilzen, *Z. Indukt. Abstamm. Vereblehre*, 31, 170–183, 1923.
19. Kniep, H., *Die Sexualität der niederen Pflanzen*, Fischer, Jena, East Germany, 1928.
20. Miles, P.G., The action of a modifier gene on the *puff* morphological mutant of *Schizophyllum commune, Can. J. Genet. Cytol.,* 12, 70–79, 1970.
21. Miles, P.G. and Chang, S.T., *Mushroom Biology, Concise Basics and Current Developments*, World Scientific, Singapore, 66, 1997.
22. Miles, P.G., Takemaru, T., and Kimura, K., Incompatibility factors in the natural population of *Schizophyllum commune*. I. Analysis of the incompatibility factors present in fruit bodies collected within a small area, *Bot. Mag.* (Tokyo), 79, 566–571, 1966.
23. Murakami, S. and Takemaru, T., "Puff" mutation induced by UV irradiation in *Lentinus edodes* (Berk.) Sing., *Rep. Tottori Mycol. Inst.* (Japan), 12, 47–51, 1975.
24. Newton, D., The bisexuality of individual strains of *Coprinus rostrupianus, Ann. Bot.,* 40, 105–128, 1926.
25. Oort, A.J.P., The sexuality of *Coprinus fimetarius, Proc. K. Ned. Acad. Wet.,* Amsterdam, 31, 1–6, 1929.
26. Papazian, H.P., Physiology of the incompatibility factors in *Schizophyllum commune, Bot. Gaz.,* 112, 143–163, 1950.
27. Papazian, H.P., The incompatibility factors and a related gene in *Schizophyllum commune, Genetics*, 36, 441–459, 1951.
28. Pukkila, P.J., Methods of genetic manipulation in *Coprinus cinereus,* in *Genetics and Breeding of Edible Mushrooms,* Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach, Philadelphia, 249–264, 1993.
29. Quintanilha, A., Le probleme de la sexualite chez les champignons, *Bol. Soc. Broter.*, 8, 1–99, 1933.
30. Quintanilha, A., Cytologie et genetique de la sexualite chez les Hymenomycetes, *Bol. Soc. Broter.*, 10, 5–48, 1935.
31. Quintanilha, A., Contribution a 1'etude genetique du phenomene de Buller, *C. R. Acad. Sci*. (Paris), 205, 745–747, 1937.
32. Raper, J.R., *Genetics of Sexuality in Higher Fungi,* Ronald Press, New York, 1966.
33. Raper, J.R., Baxter, M.G., and Middleton, R.B., The genetic structure of the incompatibility factors in *Schizophyllum commune, Proc. Natl. Acad. Sci. U.S.A*., 44, 889–900, 1958.
34. Raper, J.R., Krongelb, G.S., and Baxter, M.G., The number and distribution of incompatibility factors in *Schizophyllum commune, Am. Nat.,* 92, 221–232, 1958.
35. Raper, J.R. and Raper, C.A., Genetic regulation of sexual morphogenesis in *Schizophyllum commune, J. Elisha Mitchell Sci. Soc*., 84, 267–273, 1968.
36. Rawitscher, F., Besprechungen, *Z. Bot*., 26, 134–136, 1933.
37. Royse, D.J. and May, B., Multilocus enzyme electrophoresis for the genetic analysis of edible mushrooms, in *Genetics and Breeding of Edible Mushrooms*, Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach, Philadelphia, 265–284, 1993.

38. Royse, D.J., Bunyard, B.A., and Nicholson, M.S., Molecular genetic analysis of diversity in populations of edible mushrooms, in *Mushroom Biology and Mushroom Products,* Chang, S.T., Buswell, J.A., and Chiu, S.W., Eds., Chinese University Press, Hong Kong, 49–54, 1993.
39. Schwalb, M.N. and Miles, P.G., Morphogenesis of *Schizophyllum commune*. I. Morphological variation and mating behavior of the *thin* mutation, *Am. J. Bot*., 54, 440–446, 1967.
40. Takemaru, T., Genetics of *Collybia velutipes*. I. Mating types and barrage phenomenon, *Bot. Mag*. (Tokyo), 67, 82–86, 1954.
41. Takemuru, T., Genetics of *Collybia velutipes.* IV. "Interpolarity" occurring in the strain NL-55, *Bot. Mag.* (Tokyo), 70, 238–243, 1957.
42. Takemaru, T., Genetics of *Collybia velutipes*. V. Mating patterns between F1-mycelia of legitimate and illegitimate origins in the strain NL-55, *Bot. Mag.* (Tokyo), 70, 244–249, 1957.
43. Takemuru, T., Genetic studies on fungi. X. The mating system in Hymenomycetes and its genetical mechanism, *Biol. J. Okayama Univ*., 7, 133–211, 1961.
44. Tokimoto, K., Komatsu, M., and Takemaru, T., Incompatibility factors in the natural population of *Lentinus edodes* in Japan, *Rep. Tottori Mycol. Inst*. (Japan), 10, 371–376, 1973.
45. Vaillancourt, L.J. and Raper, C.A., Pheromones and pheromone receptors as mating-type determinants in Basidiomycetes, in *Genetic Engineering, Principles and Methods*, Vol. 18, Sellow, J.H., Ed., Plenum Press, New York, 1996.
46. Vakili, N.G., On the Genetics of the *A* Factor in *Schizophyllum commune,* Master's thesis, University of Chicago, 1953.
47. Vandendries, R., Recherches sur le determinisme sexuel des Basidiomycetes, *Mem. Acad. R. Belg. Cl. Sci*., 5, 1–98, 1923.
48. Vandendries, R., Recherches experimentales sur la bipolarite sexuelle des Basidiomycetes, *Bull. Soc. R. Bot. Belg*., 57, 75–78, 1924.
49. Vandendries, R. and Brodie, H.J., Manifestation de barrages sexuels dans le champignon tetrapolaire *Lenzites betulina* (L.) Fr., *Bull. Soc. Bot. Belg*., 65, 109–111, 1933.
50. Whitehouse, H.L.K., Multiple-allelomorph heterothallism in the fungi, *New Phytol*., 48, 212–244, 1949.

7 Mushroom Formation: Effects of Environmental, Nutritional, and Chemical Factors

I. INTRODUCTION

Two of the three basic stages in sexuality take place in the fruiting body of the Basidiomycetes. These stages are nuclear fusion (karyogamy) and the reductional nuclear division (meiosis). The fruiting body is the most conspicuous phase of a basidiomycete's life cycle, and, in addition, it is the structure that is prized for food. For any or all of these reasons, it is not surprising that mycologists have for a long time had a great interest in the development of the fruiting body of the Basidiomycetes — a fleshy structure, which is commonly called a mushroom.

II. DEVELOPMENT OF FRUITING BODIES

Thus, both for practical considerations and for basic biological knowledge, mycologists have studied mushroom development and the factors that affect development. Almost any environmental factor may affect fruiting, and a species can be found that is markedly affected by one of the environmental factors — pH, light, temperature, aeration, and gravity. Before beginning an examination of these factors, a general account of the development of fruiting bodies and some variations in this pattern are presented.

To accommodate the formation of a fruiting body, changes must be made in the vegetative hyphae. In general, this involves an aggregation of hyphae followed by modifications of the hyphae that often include a thickening of the cell wall and a reduction in the size of the lumen of the cell.

A. Role of Fruiting Bodies

It is always worthwhile before making an examination of a structure to consider that structure's role in nature. The structure that we are considering is the fruiting body or mushroom of the Basidiomycetes. The mushroom has two main roles:

1. The first of these is the protection of the structures where karyogamy, meiosis, and spore formation take place. Karyogamy and meiosis take place in the basidium; consequently, protection of the basidia is one role of the mushroom.
2. The second role of the mushroom is to provide for effective dissemination of the spores that are produced on the basidia. We also examine mushroom morphology in light of this requirement.

B. Variation in Fruiting Body Structure

A basic difficulty in making a synthesis and some generalizations about mushroom structure and development stems from the fact that there is a great variety of fruiting bodies in the class Basidiomycetes, which is not surprising as there are approximately 15,000 species in the class.

1. Mushroom with Cap, Gills, Stipe, and Volva

The most familiar type of basidiomycete fruiting body is the mushroom with cap, gills, stipe, and sometimes a volva. The cultivated paddy straw mushroom, *Volvariella volvacea*, can serve as an example (Figure 7.1 and Figure 7.2). The fruiting body when young is surrounded by a layer of tissue called the universal veil. The developing pileus, or cap, that will have gills on the lower surface (with hymenia on the gills) is supported by a stipe (stalk), which, in the case of *V. volvacea*, arises from a basal cuplike structure known as the volva.

In the genus *Agaricus* there is no volva since there is no universal veil. A partial veil extends from the margin of the pileus to the stipe, and, as the pileus enlarges and the stipe elongates, this

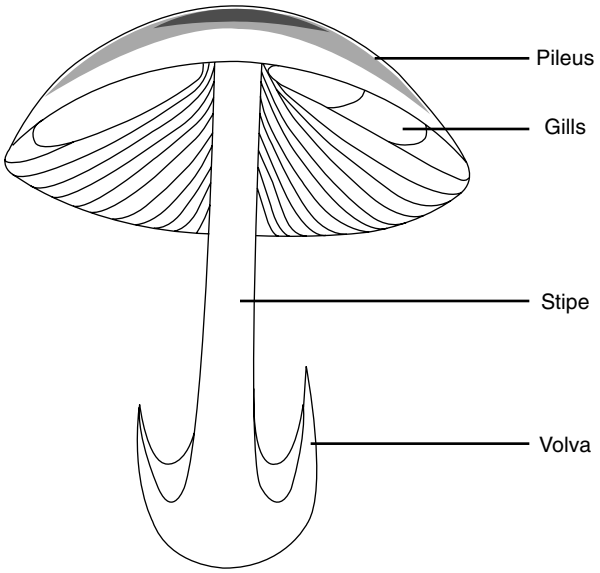


FIGURE 7.1 *Volvariella volvacea* showing pileus, gills, stipe, and volva (universal veil).

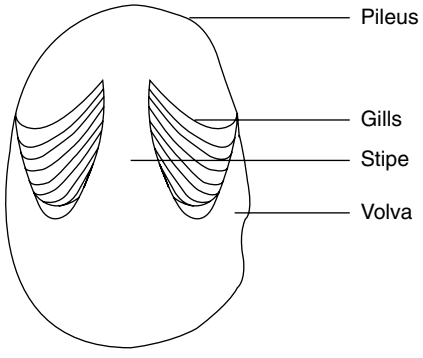


FIGURE 7.2 *Volvariella volvacea* in egg stage of development.

FIGURE 7.3 *Schizophyllum commune* showing absence of stipe.

veil ruptures leaving tissue from the veil as a ring (annulus) on the stipe. The stage of development prior to elongation of the stipe and enlargement of the pileus is known as the "button" stage. In *V. volvacea* the elongation of the stipe causes the rupture of the universal veil, which is left at the base as a volva in fruiting bodies in nature. When the mushroom first pushes through the universal veil, it has the appearance of an egg resting in an egg cup, and this is called the "egg" stage of development of *V. volvacea*.

2. Fruiting Bodies with No Stipe

Another type of fruiting body is one that lacks a stipe. The well-known experimental organism, *Schizophyllum commune* (Figure 7.3), is an example of a stipeless species although laboratory mutants that are stipitate have been described.

3. Spore-Bearing Layer (Hymenium) Not in Gills, But in Pores

Not all fruiting bodies of the Basidiomycetes have the hymenium in gills. In members of the order Polyporales, the basidia are found on the inner surface of pores or tubes. Common examples of Basidiomycetes with the hymenial surface lining tubes are the shelf or bracket fungi, such as *Ganoderma,* but the fleshy, mushroom-shaped fruiting body of *Boletus* also has pores rather than gills on the lower surface of the cap. Some individuals become ill after consuming certain species of *Boletus* (Figure 7.4); but *B. edulis* is a highly prized edible mushroom, albeit one that has not yet been cultivated commercially, possibly as a consequence of complexities due to its existence as a mycorrhizal fungus.

4. Funnel-Shaped Fruiting Body with Hymenial Layer in Folds on Underside of Body

The genus *Cantharellus* has a funnel-shaped fruiting body with folds, somewhat like gills, on the underside of the body. These folds constitute the hymenial layer. *Cantharellus cibarius* is an edible species that is much sought in the wild because of its excellent gastronomic qualities.

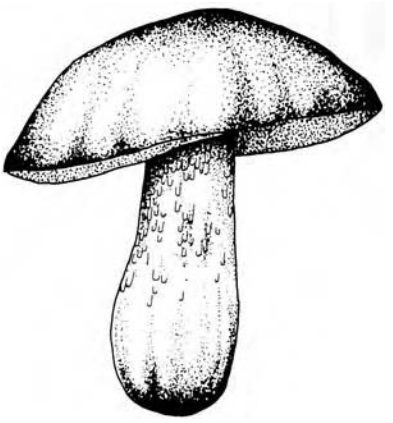


FIGURE 7.4 *Boletus*. Note pores on underneath surface of pileus.

C. Primordium Formation

The fruiting body of Basidiomycetes is completely made up of hyphae, and in most cases these hyphae are dikaryotic. The investigations of Buller on *Coprinus* suggested that the origin of the fruiting body can be traced to a single cell, and more recently this problem was investigated by Matthews and Niederpruem,[20] who developed a simple technique for inducing primordia formation in *Coprinus* in predetermined regions of the mycelium. By microscopic observations, it was determined that the initial events in primordia formation were the establishment of networks of fused hyphae; i.e., the mushroom primordium of *Coprinus* does not develop from a single cell, as suggested by Buller, but arises from a group of fused hyphae.

Branching and hyphal anastomoses followed, causing the formation of hyphal lattices, which produced aerial hyphae that formed an aggregation of dikaryotic hyphae about 0.1 to 0.2 mm in diameter.

At these early stages of primordial development, no changes in cytochemical properties of the cells or differentiation into specialized tissues were observed. With further growth of the primordia, there are differences in organization and development of the hyphal cells that were detected microscopically and by staining reactions. When the primordium reached a size of about 1.0 mm in diameter, the form of the mushroom, its pileus, gill area, and stipe could be seen; and it was recorded that the pileus region is the first to form.

Earlier studies on primordia than those just reported for *Coprinus* described two general types of primordia:

1. The "compact" primordium is tightly interwoven with hyphae more or less longitudinally oriented so that it has a subcylindrical structure. This type of primordium gives rise to stipitate mushrooms, such as *Coprinus*.
2. The "diffuse" primordium develops into a hemisphere of hyphae.

D. Primordium Development

Although we have already described briefly the development of the primordium of *Coprinus*, there have been numerous other descriptions of mushroom development because developmental characterization is valuable in establishing phylogenetic relationships. Thus, it is useful to consider at this time matters of terminology in primordium development and types of hyphae occurring in fruiting bodies.

In primordium development, generally the first step to be recognized is a loss of the characteristic branching pattern of the hyphae. The hyphae in the primordial region do not radiate outward.

The next observation may be that the hyphal walls thicken and the hyphae change in diameter. Two terms used to describe development of the primordium are **gymnocarpic** and **angiocarpic**.

1. Gymnocarpic (or exogenous) development refers to that which proceeds outward and is unenclosed.
2. Angiocarpic (or endogenous) development refers to that which proceeds inward and is enclosed, as, for example, in the familiar button stage of mushrooms.

E. Types of Hyphae in Fruiting Bodies

The types of hyphae composing fruiting bodies are as follows:

1. Generative — These are thin-walled, branched, and often clamped.
2. Skeletal — These are thick-walled, often unbranched, and often without septa.
3. Binding — These are thick walled, highly branched, and rarely septate.

An exception to the above scheme of classification devised by Corner[4] is found in the genus *Russula*. In this genus rounded, swollen, more or less spherical cells, called sphaerocysts, are present in the trama. (The trama is the tissue composing the pileus or bearing the hymenium of the members of the subclass Holobasidiomycetidae.)

On the basis of these hyphal types present in the fruiting bodies the following classification is possible:

1. Monomitric — Fruiting bodies made up of only generative hyphae
2. Dimitric — Fruiting bodies made up of both generative and skeletal hyphae
3. Trimitric — Fruiting bodies made up of generative, skeletal, and binding hyphae

F. Growth of *Agaricus*

The growth and development of the common field mushroom *Agaricus campestris* (actually *Agaricus bisporus* was used) was studied by Bonner and his students.[2] They reported that there was a pause in growth after the button stage, and then maturation proceeded at great speed. By placing marks at uniform distances along the stipe of fruiting bodies during early development, they were able to show that the region of greatest growth was at the upper part of the stipe, just below the cap. It was suggested that this may involve only cellular expansion.

III. ENVIRONMENTAL FACTORS AND FRUITING

Almost any environmental factor may affect fruiting, sometimes markedly so. The German mycologist Klebs[15] made a number of generalizations that are germane to the present topics. These have been passed along to us as "Klebs' principles," appearing in texts on fungal physiology by Lilly and Barnett[17] and Griffin.[6] Selected from these texts and briefly presented here are the main ideas embodied in Klebs' principles.

1. Growth and reproduction are life processes, which depend upon different conditions in all organisms. In the lower organisms (e.g., fungi) the external conditions mainly make the determination of whether growth or reproduction takes place.
2. Reproduction in the lower organisms does not occur as long as characteristic external conditions are favorable for growth. **The conditions that are favorable for reproduction are always less favorable for growth.**

TABLE 7.1
Cultivated Mushrooms — pH Range and Optima

Species	Mycelial Growth[a]		Fruiting
Agaricus bisporus	3.5–9.0	(6.8–7.0)	5.5–8.0 (casing)
Agaricus bitorquis		(6.0–6.4)	(7.2) (casing)
Auricularia auricula	3.5–8.5	(4.5–7.5)	
Auricularia polytricha	2.8–9.0	(5.0–5.4)	
Flammulina velutipes	4.0–8.0		5.2–7.2
Hericium erinaceus	2.4–5.4	(4.0)	
Lentinula edodes	4.4–7.5	(4.7–4.8)	(4.2–4.6)
Pholiota nameko		(5.0–7.0)	
Pleurotus ostreatus		(5.4–6.0)	
Pleurotus sajor-caju		(6.0–6.5)	
Tremella fuciformis		(5.0–6.0)	
Volvariella volvacea	5.0–8.5	(7.5)	(7.6–8.0)

Note: Values are affected by differences in media, growth conditions, and strains or stocks used. These are representative values obtained from the literature.

[a] Figures within parentheses are optimal values.

3. The processes of growth and reproduction differ in that **growth may take place under a wider range of environmental conditions than reproduction**. That is, growth may take place under conditions that inhibit reproduction.
4. Vegetative growth appears to be mostly a preliminary step for reproduction in that growth creates a suitable internal environment for reproduction. To a certain degree it is not growth in itself but the prolonged period of assimilation that accompanies growth that is decisive for reproduction.

A. Hydrogen Ion Concentration (pH)

It is well known from experimental studies that the optimal pH values for fruiting may differ from those for growth. It is also known that species differ in their optimal pH values for fruiting. During the course of an experiment the pH value of the medium may change because the fungus has produced metabolites, e.g., organic acids, that affect the hydrogen ion concentration. Media that are strongly buffered or that are periodically neutralized by the addition of base or acid may not fruit because the pH value required for the metabolic reactions necessary for fruiting is not reached. Table 7.1 illustrates the differences in pH requirements for fruiting of different mushrooms and their pH optima for mycelial growth.

B. Temperature

A generalization that can be made in reference to temperature is that the temperature range is narrower for fruiting than for mycelial growth, and the range for the optimum temperature is likewise narrower for fruiting than for growth (Table 7.2).

These temperature effects are illustrated in the study of the wild, edible, wood-rotting mushroom, *Pholiota adiposa*, by Arita et al.[1] These investigators determined the optimal and critical temperatures for mycelial growth and fruiting with a view toward possible commercial cultivation of this species. Mycelial growth was found to occur over the range of 5 to 33°C; whereas fruiting occurred only from 13 to 24°C. The optimal temperature for mycelial growth was 27°C and for

TABLE 7.2
Cultivated Mushrooms — Temperature Range and Optima

Species	Mycelial Growth[a]		Fruiting[a]	
Agaricus bisporus	3–32	(22–25)	9–22	(15–17)
Agaricus bitorquis	3–35	(28–30)	18–25	(22–24)
Auricularia auricula	15–34	(28)	15–28	(22–25)
Auricularia polytricha	10–36	(20–34)	15–28	(24–27)
Flammulina velutipes	3–34	(18–25)	6–18	(8–12)
Hericium erinaceus	12–33	(21–25)	12–24	(15–22)
Lentinula edodes	5–35	(24)	6–25	(15) Autumn (10) Winter (20) Spring
Pholiota nameko	5–32	(24–26)		(7–10) 8–20 (High temp. strains) 5–15 (Low temp. strains)
Pleurotus ostreatus	7–37	(26–28)		25–30 (High temp. strains) 16–22 (Med. temp. strains) 12–15 (Low temp. strains)
Pleurotus sajor-caju	14–32	(25–27)	10–26	(19–21)
Tremella fuciformis	5–38	(25)	20–28	(20–24)
Volvariella volvacea	15–45	(32–35)	22–38	(28–32)

Note: Values are affected by differences in media, growth conditions, and strains or stocks used. These are representative values obtained from the literature.

[a] Figures within parentheses are optimal values.

fruiting body formation 18 to 21°C. For the fruiting experiment, *P. adiposa* was precultured at 25°C for 34 days to allow the mycelium to become established and thus to negate the effect that lower temperatures have on mycelial growth in that growth is much reduced at low temperatures. Thus, cultures of comparable growth were placed at different temperatures, and fruiting was measured by the number and weight of the fruiting bodies produced as well as the time required for fruiting.

C. Aeration

Most fungi require adequate aeration for vegetative growth, and the requirements for fruiting are even more stringent. A generalization can be made that fruiting bodies of higher fungi typically form best under conditions of good aeration. The failure of fungi to fruit is frequently attributed to the accumulation of carbon dioxide from respiration. Evidence for the inhibition of pileus formation of *Agaricus bisporus* as a result of high concentrations of carbon dioxide is available, as well as a role at the earlier stage of morphogenesis involving initiation of fruiting body primordia. In the case of *A. bisporus*, it has been suggested that other volatile metabolic products such as ethylene or ammonia may have an inhibitory role in fruiting also.

Niederpruem[21] convincingly demonstrated the inhibitory effect of carbon dioxide on fruiting in the experimental basidiomycete *Schizophyllum commune*. He placed plates inoculated with dikaryotic mycelia in desiccators containing KOH to absorb CO_2carbon dioxide. The KOH kept a low concentration of CO_2 in the atmosphere of the desiccator, and it was found that the dikaryotic mycelium fruited; whereas a concentration of 5% CO_2 completely prevented primordium development of *S. commune*.

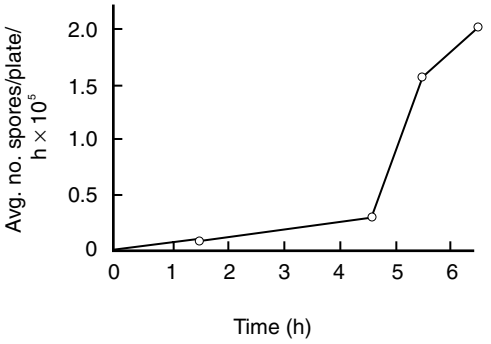


FIGURE 7.5 Recovery from dark inhibition of sporulation. Cultures were placed in light at zero time. (Data from Schwalb, M.N., in *Genetics and Morphogenesis in the Basidiomycetes*, Schwalb, M.N. and Miles, P.G., Eds., Academic Press, New York, 135–165, 1978.)

Mushroom growers frequently attribute malformation of fruiting bodies and poor primordia formation to a high concentration of CO_2 brought about by improper management of the aeration in the mushroom house.

D. Light

Many fungi are apparently uninfluenced in reproduction by light in the visible range; i.e., they do equally well in darkness, continuous light, or alternating darkness and light.

There are some fungi that do not fruit without light, however. For example, among the Ascomycetes, *Pyronema* fails to form antheridia and ascogonia without light. Among the Basidiomycetes, *Coprinus cinereus* (frequently referred to in the earlier literature as *C. lagopus*) requires a short exposure to light for fruiting body formation. This is also the case with *S. commune*, which requires light for the initiation of fruiting body primordia but, according to some reports, not for subsequent stages of development and growth of the fruiting bodies.

One exception to this latter statement concerning *S. commune* is of interest. Bromberg and Schwalb[3] have demonstrated that light is required in *S. commune* for the formation of primordia and also for the early stages of fruiting body development in which short cylindrical stipes with terminal apical pits are formed. Light is not required, however, for the appearance of gills and subsequent growth and expansion to the mature fruiting body stage, but the functioning of the fruiting body by production of spores ceases if the fruiting body is placed in the dark. A period of light of 5 to 6 hours is then required for recovery from the dark inhibition of sporulation (Figure 7.5).

The opposite extreme of a requirement for light for fruiting body formation is that of inhibition by light. In some species, of which *Agaricus bisporus* is the best-known example, light is inhibitory to the development of fruiting body primordia and also has been demonstrated to inhibit stipe elongation and pileus expansion.

There are a number of fungi that require light for the normal development of the fruiting body. In the ascomycete *Sordaria fimicola*, perithecia are produced in both light and darkness, but the perithecia produced in darkness are smaller. In the basidiomycete *Flammulina velutipes*, light is required for the normal expansion of the pileus.

The studies of Plunkett[22] indicate an effect of light for pileus expansion of *Polyporus brumalis* similar to that just mentioned for *F. velutipes*.

Kitamoto and co-workers[10–13] have reported on a series of studies dealing with the details of the effects of light on pileus formation in *Favolus arcularius*. In this basidiomycete, pileus formation is induced by light, as is also true for *Panus fragilis* and *Boletus rubinellus*. In *F. arcularius* light is required for the induction of fruiting body primordia, but if these primordia are transferred to the dark, stipes will form, but no pilei. It was found by Kitamoto and colleagues that the stipes

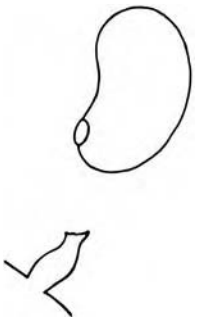


FIGURE 7.6 Discharge from the sterigma of basidiospore with attached bubble.

had to reach a length of about 5 mm before pilei could be photoinduced. Young stipes were not induced to form pilei by light.

A technique was developed that permitted synchronous pileus formation so that the effect of light on the initiation of pileus formation could be studied. The method was to use stipes (lacking pilei) which had been preincubated in darkness for 48 to 72 hours. It was found that pileus formation then would begin about 24 hours after the start of illumination, but constant illumination was not required; pileus formation occurred if a dark period was interposed between 1 and 8 hours after the start of illumination. The initiation of pileus formation was discovered to involve two light-requiring steps: the first, which occurred during the first hour of illumination, was saturated with 5 lux of light, and the second light process caused earlier pileus-primordium formation the more the light intensity was increased up to 150 lux.

Perhaps the most familiar light effects on fungi are the phototropic responses. **Phototropism** is a growth response that causes a bending toward (positive) or away from (negative) light. Although the studies of the phototropic response of the sporangiophore of *Phycomyces* are classical, our present concern has to do with phototropism in relation to the development of fruiting bodies of basidiomycetes. Earlier in the chapter we mentioned that one of the roles of the mushroom is to provide for effective dissemination of the spores that are produced on the basidia. Phototropism plays a prominent role in spore dissemination in many basidiomycetes.

Basidiospores are forcibly ejected from the basidium by a discharge mechanism that involves the bursting of a bubble that forms near the point where the basidiospore is attached to the sterigma (Figure 7.6). The basidiospores are only discharged a short distance by this method, but it is a distance sufficient to place the spore in a position that, when the forces of gravity take over, permits the spore to fall free of the hymenial surface — provided that the gills of the agaric or tubes of the polypore are properly aligned by the positioning of the stipe and pileus. This, then, is accomplished to an extent by phototropic responses in the stipes of a number of mushrooms, including species of *Coprinus, Flammulina velutipes, Polyporus brumalis*, and many other species, including *Schizophyllum commune*. The stipe of *S. commune* is so small that the species is commonly described as nonstipitate, but Schwalb and Shanler[28] have studied phototropism in a mutant stock that has stipes and is positively phototropic at wavelengths of 420 and 540 nm at intensities greater than 11 lux.

Plunkett[23] demonstrated experimentally the positive phototropism of *P. brumalis* stipes, but the most interesting aspect of this study involves its relationship with geotropic responses, which we take up now.

E. Gravity

In contrast to the positive growth response of fruiting bodies to light, the growth responses to gravity are negative. In Plunkett's study[23] of *Polyporus brumalis*, it was shown that geotropism operates at both the prepiliate (i.e., epileate) and pileate stages of basidiocarp development, but this geotropic effect can be overcome by phototropism.

With the onset of pileus expansion (induced by unidirectional light), the stipe is shaded and the region of phototropic response is no longer subjected to unilateral illumination. The mushroom then grows in response to negative geotropism; however, if the source of light is changed to come from a direction not shielded by the pileus so that it falls on the stipe, the positive phototropic response takes over. This demonstrates the relationship of phototropism and geotropism in *P. brumalis*. In nature, both phenomena operate to provide a proper orientation of the fruit body for basidiospore dissemination.

Geotropism was also studied by Schwalb and Shanler[28] in *Schizophyllum commune*. Studying a stipitate mutant, they found that the stipe was not affected by gravity. However, the apical pit stage was found to be positively geotropic, and the next stage, involving development of the gills, was also found to occur in response to gravity, with the gilled surface becoming oriented to face downward. Finally, the expansion of the pileus was negatively geotropic.

IV. NUTRITIONAL FACTORS AND FRUITING

It has been pointed out in the previous section, Environmental Factors and Fruiting, that the factors required for fruiting may differ widely from those for vegetative mycelial growth. Hawker[7] wrote, "The total amount, nature, and concentration of food materials necessary for reproduction may all be different from those favoring vegetative growth."

From this statement of Hawker we see that the same principle that vegetative growth and reproduction may respond differently to controlling factors holds for both environmental and nutritional factors. Let us first consider the effect of concentration of nutrients on the fruiting process.

A. Concentration of Nutrients

It is well known that too high a concentration of nutrients encourages vegetative growth, and it is often possible to induce spore formation or fruiting body formation by transferring such a well-nourished culture to weak medium. (Recall Klebs' principles discussed earlier in the chapter.)

It seems obvious that good mycelial growth (spawn running or mycelial running, in the terminology of the commercial mushroom grower) must take place if mushrooms are to be produced in any quantity, but there is substantial evidence that the fruiting process in many fungi is triggered by the cessation of vegetative growth. Is this to be interpreted as indicating that fruiting will not occur so long as vegetative growth is taking place? Does it mean that exogenous nutrients are not required for mushroom production once the fruiting body primordia have been initiated? There are several studies that relate to the above questions.

In *Coprinus lagopus* (= *C. cinereus*) it was shown by Madelin[19] that the amount of mushrooms produced was proportional to the amount of nutrients made available, and pure culture studies demonstrated that a decrease in the dry weight of mycelium was correlated with the formation of mushrooms. From these observations we can infer that the mushrooms of *C. cinereus* obtain their nutrients from both the previously established vegetative mycelium and from exogenous sources.

Studies of Kitamoto and co-workers,[10,11] using replacement cultures for studying the nutritional requirements of fruiting body formation of *Favolus arcularius*, found that the main source of nutrients for growth of the mushrooms was the vegetative mycelial cells, but that some nutrients for growth of the mushrooms were also obtained directly from the medium.

Studies of fruiting body formation of a similar nature involving *Psilocybe panaeoliformis* were also reported from the laboratory of Kitamoto.[14] The results differed from those obtained in the studies of *F. arcularius*. With *Psilocybe*, mushroom growth depended mainly on the nutrients of the medium and less on nutrients obtained from the vegetative mycelium.

Schizophyllum commune has been studied extensively from the standpoint of morphogenesis of fruiting. There is general agreement that fruiting in this fungus is encouraged by removal or exhaustion of nutrients. Dikaryotic stocks, which are poor fruiters, as well as stocks with good

genetic fruiting capabilities, can frequently be induced to fruit by cutting the vegetative mycelium with a scalpel. The fruiting bodies then appear on the cut edge. Commonly, fruiting bodies will form at the edge of the petri dish.

Wessels[31] induced fruiting body primordia by supplying thiamine, a known requirement for primordia formation. In his experimental studies, a carbon source was found to be required for growth of the primordia, but for formation of pilei no nutrients were required. In fact, if glucose were added, the development of pilei was reported to be inhibited. In experiments in which fruiting was studied in synchronously developing cultures, Schwalb[24] found that fruiting was not inhibited with continuous supplies of glucose. However, in *C. macrohizus* (= *C. cinereus*) Uno and Ishikawa[30] reported that high concentrations of glucose did inhibit fruiting. Thus, the effects of glucose on fruiting vary with species and require further research.

B. Nature of Carbohydrate

A carbon source that yields very high vegetative growth may not be the best for fruiting. Generally, hexoses are better than polysaccharides for mycelial growth, but the opposite may be true for mushroom production.

In the studies by Kitamoto et al.[10,14] mentioned previously, the effect of carbohydrates on fruiting was also investigated. In the case of *Psilocybe panaeoliformis*, based on weight and time of appearance of the fruiting bodies, they reported glucose, fructose, and trehalose as good substrates for producing high yields of fruiting bodies. (In the tabulated data, soluble starch had the highest value and maltose had a higher value than all except trehalose, but, for some reason, soluble starch and maltose were not mentioned in the discussion.)

Certain carbon sources, especially glycerol, xylose, sucrose, and fructose, produced abnormal fruiting bodies. The best carbon sources for mycelial growth were glycerol, fructose, xylose, and glucose, but of these only glucose and fructose gave good fruiting body production, and 20 to 40% of the fruiting bodies produced by fructose were abnormal. This serves as an example for the statement made earlier that a carbon source that gives high mycelial growth may not be good for fruiting.

Studies in which the concentration of glucose was varied indicated that 2% was the best concentration for fruiting body production of *P. panaeoliformis*, but 0.5 and 4.0% concentrations were both better than 2% for production of mycelium.

The studies of fruiting of *Favolus arcularius* by Kitamoto et al.[10] indicated that large amounts of carbohydrate were needed in the early phases of fruiting body development. Of the carbohydrates, trehalose was the best, followed by fructose and glucose. The optimal concentration of glucose for fruiting was 1%.

C. Nitrogen

Any consideration of nutrition must include a discussion of nitrogen requirements. The generalization in reference to nitrogen requirement is similar to some made previously. Namely, species differ in their ability to utilize nitrogen sources for fruiting as well as for mycelial growth, and the minimum concentration of nitrogen necessary for fruiting body formation may be slightly greater than the concentration supporting mycelial growth. Furthermore, a nitrogen compound that gives good mycelial growth may not provide for good fruiting.

A high concentration of nitrogen encourages mycelial growth and decreases sporulation (= formation of fruiting bodies in the Basidiomycetes). This is brought about through the accumulation of toxic metabolic products or exhaustion of some essential metabolite due to the excessive mycelial growth.

The effect of nitrogen is less specific than that of carbon. The carbon-to-nitrogen ratio (C:N ratio) is important in fruiting body formation. The C:N ratio obtained by chemical analysis of fungal cells is approximately 10:1, but substrate carbon is also used for energy and is respired as

CO_2. It is thus estimated that an amount is converted to cellular material that is similar to the amount respired as CO_2; consequently, for growth, a C:N ratio of 20:1 is suitable.

D. Mineral Nutrition

In the matter of mineral nutrition, there seem to be no reports of qualitative differences between vegetative growth and sporulation, with the single exception of the element calcium. Calcium is required for the production of perithecia in the ascomycete *Chaetomium*, and Lu[18] has demonstrated that there is a requirement for calcium for the production of fruiting bodies in *Cyathus stercoreus*. Lu used liquid media to investigate fruiting in this gasteromycete, which is one of the so-called bird's nest fungi, and he obtained experimental evidence that conclusively revealed the necessity of calcium in the medium if fruiting were to occur.

In regard to quantitative differences of minerals, it should be pointed out that a concentration of a mineral sufficient for vegetative growth may be insufficient for sporulation.

E. Vitamins

A number of species have requirements for vitamins that are generally the same for sporulation as for growth except in a quantitative sense — the concentration of the vitamin for primordia or fruiting body formation commonly is higher than for vegetative growth. The most common requirements that filamentous fungi have are for thiamine (vitamin B_1) and biotin (vitamin H or vitamin B_7). *Sordaria*, an ascomycete used in many basic genetic studies, requires biotin for growth, but some strains of *Sordaria* need thiamine too for perithecial formation. Thiamine has its role as cocarboxylase, the coenzyme of the enzyme carboxylase that functions in the conversion of pyruvic acid to acetaldehyde and carbon dioxide. Thus, thiamine plays a critical role in the regulation of carbohydrate metabolism, which was previously indicated to be important in the development of fruiting bodies.

Many of the cultivated mushrooms have a thiamine requirement for growth and the formation of fruiting body primordia and/or fruiting bodies. Among these genera are *Auricularia*, *Coprinus, Flammulina, Lentinula, Pholiota, Pleurotus*, and *Volvariella*. In some cases the vitamin serves to enhance growth, and it may not be an absolute requirement; that is, the fungus is able to manufacture this vitamin, but not in sufficient quantities under the test conditions to permit optimal growth or fruiting. Vitamins other than thiamine and biotin may have a similar enhancement role. Many compounds have been reported to be required for sporulation. Lilly and Barnett[17] have listed 25 growth factors that may initiate or stimulate sporulation of fungi.

V. CHEMICAL FACTORS AND FRUITING

In the Ascomycetes, studies of the biochemistry of fruiting have frequently focused on the events surrounding melanin synthesis. This has been the case for *Neurospora crassa, Podospora anserina*, and *Sordaria fimicola* — three species of importance in experimental genetics. Some studies with basidiomycetes have also suggested a role for the phenol oxidase, tyrosinase, which is required in the polymerization reaction that makes melanin. A brief examination of these studies with ascomycetes may be worthwhile, even though our chief concern is with fruiting of basidiomycetes.

In *N. crassa* it was suggested by Hirsch[8] that the melanin pigments, or tyrosinase, play a role in the formation of protoperithecia. Furthermore, it was later shown that a mutant that did not form protoperithecia also did not form tyrosinase (or at least it formed very, very little of it), thus supporting the hypothesis of Hirsch that melanin pigments, or the enzyme tyrosinase, play a direct role in the formation of protoperithecia.

This hypothesis was disproved by the experiments of Horowitz and colleagues,[9] who induced tyrosinase in such mutants, and the mutants still failed to form perithecia. From this experimental

TABLE 7.3
Phenol Oxidase Content and CRM Values of Strains with Different Combinations of Minor-1 (*m-1*) and Suppressor (*su-m*) Alleles

	Wild Type *m-1*$^+$, *su-m*$^+$	Mutant *m-1*, *su-m*$^+$	Suppressed Mutant *m-1*, *su-m*	Wild Type with Suppressor *m-1*$^+$, *su-m*
Laccase and tyrosinase	1.00	0.51	1.13	0.93
Laccase	1.00	0.47	1.15	0.84
Tyrosinase	1.00	4.35	0.74	1.08
CRM value	1.00	0.34	0.87	0.81

Source: Adapted from Esser.[5]

study, it was concluded that there was probably not a direct, causal relationship between melanin synthesis, or tyrosinase activity, and the development of perithecia.

A. Melanin Production and Perithecial Development in *Podospora*

The failure to demonstrate a direct, causal relationship between melanin production and perithecial formation in *Neurospora* does not, of course, mean that such a relationship cannot exist in other organisms. In studying mutants of *P. anserina*, Esser[5] found that two allelic genes, *m-1* and *m-2,* caused (1) a drastic reduction in perithecial size, and (2) a reduction in the formation of melanin pigments in the mycelia. It was then found that a suppressor gene, *su-m*, removed the mutant phenotype; i.e., strains carrying *su-m* had wild-type phenotype.

Enzymatic analyses for the phenol oxidases known as tyrosinase and laccase were made for the following (the genotypes are given in parentheses):

- Wild-type strains (*m-1*$^+$, *su-m*$^+$)
- *m-1* strain (*m-1*, *su-m*$^+$)
- *m-1* strain with suppressor (*m-1*, *su-m*)
- Wild-type strain with suppressor (*m-1*$^+$, *su-m*)

Serological studies were also made, and the values of the cross-reacting materials (CRM) obtained. (The CRM value is the quotient of enzyme units and antienzyme units. By definition, this is 1 for wild type, and deviations from this value indicate a structural change of the enzyme.) The results obtained by Esser[5] are given in Table 7.3.

From the table it can be seen that the minor (*m-1*) mutant has half the phenol oxidase activity of wild type, and that it is the activity of laccase that is diminished, whereas the activity of tyrosinase in the mutant is actually higher. A structural alteration of the laccase molecule rather than reduced synthesis of the enzyme is indicated by the reduced CRM value of the *m-1* mutant — the mutant enzyme particles bound by the antiserum have only approximately one third the activity of the other strains.

The suppressor gene, *su-m,* affects not only perithecial production, but it also restores the level of enzyme activity to that of wild type. The mutant gene, minor (*m-1*), causes a defect in morphogenesis (reduction in perithecial size and in formation of melanin pigments) and in the structure of laccase. Hence, there is in this case in *P. anserina* a direct relationship between enzyme and morphogenetic events.

B. Morphogenesis in *Schizophyllum commune*

Some of the findings of Wessels[31] were mentioned earlier in the sections dealing with environmental and nutritional factors and fruiting. Because these studies have been reported in a number of reviews,

we indicate the general approach to the problem without providing a critique and detailed analysis of the results.

The objective of the research by Wessels[31] was to study the biochemistry of fruiting body development. Of course, any such an investigation requires that there be synchrony in development if analysis of enzyme activity or products of enzyme activity are to be compared at different stages of development and involve more than a single individual developing structure. That is, data collected from a sample in which more than one development stage is present have little value at the biochemical level.

Consequently, the first stage in the study involved dividing development of *S. commune* into distinguishable stages so that biochemical events occurring at the various stages could be assayed. In this study, determinations were made of total nitrogen, total carbohydrates, principal polysaccharides, changes in nucleic acid and protein metabolism, as well as the effect of thiamine on primordia induction.

It was found that fruiting did not proceed beyond the primordial stage at low levels of glucose in cultures grown in sand in special flasks that permitted the changing of the stale medium with fresh medium. That is, when a constant low level of glucose is maintained by regular replacement of the culture medium, primordia failed to develop pilei.

When primordia grew normally and developed pilei, from where did the material that formed the pilei come? Wessels measured the glucans of the cell walls, both of the mycelium and of the fruiting bodies at different times. (The glucans constitute a high percentage of the cell wall polysaccharides of *S. commune*.) It was found that there was a loss in glucans in the mycelium during the stage from mycelium to fruiting, and a decrease in the total amount of glucans, suggesting that the material forming the pilei came from the cell wall glucans of the mycelium.

Studies of R-glucanase activity (R-glucan is the alkali-resistant polysaccharide of *S. commune*) have revealed that there is an increase in R-glucanase activity in mycelial extracts and the culture fluid when glucose is exhausted. This increase in R-glucanase activity explains the loss of R-glucan during the stage from mycelium to pileus formation.

Although all the findings of Wessels[31] have not been confirmed (e.g., a contrary account to his report that external glucose inhibited pileus growth has been presented by Schwalb),[24] his study has provided a framework for biochemical investigations of morphogenesis of fruiting in basidiomycetes, and this has stimulated a number of investigations on this very important topic.

Chemical substances have been found to induce fruiting in *S. commune*. Previously, we have shown that certain environmental and nutritional factors operate in the induction of fruiting as well as the chemical factors involved in morphogenesis just described. The chemical factor that is now described is different in that it induces fruiting in monokaryotic (haploid) mycelia.

The phenomenon of monokaryotic (or haploid, or homokaryotic) fruiting is not uncommon, but it has generally attracted limited interest because, ordinarily, the phenomenon does not lead anywhere in a biological sense. In most examples of monokaryotic fruiting, the fruiting bodies are small or atypical, the spores are produced in smaller number and, of course, are all of one genotype, and commonly the spores germinate poorly. The chance observation of a monokaryotic strain of *S. commune* fruiting as a result of an intermycelial reaction with the imperfect fungus, *Hormodendrum cladosporioides*, led Leonard and Dick[16] to a series of investigations in which they demonstrated the presence of a fruiting-inducing substance (abbreviated FIS) in cell-free extracts of *Hormodendrum*, in *S. commune* at certain developmental stages, and in *Agaricus bisporus*. The monokaryotic fruiting bodies induced by FIS produced abundant spores, which germinated in high percentage. The possibility that FIS acts as a regulatory hormone in fruiting seems quite likely.

C. Effect of Cyclic AMP

In *Coprinus macrorhizus* (= *C. cinereus*), it has been found by Uno and Ishikawa[29] that there is an FIS, and this substance is cyclic adenosine monophosphate (cAMP or cyclic AMP). In this fungus

there is a direct relationship between the level of cyclic AMP and fruiting body formation. Cyclic AMP acts as an FIS and accumulates only during fruiting.

The possibility that the FIS of *S. commune* might be cyclic AMP was tested by Schwalb,[25] but no evidence was obtained that this was the case. In the course of these experiments, it was found that cyclic AMP affected the morphology of developing fruiting bodies.[26,27] Wild-type stocks in the presence of 10^{-3} *M* cAMP produced small, gill-less fruiting bodies. The dominant fruiting body mutation known as bug's ear (*bse*) is phenotypically similar to the fruiting body from the wild-type stock treated with 10^{-3} *M* cAMP. In stocks bearing *bse* treated with cAMP, the stock that was heterozygous for bugs ear (bse^+ bse^+) produced fruiting bodies with smooth, spore-producing hymenial areas on undifferentiated masses of tightly knit hyphae. That is, the cyclic AMP causes stocks heterozygous for *bse* to mimic stocks homozygous for *bse*.

Further studies revealed that cAMP levels were higher in homokaryotic strains and in dikaryotic stocks carrying the *bse* gene. The mature fruiting bodies, with and without *bse*, were higher than mycelia in cAMP. It is thus likely that cAMP is a regulator of morphogenesis in fungi.

An interesting offshoot of the bug's ear experimentation was the consequence of procedures used to make bug's ear coisogenic with other strains. After backcrossing bug's ear for four generations, the isolates bearing *bse* were all monokaryotic fruiters, but the bse^+ strains were not! Because strains with *bse* have high levels of cyclic AMP and are monokaryotic fruiters, it indicates that cyclic AMP is a fruiting inducer in *S. commune* as well as in *C. cinereus*. The proof for this is not positive, however, as the application of cAMP to wild-type strains did not induce fruiting.

VI. SUMMARY

The multicellular fruiting body that we commonly refer to as a mushroom is a relatively simple biological structure; yet it is apparent from the foregoing account that a variety of environmental, nutritional, and chemical factors are involved in its initiation from vegetative mycelium and the various stages of its subsequent development into a mature fruiting body that produces and disseminates basidiospores.

In the next chapter, the genetic factors that bear on the fruiting process are examined.

REFERENCES

1. Arita, I., Teratani, A., and Shione, Y., The optimal and critical temperatures for growth of *Pholiota adiposa*, *Rep. Tottori Mycol. Inst.* (Japan), 18, 107–113, 1980.
2. Bonner, J.T., Kane, K.K., and Levey, R.H., Studies on the mechanics of growth in the common mushroom, *Agaricus campestris*, *Mycologia*, 48, 13–19, 1956.
3. Bromberg, S.K. and Schwalb, M.N., Studies on basidiospore development in *Schizophyllum commune*, *J. Gen. Microbiol*., 96, 409–413, 1976.
4. Corner, E.J.H., The construction of polypores. I. Introduction: *Polyporus sulfureus*, *P. squamosus, P. betulinus* and *Polystictus microcyclus*, *Phytomorphology,* 3, 152–167, 1953.
5. Esser, K., Phenol oxidases and morphogenesis in *Podospora anserina*, *Genetics*, 60, 281–288, 1968.
6. Griffin, D.H., *Fungal Physiology*, John Wiley & Sons, New York, 1981.
7. Hawker, L., Environmental influences on reproduction, in *The Fungi*, Vol. 2, Ainsworth, G.C. and Sussman, A.S., Eds., Academic Press, New York, 1966.
8. Hirsch, H.M., Environmental factors influencing the differentiation of protoperithecia and their relation to tyrosinase and melanin formation in *Neurospora crassa*, *Physiol. Plant.*, 7, 72–97, 1954.
9. Horowitz, N.H., Fling, M., MacLeod, H., and Sueoka, N., A genetic study of two new structural forms of tyrosinase in *Neurospora*, *Genetics*, 46, 1015–1024, 1961.
10. Kitamoto, Y., Horikoshi, T., and Kasai, Z., Growth of fruit-bodies in *Favolus arcularius*, *Bot. Mag.* (Tokyo), 87, 41–49, 1974.
11. Kitamoto, Y., Horikoshi, T., and Suzuki, A., An action spectrum for photoinduction of pileus formation in a basidiomycete, *Favolus arcularius*, *Planta,* 119, 81–84, 1974.

12. Kitamoto, Y., Takahashi, M., and Kasai, Z., Light-induced formation of fruit-bodies in a basidiomycete, *Favolus arcularius* (Fr.) Ames, *Plant Cell Physiol*., 9, 797–805, 1968.
13. Kitamoto, Y., Suzuki, A., and Furukawa, S., An action spectrum for light-induced primordium formation in a basidiomycete, *Favolus arcularius* (Fr.) Ames, *Plant Physiol*., 49, 338–349, 1972.
14. Kitamoto, Y., Horikoshi, T., Hosoi, N., and Ichikawa, Y., Nutritional study of fruit-body formation in *Psilocybe panaeoliformis*, *Trans. Mycol. Soc.* (Japan), 16(3), 268–281, 1975.
15. Klebs, G., Zur Physiologie der Fortflanzung einiger Pilze. III. Allegemeine Betrachtungen, *Jahrb. Wiss. Bot*., 35, 80–203, 1900.
16. Leonard, T.J. and Dick, S., Chemical induction of haploid fruit bodies in *Schizophyllum commune, Proc. Nat. Acad. Sci. U.S.A.*, 59, 745–751, 1968.
17. Lilly, V.G. and Barnett, H.L., *Physiology of the Fungi*, McGraw-Hill, New York, 1951.
18. Lu, S.H., Effect of calcium on fruiting of *Cyathus stercoreus*, *Mycologia*, 65, 329–334, 1973.
19. Madelin, M.F., Studies on the nutrition of *Coprinus lagopus* Fr., especially as affecting fruiting, *Ann. Bot.* (London, New Series), 20, 307–330, 1956.
20. Matthews, T. and Niederpruem, D.J., Differentiation in *Coprinus lagopus*. I. Control of fruiting and cytology of initial events, *Arch. Microbiol*., 87, 257–268, 1972.
21. Niederpruem, D.J., Role of carbon dioxide in the control of fruiting of *Schizophyllum commune*, *J. Bacteriol*., 85, 1300–1308, 1963.
22. Plunkett, B.E., The influence of factors of the aeration complex and light upon fruit-body form in pure culture of an agaric and a polypore, *Ann. Bot.* (London), 20, 563–586, 1956.
23. Plunkett, B.E., The change of tropism of *Polyporus brumalis* stipes and the effect of directional stimuli on pileus differentiation, *Ann. Bot. London*, 25, 206–223, 1961.
24. Schwalb, M.N., Commitment to fruiting in synchronously developing cultures of the basidiomycete *Schizophyllum commune*, *Arch. Microbiol*., 79, 102–107, 1971.
25. Schwalb, M.N., Effect of adenosine 3′,5′-cyclic monophosphate on the morphogenesis of fruit bodies of *Schizophyllum commune*, *Arch. Microbiol*., 96, 17–25, 1974.
26. Schwalb, M.N., A developmental mutant affecting 3′,5′-cyclic AMP metabolism in the basidiomycete *Schizophyllum commune*, *FEMS Microbiol. Lett*., 3, 107–110, 1978.
27. Schwalb, M.N., Regulation of fruiting, in *Genetics and Morphogenesis in the Basidiomycetes*, Schwalb, M.N. and Miles, P.G., Eds., Academic Press, New York, 135–165, 1978.
28. Schwalb, M.N. and Shanler, A., Phototropic and geotropic responses during the development of normal and mutant fruit bodies of the basidiomycete *Schizophyllum commune*, *J. Gen. Microbiol*., 82, 209–212, 1974.
29. Uno, I. and Ishikawa, T., Purification and identification of the fruiting-inducing substances in *Coprinus macrorhizus*, *J. Bacteriol*., 113, 1240–1248, 1973.
30. Uno, I. and Ishikawa, T., Effect of glucose on the fruiting body formation and adenosine 3′,5′-cyclic monophosphate levels in *Coprinus macrorhizus*, *J. Bacteriol*., 120, 96–100, 1974.
31. Wessels, J.G.H., Morphogenesis and biochemical processes in *Schizophyllum commune* Fr., *Wentia,* 13, 1–113, 1965.

8 Mushroom Formation: Effects of Genetic Factors; Breeding

I. INTRODUCTION

It is natural that there has long been interest in fruiting members of the Basidiomycetes, as the mushroom represents the consummation of the life cycle and is thus important in basic scientific studies, including genetics, of these organisms. In addition, the mushroom is the structure that is prized as food in edible species. Most of the early attempts to produce fruiting in culture emphasized nutrition and environmental conditions. As discussed in the previous chapter, these factors are definitely important, and it is certainly an appropriate and reasonable approach to the problem to attempt to reproduce in the laboratory the conditions in nature that exist when and where fruiting takes place.

Two edible fungi whose fruiting bodies are valued for their gastronomic appeal to such an extent that they are farmed by half-culture or semiculture techniques are *Tuber* (truffle) and *Tricholoma matsutake* (the pine mushroom or *matsutake*). Although these have not yet been induced to fruit in culture, probably as a consequence of complex interactions involving their existence in close association with higher plants as mycorrhizal fungi, extensive ecological studies have been performed. As a result of these ecological studies, it is now possible to obtain fruiting bodies of both of these fungi on a regular basis in fair quantity. In the semiculture technique, the higher plant partner is planted along with the mycorrhizal fungus. This must be done in areas in which the edaphic and climatic situations are suitable for fruiting, as revealed by ecological studies.

Many early attempts to fruit various basidiomycetes either resulted in failure or gave inconsistent results. Following the studies of Kniep and of Bensaude, it was recognized that normally it was dikaryotic mycelium that formed fruiting bodies, and so it was assumed that a dikaryotic mycelium provided the proper genes for fruiting body formation. Mycologists thus realized that they should use dikaryotic mycelia for their studies of the nutritional and physiological factors involved in fruiting. Too frequently, however, a single dikaryotic stock was employed in their experimental studies, and it was not generally understood that dikaryons might vary in their capabilities to form fruiting bodies.

II. GENETIC FACTORS FOR FRUITING IMPOSED ON THE MATING TYPE REQUIREMENTS

A. *Schizophyllum commune*

Schizophyllum commune is normally fruited easily in culture. This is one of the reasons that it has become the organism of choice for basidiomycete genetic studies. Shortly after Raper initiated his study of the genetics of the mating type loci, in which dikaryotic stocks from all over the world were employed, it became evident that there was great variation in fruiting ability of different

dikaryons. Preliminary studies indicated that this was probably under genetic control, and the system controlling fruiting was superimposed on the mating type control of dikaryosis.

1. Multigenic Fruiting Factors

Raper and Krongelb[19] reported on an extensive study of fruiting in *S. commune*. The worldwide sample contained 80 homokaryotic (= monokaryotic) strains, which were mated in all possible combinations. This should have resulted in 3160 dikaryons, except that there were 60 cases in which the strains mated had a common *A* or a common *B* factor so that dikaryons resulted in only 3100 matings. These 3100 distinct dikaryons were then studied for their ability to fruit under standard conditions of nutrition and environment. Examination for fruiting was made at intervals of time over a period of 6 weeks.

From observations of fruiting of this large sample of dikaryons, some generalizations could be made:

- Most of the dikaryons fruited during this 6-week period.
- The dikaryons displayed variability in the time of fruiting and morphology of the fruiting bodies.
- Early and abundant fruiting was a characteristic of dikaryons composed of certain strains.
- Late and poor fruiting was a characteristic of dikaryons composed of certain other strains.
- When dikaryons were composed of one strain, which in other combinations gave early and good fruiting, and another strain, which in other combinations gave late and poor fruiting, the fruiting of such dikaryons was frequently good.

It seemed likely, therefore, that fruiting competence was genetically inherited, and that good fruiting was dominant to poor. This hypothesis was tested, and it was found that fruiting competence in *S. commune* was definitely an inherited trait, but it could not be explained on the basis of a single gene for fruiting with alternate dominant and recessive alleles for good and poor fruiting, respectively. The genetic control of fruiting in this fungus is polygenic, with the genes of the "good fruiting" strain masking the genes of a "poor fruiting" strains. Raper and Krongelb categorized the homokaryotic strains used in their study as good fruiting, intermediate fruiting, and poor fruiting. Any mating involving a good fruiting strain would fruit early and well, and any mating involving two poor fruiting strains would fruit either late and meagerly or not at all.

In reviewing this work on the genetic control of fruiting in *S. commune*, Raper[18] summed up the situation in the following way: "The progeny of any dikaryon displays a wide range of fruiting competence, but the average value of competence directly reflects the competence of the parental dikaryon." Because this is the situation with *S. commune*, the basidiomycete that has been most thoroughly studied genetically, and because the basic features of the life cycle of this fungus have been confirmed in other species, it is probable that polygenic control of fruiting is a characteristic of many basidiomycetes, including edible species. The many failures to fruit species by variation of nutritional and environmental conditions may have resulted from use of a poor fruiting dikaryon and not from lack of some exotic fruiting substance or combination of physiological factors. This is a compelling argument for the use of a dikaryotic mycelium that is obtained from the tissue of a fruiting body rather than from the mating of compatible strains. The mycelium obtained as tissue from the fruiting body definitely had the genetic capability to fruit.

2. Morphological Fruiting Mutants

There are a number of morphological fruiting mutants of *S. commune*. Probably the first of these to be reported and analyzed was the mutant "gnarled" studied by Zattler.[28] Gnarled is a one-gene,

TABLE 8.1
Fruiting Body Abnormalities in *Schizophyllum commune*

Mutant Name	Genetic Control	Description	Additional Features
"Gnarled"	One-gene, recessive	Misshapen, twisted	Zattler[28]
"Cauliflower"	Polygenic	Looks like cauliflower in miniature; amorphous mass with hymenium on exposed surfaces	
"Medusoid"	Polygenic	Fruiting bodies are long peglike stalks that may produce gilled structures at the end of the stalk	
"Coralloid"	One-gene, dominant	Resembles brain corals in miniature	Homokaryon bearing this gene is of aberrant morphology and also produces indigo
"Bug's Ear"	One-gene, dominant	Fruit bodies numerous, but each is tiny, has a vertical, ear-shaped fold of tissue bearing the hymenium on one side	

recessive trait that in the homozygous condition produces fruiting bodies that produce spores, but the fruiting bodies are greatly malformed, or, as the name states, gnarled.

Numerous other aberrant fruiting body forms occur in *S. commune*. Those that have been described by Raper and Krongelb[19] and their genetic control are listed in Table 8.1. It can be noted that these abnormalities of the fruiting body structure include both recessive and dominant single-gene mutations and mutants under polygenic control. As indicated in the previous chapter, some have been useful in morphogenetic studies.

B. *LENTINULA*

Fruiting body abnormalities have been reported in *Lentinula edodes*, by Komatsu and Kimura,[10] but these have been less extensively studied than in *S. commune,* because *L. edodes* does not fruit as readily or as quickly on agar medium. We suggest that, although there is apparently little restriction of fruiting of dikaryons inoculated in bed-logs for woodlot cultivation, the requirements for fruiting in the laboratory may vary in a manner similar to that for *S. commune.* In support of this concept, we offer these experimental findings from our laboratory.

1. Various Stocks Display Fruiting Differences

Ten different stocks of *L. edodes* of diverse origin were used to study fruiting in chemically defined liquid media of various formulations, both with and without the presence in the containers of organic or inorganic substances for the support of the developing fruiting bodies. Some of these media nutritionally supported the formation of primordia and/or further development of fruiting bodies of certain stocks. Of the ten stocks, primordia were formed on six, and fruiting bodies developed on five of these. There was a difference in the time of fruiting body formation, which indicated that there may be a similarity with *S. commune* in that some strains are good fruiters, some intermediate fruiters, and some poor fruiters. However, it should be noted that the same strain was not a member of more than one dikaryotic stock in this sample; and the testing of the assumption of similarity to *S. commune* in regard to the genetic control of fruiting requires the use of dikaryotic stocks in which the same homokaryotic strain is included in the constitution of several different dikaryons.

For breeding work in edible species it is desirable to know how fruiting is controlled — genetically as well as nutritionally and physiologically.

III. GENETICS OF FRUITING OF *POLYPORUS CILIATUS*

A. No Subunits of Incompatibility Factors

Polyporus ciliatus is a tetrapolar basidiomycete. The genetic control of fruiting of this fungus has been studied by Esser and Stahl.[8] An interesting difference exists between the structure of the mating type factors of *P. ciliatus* and *S. commune* (and most other tetrapolar species that have been studied in this way) in that there were no subunits of the mating type factors found in *P. ciliatus*. The sample size of *P. ciliatus* monokaryons was sufficiently large (526 monokaryotic strains) to have detected subunit recombination, if it existed, with a map unit distance equal to or greater than 0.2. This map distance is less than that reported for the subunits of *S. commune*.

B. Genetic Control of Monokaryotic Fruiting

Dikaryotic mycelium of *P. ciliatus* normally fruits under appropriate conditions of nutrition and environment. However, in this species, as well as many others, certain monokaryotic strains may also fruit. Esser and Stahl[8] report that approximately 60% of all the single-spore, monokaryotic, isolates of *P. ciliatus, P. brumalis*, and *Agrocybe aergerita* behave as monokaryotic fruiters. Although these monokaryotic fruiting bodies have abnormal shape, most of them do form basidia. These basidia are two-spored, however, rather than the typically four-spored basidia of dikaryotic fruiting bodies. There is no meiosis in the monkaryotic basidia — just a single mitotic division.

The nuclear events in *P. ciliatus* are interesting. In the monokaryotic basidium, following the mitotic division, the two nuclei migrate, one each into the young basidiospores. In each basidiospore a mitotic division of the nucleus occurs, and one of the daughter nuclei from each spore moves back to the basidium. In dikaryotic basidia, the events are similar in that a mitotic division takes place in each basidiospore, and one nucleus from each basidiospore moves back into the basidium.

The important feature to realize here is that **monokaryotic fruiting does not involve sexuality**. Neither plasmogamy, nor karyogamy, nor meiosis is required. Monokaryotic fruiting is strictly a vegetative phenomenon.

By invoking the sexual cycle, however, Esser and Stahl[8] were able to study the genetic control of monokaryotic fruiting. They classified the monokaryotic isolates into various categories ranging from nonfruiting (Group VI), i.e., without any morphological evidence of fruiting body structures, to isolates that had a stroma (Group I), to those that had primordia without hymenia (Group II), to those that had stipes with hymenia (Group III), to some that produced small fruiting bodies (Group V), and finally some that had resupinate hymenia, i.e., lacked stipe and cap (Group IV). From those groups that produced spores (Groups III, IV, and V) single spores were isolated, and the following information resulted:

1. Germination of spores from monokaryotic fruiters was lower than that of spores from dikaryotic fruiters.
2. Descendants were phenotypically the same as the parental strain.
3. All progeny had the same mating type as the parental strain.

Crosses between a nonfruiter (Group VI) with compatible representatives of some of the other groups were made, and analyses of the crosses have permitted the following generalizations:

1. Monokaryotic fruiting is the result of a single gene (definite segregation patterns occurred in all crosses).

2. A single gene is present for monokaryotic fruiting initiation. The allele *fi*$^+$ is present in monokaryotic fruiters; the allele *fi* is present in nonfruiters.
3. Because crosses between a nonfruiter and a small fruiting body strain produced two fruiting phenotypes (stipe and small fruiting body), it appeared that another gene is present. This gene was called *fruit body* with the allelic designation (*fb*$^+$*/fb*).

Segregation patterns indicated that the two genes (*fi*$^+$*/fi*) and (*fb*$^+$*/fb*) are unlinked. Thus, the phenotypes and genotypes of this cross are

Parentals		Recombinants	
Small fruit bodies	*fi*$^+$ *fb*$^+$	Stipe	*fi*$^+$ *fb*
Nonfruiter	*fi fb*	Nonfruiter	*fi fb*$^+$

From this it can be seen that *fb*$^+$ operates only in the presence of *fi*$^+$. The allele *fi*, as the name implies, initiates the monokaryotic fruiting process, and the allele *fb*$^+$ is responsible for the morphology.

The cross of a nonfruiting strain with a strain of Group I (stromatic proliferations) produced a segregation pattern, which called for a third gene, to be operative. This gene was unlinked to either of the other two, and was called *modifier*, with the allelic designation (*mod*$^+$*/mod*) because it interacted with both alleles *fi*$^+$ and *fb*$^+$. The genotype of the strains designated *stromatic proliferations* was *fi*$^+$*fb*$^+$*mod*$^+$.

The findings expressed previously were originally set forth as hypotheses to explain the data, but were confirmed by backcrosses and test crosses by Stahl and Esser.[23]

To review, the genetic control of monokaryotic fruiting in *P. ciliatus* may be summed up as follows:

1. Monokaryotic fruiting is initiated by *fi*$^+$.
2. In the presence of *fi*$^+$, the allele *fb*$^+$ leads to the production of small fertile fruiting bodies (with *fb* the fruiting body is predominantly capless but fertile stipes are formed).
3. In the presence of *fi*$^+$, the allele *mod*$^+$ produces sterile stromatic proliferations even with *fb*$^+$ present.

IV. MONOKARYOTIC FRUITING

A. Species in Which Monokaryotic Fruiting Has Been Reported

Monokaryotic fruiting is not unique to the species for which the mechanism of genetic control, studied by Esser and Stahl, has just been described. The process, which is also known as homokaryotic fruiting or haploid fruiting, has been found to occur in many species. Stahl and Esser[23] list over 34 species, and in Table 8.2 are listed some edible fungi in which monokaryotic fruiting has been reported.

B. Induction

A variety of treatments have been reported to induce the formation of monokaryotic fruiting bodies. Once again, *Schizophyllum commune* has been the organism of choice for much of the experimental work. In this organism the following treatments were found to induce monokaryotic fruiting:

1. Mycelial aging
2. Exhaustion of nutrients
3. Mechanical injury
4. Chemical substances

TABLE 8.2
Edible Species in Which Monokaryotic Fruiting Has Been Reported

Species	Genetic Control	Ref.
Agrocybe aegerita	*fi*$^+$, *fb*$^+$	Esser and Meinhardt[7]
Coprinus macrorhizus (= *C. cinereus*)	Polygenic	Uno and Ishikawa[26]
Flammulina velutipes	One-gene	Takemaru[24]
Mycena spp.		Smith[22]
Pholiota nameko		Arita[1]
Pleurotus flabellatus		Samsudin and Graham[20]
Pleurotus ostreatis		Eger[4]

Leslie and Leonard[13] have studied monokaryotic fruiting initiation in *S. commune* and report that at least two genes are responsible. Esser et al.[9] propose that two genes, *fi1*$^+$ and *fi2*$^+$, are responsible in this organism for initiation of fruiting.

In addition to the fruiting initiator genes, Leslie and Leonard[13] found evidence for a series of four genes for injury-induced fruiting, and two or more genes for fruiting in response to chemical additives. Chemical induction of haploid fruiting bodies in *S. commune* was first reported by Leonard and Dick.[12] A fruiting-inducing substance (FIS) was found in a number of fungi, including the imperfect fungus *Hormodendrum cladosporioides*. FIS was found to be present in abundance in the fruiting bodies of *S. commune*.

C. Relationship with Dikaryotic Fruiting

In *S. commune* the early studies suggested the possibility that because dikaryotic fruiting was under polygenic control, a monokaryotic fruiter might possess a superior complement of genes for good fruiting of dikaryons. When examined in this way, it was found by Raper and Krongelb[19] that there was no relationship between monokaryotic fruiting and good fruiting of dikaryons.

The above findings in *S. commune* differ from the results obtained by Stahl and Esser[23] with *Polyporus ciliatus*. In *P. ciliatus* in the dikaryon ($A \neq B \neq$) monokaryotic fruiting is suppressed, and only normal dikaryotic fruiting bodies are produced. The heterokaryon, which is heteroallelic for the *B* mating type factor (i.e., $A = B \neq$), also suppresses monokaryotic fruiting, but in the heterokaryons that are homoallelic for *B* (i.e., $A \neq B =$, and $A = B =$) the gene *fi*$^+$ for fruiting body initiation is expressed and monokaryotic fruiting occurs. From these and further tests it can be stated that **the expression of the genes responsible for monokaryotic fruiting is suppressed in mycelia that are heteroallelic for the *B* incompatibility factor**.

A further point of interest in reference to the relationship of monokaryotic and dikaryotic fruiting stems from the studies of Esser and Meinhardt[7] on *Agrocybe aegerita*. In *A. aegerita* the *fi*$^+$ allele enhances fruiting body production in the dikaryon, and the *fi* allele may completely inhibit fruiting body production.

D. Potential in Mushroom Cultivation

In both *Pleurotus ostreatus*[4] and *P. flabellatus*[20] a correlation has been shown between monokaryotic fruiting and good yield in dikaryotic fruiting. Because it is relatively simple to screen single-spore isolates for the ability to form fruiting bodies, a breeding program that uses this characteristic has advantages in saving time and effort.

A monokaryotic fruiting strain that has the necessary desirable traits for commercial production, such as taste, texture, yield, etc., would also have some advantages over sexually

fruiting stocks. Simplification of screening and selection procedures in a breeding program involving the use of mutagenic techniques is one advantage. In the case of *Pleurotus* species in which the production of spores in great numbers is a health hazard to workers in the mushroom houses, the lowered spore production by monokaryotic fruiting bodies is also an advantage.

V. BREEDING FOR DESIRED MUSHROOM FEATURES

If we start with a mushroom species that has consumer acceptability on the basis of its taste and texture, and for which fruiting cultures are available, what are the characteristics that might be improved by breeding techniques? Of course, taste and texture are always subject to improvement, but what are some of the other characteristics that we would expect the breeder to consider?

Although nutritional value is probably seldom of primary concern in the mind of the purchaser of mushrooms, the public is becoming increasingly aware of the good nutritional value of mushrooms and their use as a main ingredient of dishes rather than as simply a garnish for various dishes. Thus, breeding for even better nutritional values is a worthy objective.

From the standpoint of the grower, increases in yield will be reflected in greater profits, and in this connection breeding for consistency of yield is important. The grower must be able to supply the mushrooms when there is a demand for them in the market. Thus, yield is not just a matter of the greatest amount, but the breeder must provide fruiting cultures that fruit with regularity and uniformity. These are all characteristics the breeder will wish to introduce into the stocks used for spawn. The spawn has characteristics of its own that the breeder must consider; for example, the form of the mycelium of the spawn, its storage properties, its stability in regard to mutation, and degeneration.

Other goals for the mushroom breeder may be such things as extension of the range of temperature over which the mushroom mycelium will grow and fruit, or improvement of its ability to use various substrates.

A. Extension of Temperature Range

Some species of edible fungi are restricted by temperature regarding where they can be cultivated commercially under natural conditions. A fungus with a requirement for a low-temperature for fruiting, e.g., *Flammulina velutipes*, could normally be cultivated in the tropics only with the expenditure of energy for cooling the buildings in which the mushroom is cultivated.

There is great interest in obtaining high-temperature fruiting stocks of *Lentinula edodes*, so that this mushroom can be grown commercially in warmer climates without having to reduce the temperature by artificial means. There are some cultivars that produce primordia at 22 to 32°C, but in practice the best cultivars seem to be those that require a lower range of temperatures (3 to 12°C) to trigger fruiting. The *Lentinula* mushrooms that are produced in tropical or subtropical regions are either grown in temperature-controlled buildings or in mountainous areas where the temperature is lower, or the fruiting is seasonal and follows a period of low temperature.

The cardinal temperatures for mycelial growth and fruiting are different. Recall Klebs' principle that says in essence that the conditions that are favorable for reproduction are always less favorable for growth. However, good mycelial growth is a prerequisite for good fruiting, and, if mycelial running is not good at high temperatures, good fruiting will not be obtained even if the conditions for stimulation of fruiting are satisfied. Therefore, it is important to study mycelial growth at high temperatures, as well as fruiting, and to devise techniques for selection of high-temperature dikaryotic stocks for both phases.

A study of *Pleurotus* by Wang and Anderson[27] indicates a correlation in time to initiation of primordia and the eventual yield. Using this finding, it should be possible to screen for high-yielding

stock at various temperatures without carrying out an experiment to assay the complete yield from each stock. This could be done with stocks previously determined to grow well at high temperatures. Such stocks might be obtained by mutagenic treatment of mycelium followed by the selection of a high-temperature stock or strain arising from the mutagenically treated mycelium incubated at an elevated temperature.

It is evident that for the edible species there is need for much basic study in such things as the quantitative determination of the combining ability of various monokaryons in dikaryotic growth rate, as has been done by Simchen and Jinks[21] in *Schizophyllum commune*.

B. Utilization of Substrates

1. Use of Waste Substrates

One of the most attractive features in the use of mushrooms as a source of food for humans is that they can be grown on a variety of agricultural, industrial, and household waste materials. The main part of the substrates consists of the polysaccharides cellulose, hemicellulose, and lignin. These are degraded into soluble forms that can be absorbed by the mycelium and then used for the various life processes of the mushroom. This is a saprophytic mode of nutrition. Conversion of the insoluble substrates to soluble materials involves two systems in those cultivated fungi that are produced on compost. One system, which is common to all the cultivated fungi, involves extracellular enzymes produced by the edible fungi. The other system involves enzymes produced by the microflora of the compost. These include both mesophilic and thermophilic bacteria, actinomycetes, and fungi.

2. Increased Yield

The different substrates are composed of polysaccharides that may vary in their linkages and branch points and thus to the readiness with which they will be broken down by the enzymes secreted by the mushroom mycelium. A breeding program that selects stocks with better enzyme activities for the substrate polysaccharides has the potential of increasing yield. The possibility also exists of inoculating the compost with thermorphilic microorganisms that have been selected or even "bioengineered" to break down particular substrates of the compost efficiently.

C. Sporeless Fruiting Bodies

1. Why Desirable?

Because fruiting bodies of *Pleurotus* species shed spores early in the development of the mature mushroom and continue to do so up to harvesting, spore density in the air in mushroom growing houses can become very heavy. As pointed out in Chapter 2, mushroom workers have suffered respiratory tract problems and allergic reactions to spores of *Pleurotus*. For this reason there is great interest in sporeless mutants, and it is a goal of mushroom breeders to produce a sporeless mutant whose fruiting body has qualities equivalent to those of accepted commercial spore-forming stocks in yield, flavor, texture, fruiting time, and nutrient value. Two sporeless cultures of *P. ostreatus*, produced by Eger in Germany, are now commercially available.

There are reports of sporeless mutants in other species:

1. *Pleurotus pulmonarius* by Ohira[16]
2. *Coprinus macrorhizus* (= *C. cinereus*) by Takemaru and Kamada[25]
3. *Schizophyllum commune* by Bromberg and Schwalb[2]
4. *Pleurotus florida* by Chang et al.[3]

TABLE 8.3
Use of Dedikaryotization Procedure to Determine Mode of Inheritance of Trait Appearing in Dikaryon

1. Sporeless stock treated with dedikaryotizing agent
   ↓
   Neohaplont #1 + Neohaplont #2
2. Neohaplont #1 × Neohaplont #2 restores sporeless stock
3. Neohaplont #1 × Compatible WT
   and
   Neohaplont #2 × Compatible WT

Interpretation of results:

1. If either neohaplont #1 or #2 when mated with a compatible wild-type strain gives a dikaryon that produces sporeless fruiting bodies, the character is due to a single dominant gene.
2. If neither neohaplont #1 nor #2 when mated with a compatible wild-type strain gives a dikaryon that produces sporeless fruiting bodies, the sporeless character is probably the result of a single gene recessive mutation.

2. Methods Used to Obtain

The sporeless mutant of *P. florida* arose from a mycelium that came from a mixed basidiospore inoculation. The genetic control of the sporeless mutant is unknown. To perform any genetic analysis of the sporeless mutant, it is necessary to separate out into monokaryotic mycelia the nuclei of the dikaryon of the sporeless stock. Once these two monokaryotic mycelia have been obtained, each must be mated with compatible strains of known genetic constitution for the characters being studied. There are two means by which each nucleus of the sporeless dikaryon can be combined with a nucleus of known genetic constitution for the sporeless character. One method involves dedikaryotization of the sporeless stock; the other involves selection of a nucleus of the sporeless stock by use of the Buller phenomenon, or di-mon mating.

Dedikaryotization is the separation of the dikaryon into its homokaryotic components. These homokaryotic components are commonly referred to as neohaplonts. There are several methods of accomplishing dedikaryotization (also sometimes referred to as monokaryotization or haploidization). Certain chemicals facilitate dedikaryotization when added to the growth medium of dikaryotic mycelium. An example of this is cholic acid.[15] Another method involving a use of a dedikaryotization solution was developed by Leal-Lara and Eger-Hummel.[11] Protoplast isolation from dikaryotic mycelium and the subsequent regeneration of the protoplasts often results in mycelia arising from the dissociation of the component nuclei. Protoplast technology, which is now quite commonly used, has been described carefully in relationship to its utility with edible mushrooms by Peberdy and Fox.[17]

The homokaryotic mycelium will be recognized following dedikaryotization treatment by the absence of clamp connections (the parental sporeless mutant stock of *P. florida*, for example, has clamp connections). If compatible strains that are wild type for the sporeless character are available, they can be mated with the neohaplonts for study of the inheritance of the sporeless stock. The procedure is outlined in Table 8.3.

The use of the Buller phenomenon (di-mon mating) may be relatively simple provided that the mating types of the homokaryons that made up the dikaryon that is sporeless are known, and wild-type strains of these mating types are available. If, however, the mating types of the component nuclei are not known, but the mating type alleles present in the stock are known, and if homokaryotic strains bearing these alleles in all four possible combinations are available, the Buller phenomenon approach can be used, but the analysis is much more complicated.

In this section we have emphasized that in addition to the incompatibility system there are other factors for fruiting that must be considered in breeding programs for edible basidiomycetes.

We have also examined briefly some special topics related to the genetics of fruiting including monokaryotic fruiting, sporeless fruiting bodies, and the role of dedikaryotization and di-mon matings (= Buller phenomenon) in basidiomycete genetic studies. We have also suggested some procedures that might be useful in breeding to extend the temperature range and to make better use of substrates.

D. General Techniques of Breeding for Strain Improvement

Any breeding program for strain improvement of edible mushrooms is dependent on the existence of detailed biological knowledge of the species to be improved. For example, if the life cycle is not well understood so that the breeder can manipulate the fungus genetically, the opportunity for strain improvement is limited to the selection of mutant varieties, either induced by mutagenic techniques or of spontaneous occurrence. Improvement by selection of such varieties was useful with *Agaricus bisporus* even before the life cycle was completely understood, but breeding techniques have not yet brought forth significantly improved strains of the straw mushroom, *Volvariella volvacea*, whose life cycle is still imperfectly understood.

The breeder must know whether the species is homothallic, heterothallic, or secondarily homothallic. Recall from Section IX of Chapter 4 that in homothallic species the mycelium from a single basidiospore will lead to the completion of the life cycle with the formation of the mushroom and its basidiospores, but in heterothallic species the mycelia from single spores are sterile. For the completion of the life cycle and formation of the mushroom, mycelia from compatible mycelia must be brought together. In secondary homothallism, some of the single basidiospores form mycelia that will complete the life cycle; others are self-sterile, but when their mycelia are brought together in compatible combinations, mushrooms may be produced. Genetic manipulation for breeding obviously requires that such details of the life cycle be known.

A clue as to some basic aspects of the biology of the edible fungus may be gained from knowledge of the habitat in which it grows in nature, the substrates that will support that growth, the temperature range under which it will grow and develop into a mushroom, the role of light, if any, and its relationship with other organisms. In short, knowledge of the ecology of the fungus will give very good insight into the biology of the organism and thus its requirements for growth in the laboratory.

In addition to the knowledge whether the life cycle is homothallic or heterothallic, the breeder must also have information regarding the genetic control of sexuality. It will also be recalled from Chapter 6 that in the Basidiomycetes the genetic control of sexuality may be quite complicated, and from what has been presented earlier in this chapter we have seen that the formation of fruiting bodies is under the control of different genes. Furthermore, the genetic control of fruiting has been found to be multigenic in the cases that have been most thoroughly studied. It is important to recognize that there is this requirement of genes for fruiting as well as genes for the control of incompatibility; i.e., dikaryotic stocks may differ markedly in their fruiting competence, and good fruiting competence is an essential final result in any commercial breeding program.

Other desirable basic biological knowledge that the breeder should have about the species would include the nutritional and environmental requirements for both mycelial growth and fruiting. Studies should be made of the utilization of different carbon and nitrogen sources to determine those that provide the best mycelial growth and fruiting, as well as to determine their optimal concentrations for both the mycelial and fruiting phases and the best C:N ratio for the relative amounts for these two elements. In addition to these nutritional factors, optimal conditions for various environmental factors such as temperature, light, moisture, pH, and aeration, for both the mycelial and fruiting phases, should be known.

Unfortunately, several of the most extensively cultivated mushrooms have certain drawbacks to the establishment of breeding programs. *Agaricus bisporus* is a secondarily homothallic

species, which makes controlled matings more difficult to achieve. Breeding advances with *Lentinula edodes* have been slow because of the long time required for fruiting when the log cultivation method is used; but with the extensive use of cultivation methods in which the substrate is placed in plastic bags, the time required for fruiting has been shortened, and the number of genetic studies has increased. As previously mentioned, breeding of *V. volvacea* is limited by uncertainties regarding its homothallic life cycle. The goal of the mushroom breeder, no matter what the species, is to bring together the best possible combination of genes that control characteristics of commercial importance. Elliott[6] has also emphasized that the mushroom breeder's job is more difficult than that of the breeder of a microorganism seeking improvement in the industrial production of a specific metabolite because the microorganism for this purpose need not involve a complex structure such as the mushroom, which, additionally, most commonly results from a sexual process.

There are certain requirements that are essential for the establishment of a mushroom breeding program.[14] One is to have knowledge of the natural breeding system of the fungus, for, if improvement is sought by hybridization of strains with contrasting characteristics, the most straightforward means is to use a sexual reaction between compatible strains. The procedure is to mate the strain with the desired characteristic with a compatible strain of a cultivar with good commercial qualities. Selection and backcrosses with this cultivar will eventually place the characteristic into the genome of the cultivar — provided that the characteristic in question is simply inherited. What will have occurred is recombination through meiosis, which takes place in the basidia of the fruiting body. Thus, fruiting competence is essential and must be considered as another general requirement for breeding.

Because the haploid products of meiosis that bear the desired recombinant genomes are located in the basidiospores (one nucleus per basidiospore), it is essential that the basidiospores germinate if the genetic recombination is to be expressed. Therefore, another general requirement for mushroom breeding is that the basidiospores germinate in high percentage.

At this point we mention that we are giving attention to the methods in breeding of heterothallic species of Basidiomycetes. Because only 10% of the Basidiomycetes are homothallic and the remaining 90% are heterothallic, with 65% bifactorial and 25% unifactorial, it seems desirable to use as an example the methodology of breeding bifactorial, heterothallic species.

1. Establishment of Cultures

From fruiting bodies collected in nature from widespread geographical areas and diverse ecological situations, a large number of pure cultures should be obtained to provide a wide genetic base and variation in agronomically important traits.

2. Maintenance of Cultures

The cultures established for breeding work should be maintained in a manner that will maintain genetic stability to the greatest extent possible. Attenuation of stocks commonly occurs with constant growth of the fungus, so maintenance of cultures by means that minimize growth will also keep genetic changes at a low level. The most effective means of maintaining cultures in this way is to store them in liquid nitrogen (see Chapter 10).

3. Characterization of Monosporous Mycelia

If monosporous mycelia (i.e., mycelia derived from single spores) are to be useful in recombination experiments, their mating types must be known. Mating types are ascertained by the determination of compatible or noncompatible reactions when different monosporous mycelia are confronted in pairs. There are various means for making the mating type determinations, but the general procedure for a bifactorial heterothallic species is the following:

1. Select one isolate as a tester and give it a mating type designation, e.g., *AxBx*, and mate each of the other isolates with this tester (tester 1). Those isolates that give a compatible reaction (indicated in many species by the occurrence of clamp connections) differ in both the *A* and *B* mating type alleles, and so they can be designated *AyBy*.
2. Select an *AyBy* isolate as tester (tester 2) and mate it with all the isolates that did not give compatible reactions with tester 1. Compatible reactions will occur with those isolates whose mating type is *AxBx*.
3. Select an isolate that has not given a compatible reaction with either tester 1 or tester 2 and arbitrarily designate its mating type as *AxBy* (tester 3); then confront tester 3 with all the other isolates that failed to give a compatible reaction with either tester 1 or tester 2. When a compatible reaction occurs in a mating with tester 3, the isolate is of mating type *AyBx*; and when there is an absence of a compatible reaction in such matings, the isolate is mating type *AxBy*. Knowledge of the mating types of isolates is essential for breeding work.

4. Selection of Recombinants

Because the goal of a breeding program is to bring together genes controlling characteristics that are desirable in commercial mushroom production, the selection of recombinants from a mating between different strains, each of which has certain desirable traits, is a reasonable approach. Certain techniques are available to facilitate the selection of recombinants. Genetic markers linked to the genes to be combined can be used, and these may be auxotrophic mutants or even mycelial morphological mutants. Of great promise for breeding cultivated mushrooms is the suggestion by Elliott[5] that two strains with contrasting agronomic characteristics can be brought together in a heterokaryon if each strain is resistant to a different antimetabolite (e.g., a fungicide or antibiotic). This operates because in the presence of the two antimetabolites neither strain will grow, but the hybrid will grow because it is resistant to the two antimetabolites by virtue of the nucleus of strain one being resistant to the antimetabolite to which strain two is sensitive, and vice versa.

Desirable traits for which the mushroom breeder may wish to select mutations, either spontaneous or induced, include such qualities as taste, color, nutritive value, odor of the mushroom, temperature tolerance for both mycelial growth and fruiting, yield, a shortened time from spawning to cropping and disease resistance.

Although there is much interest in the use of modern techniques of genetic engineering to develop improved strains of edible mushrooms, this remains a difficult problem because the mushroom is a complex organism with a range of characteristics, many of which are certainly under multigenic control. Thus, it is still useful to use the natural breeding system, supplemented by such means as parasexuality and protoplast fusion techniques for bringing about genetic recombination.

REFERENCES

1. Arita, I., Studies on the life cycles of *Pholiota nameko* (T. Ito) S. Ito et Imai, I., *Rep. Tottori Mycol. Inst.* (Japan), 6, 49–57, 1968.
2. Bromberg, S.K. and Schwalb, M.N., Isolation and characterization of temperature sensitive sporulationless mutants of the basidiomycete, *Schizophyllum commune*, *Can. J. Genet. Cytol.*, 19, 477–481, 1977.
3. Chang, S.T., Yu, M.L., and Lau, W.C., A sporeless strain in *Pleurotus florida*, *Mushroom Newsl. Trop.*, 5(3), 19–21, 1985.
4. Eger, G., Rapid method for breeding *Pleurotus ostreatus*, *Mushroom Sci.*, 9(Part 1), 567–573, 1974.
5. Elliott, T.J., Breeding strategies in *Agaricus bisporus*, *Mushroom Sci.*, 10(Part 1), 73–81, 1978.

6. Elliott, T.J., Genetics and breeding of cultivated mushrooms, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 11–30, 1952.
7. Esser, K. and Meinhardt, F., A common genetic control of dikaryotic and monokaryotic fruiting in the basidiomycete *Agrocybe aergerita*, *Mol. Gen. Genet*., 155, 113–115, 1977.
8. Esser, K. and Stahl, U., A genetic correlation between dikaryotic and monokaryotic fruiting in Basidiomycetes, *Proc. First Intersect. Congr. IAMS*, 1, 294–300, 1975.
9. Esser, K., Saleh, F., and Meinhardt, F., Genetics of fruit body production in higher Basidiomycetes. 2. Monokaryotic and dikaryotic fruiting in *Schizophyllum commune*, *Curr. Genet.*, 1(1), 85, 1979.
10. Komatsu, M. and Kimura, K., Studies on abnormal fruit-bodies of hymenomycetous fungi. V. Fruit-bodies with white pilei of *Lentinus edodes* (Berk.) sing., *Rep. Tottori Mycol. Inst*. (Japan), 6, 9–17, 1968.
11. Leal-Lara, H. and Eger-Hummel, G., A monokaryotization method and its use for genetic studies in wood-rotting Basidiomycetes, *Theor. Appl. Genet.*, 61, 65–68, 1982.
12. Leonard, T.J. and Dick, S., Chemical induction of haploid fruit bodies in *Schizophyllum commune*; Genetic control of the response to mechanical injury, *Proc. Natl. Acad. Sci. U.S.A*., 59, 745–754, 1968.
13. Leslie, J.F. and Leonard, T.J., Monokaryotic fruiting in *Schizophyllum commune*, *Mol. Gen. Genet*., 175, 5–12, 1979.
14. Miles, P.G. and Chang, S.T., Application of biotechnology in strain selection and development of edible mushroom, *ASEAN Food J*., 2(1), 3–10, 1986.
15. Miles, P.G. and Raper, J., Recovery of the component strains from dikaryotic mycelia, *Mycologia*, 48, 484, 1956.
16. Ohira, I., Sporulation-deficient mutant in *Pleurotus pulmonarius* Fr., *Trans. Mycol. Soc*. (Japan), 20, 107–114, 1979.
17. Peberdy, J.F. and Fox, H.M., Protoplast technology and edible mushrooms, in *Genetics and Breeding of Edible Mushrooms*, Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach, Philadelphia, 125–147, 1993.
18. Raper, J.R., *Genetics of Sexuality in Higher Fungi*, Ronald Press, New York, 1966.
19. Raper, J.R. and Krongelb, G.S., Genetic and environmental aspects of fruiting in *Schizophyllum commune* Fr., *Mycologia*, 50, 707–740, 1958.
20. Samsudin, S. and Graham, K.M., Monokaryotic fruiting in *Pleurotus flabellatus* (Berk. et Br.) Sacc., *Malays. Appl. Biol*., 13(1), 61–65, 1984.
21. Simchen, G. and Jinks, J.L., The determination of dikaryotic growth rate in the basidiomycete *Schizophyllum commune*, a biometrical analysis, *Heredity*, 19, 629–649, 1964.
22. Smith, A.H., Investigations of two spored forms in the genus *Mycena, Mycologia*, 26, 305–311, 1934.
23. Stahl, U. and Esser, K., Genetics of fruit-body production in higher Basidiomycetes. I. Monokaryotic fruiting and its correlation with dikaryotic fruiting in *Polyporus ciliatus*, *Mol. Gen. Genet*., 148, 183–197, 1976.
24. Takemaru, T., Genetical studies on fungi. IX. The mating system in *Lentinus edodes* (Berk.) Sing., *Rep. Tottori Mycol. Inst*. (Japan), 1, 61–68, 1961.
25. Takemaru, T. and Kamada, T., Gene control of basidiocarp development in *Coprinus macrohizus*, *Rep. Tottori Mycol. Inst*. (Japan), 9, 21–35, 1971.
26. Uno, I. and Ishikawa, T., Effect of glucose on the fruiting body formation and adenosine 3*N,*5*N*-cyclic monophosphate levels in *Coprinus macrorhizus*, *J. Bacteriol*., 120, 96–100, 1974.
27. Wang, S.S. and Anderson, N.A., A genetic analysis of sporocarp production in *Pleurotus sapidus*, *Mycologia*, 64, 521–528, 1972.
28. Zattler, F., Verebungsstudien an Hutpilzer (Basidomyceten), *Z. Bot.,* 16, 433–439, 1924.

9 Mushroom Formation: Effect of Pests and Diseases in Mushroom Cultivation

I. INTRODUCTION

Although we have no historical documentation of early human use of mushrooms collected in the wild as foods, tonics, or medicinals, such usage is generally accepted *a priori*. Concerning the intentional cultivation of mushrooms, we do have historical information. This is described in more detail in later chapters, but some of this information is relevant to our present concern with pests and diseases in mushroom cultivation and is presented now.

A. History of Mushroom Cultivation and Diseases

1. Outdoor Cultivation

The first mushroom to be intentionally grown (cultivated) was probably *Auricularia auricula* circa A.D. 600, followed by *Flammulina velutipes* circa A.D. 800. The historical record for *Lentinula edodes* is much more extensive and indicates that *L. edodes* was first cultivated circa A.D. 1000 according to the famous *Chinese Book of Agriculture* published in 1313. These mushrooms were cultivated outdoors without the use of a specially prepared spawn.[9]

By A.D. 1600, *Agaricus bisporus* was cultivated in France; other mushrooms cultivated outdoors were *Ganoderma* in 1621, *Volvariella volvacea* in 1700, and *Tremella fuciformis* in 1800. In these cases a spawning might involve the placing of fruiting bodies on the substrate, or, as in the case of *Agaricus,* the insertion of small masses of infected manure from the producing mushroom beds onto fresh manure. This spawning technique for *Agaricus* was developed in the latter part of the seventeenth century.[62]

2. Indoor Cultivation with Pure Culture Spawn

Later developments in mushroom cultivation included the growing of mushrooms indoors and the use of a pure culture spawn. A pure culture spawn contains only living mycelium of the desired mushroom species. Obviously, the block of manure containing the mycelium of *Agaricus* that was used for spawning would contain a whole host of other microorganisms, which could interfere with the development of the mycelium and fruiting bodies of *Agaricus*. Thus, there would be advantages to using pure culture spawn. As described by Elliott,[17] pure culture mushroom spawn for *Agaricus* was first achieved in 1886 in the United Kingdom, in 1894 in France, and in 1902 in the United States. These spawns were produced from germinating spores. In 1905, Duggar[15] produced spawn from mushroom tissue. This had the advantage that a particular strain could be maintained for use as spawn.

A consequence of the advent of pure culture spawn was the opportunity to recognize mushroom diseases. At various stages of mushroom production, such as composting, spawning, spawn running,

and mushroom development, there are opportunities for the organisms to enter and grow in the substrate, or on the mycelium, or on the developing sporophore, and these occurrences consequently cause diseases. Included here are viruses, bacteria, fungi, nematodes, and insects. The study of diseases of mushrooms has thus become a very important aspect of mushroom research, because these diseases can cause decreased yield and even loss of the crop with the subsequent loss of income to the growers. Additional costs involve the measures that are necessary to prevent diseases.

II. VIRAL DISEASES

A. History

An excellent account of the virus diseases of *Agaricus bisporus* by Atkey[2] is in *The Biology and Technology of the Cultivated Mushroom*, edited by Flegg et al., published in 1985. Atkey points out that the conditions in mushroom cultivation after World War II brought forth substantial increases in yields over those pre-war yields, but this also set the stage for the spread of diseases. Large farms, which used similar substrates, techniques, and common mushroom strains, provided the conditions by which a contaminating organism could enter, grow, and reproduce on the substrate, mycelium, or fruiting body of the mushroom. Such a situation occurred in Pennsylvania in 1948 when a farm suffered severe damage from a disease whose symptoms included a brown coloration of the stipe and waterlogged patches of the diseased beds. This latter condition was responsible for the watery stipe that was also a symptom of the disease. When it was first reported in Pennsylvania, it was called the LaFrance disease after the owners of the farm on which it first appeared. In England, the disease was known as "brown disease" because of the browning of the stipe. The name "watery stipe" was also used, and "x" disease was used to indicate that the cause of the disease was unknown. Studies soon indicated that there was an infectious agent for this disease. Elimination of the other infectious agents turned the search in the direction of viruses. In 1962, Gandy and Hollings[28] described what they called the "die-back" disease because the dead areas on the mushroom bed increased in size with time. The important thing, however, was that they demonstrated by using electron microscopic studies, that mushrooms from infected beds contained some isodiametric viruslike particles. It had previously been shown by Gandy[26] that when healthy mushroom beds were inoculated with mycelium from diseased beds, the die-back symptoms appeared. Now it was demonstrated that partially purified virus material from infected mushrooms produced the die-back symptoms when inoculated into healthy beds.

B. Symptoms

Viral diseases may be difficult to detect by the symptoms displayed, but detection is fairly easy in extreme cases. An example of this is when all sporophore initiation is inhibited and the vigor of the mycelium is greatly reduced. There are a number of factors that influence the symptoms. These include the strain of spawn used, the cultural conditions employed in the mushroom house, the time of infection, and especially the concentration of the virus. Detected most easily are the symptoms expressed on individual fruiting bodies. The appearance of these individual, diseased sporophores has given use to some colorful names. The discussed sporophores are sometimes referred to as "drumsticks" or "German helmets." In these sporophores, the cap or pileus is of smaller diameter than that of the normal fruiting body, and the stipe may be elongated and curved. There are other forms that the sporophore may take. For example, there may be an enlarged barrel-like stipe which may have brown patches on it, or patches of the stipe or pileus may become waterlogged. The diseased sporophore has a distinctive coloration: grayish white rather than pinkish white. Reduction in weight of the diseased mushroom is another characteristic. Also, there is a sparse mycelial attachment of the diseased mushroom to the substrate, which may result in the toppling over of the mushroom. Atkey[2] points out that the occurrence of foul-smelling mushrooms

may actually be the consequence of bacterial rotting. Not only viral infections cause the sporophore malformations, but they may be caused also by genetic or cultural factors so that the appearance of a misshapen sporophore alone should not lead one to conclude that a virus infection is present.

Symptoms of viral infection may be expressed by reduction in mushroom number and size. This reduction is augmented by an increase in virus concentration. Progression of the disease results in bare patches on the substrate, and the area of these bare patches increases with successive flushes.

C. Diagnosis

It should be emphasized that virus diseases cannot be identified reliably by just the symptoms. The symptoms may result from other stimuli. For example, although viral infection may cause an elongation of the stipe, such elongation is also caused by high carbon dioxide concentration. This is a treatment that is used by the growers of *Flammulina velutipes* to obtain the commercially desirable feature for this mushroom of a long stipe and a small pileus. Overcrowding of mushrooms on the bed also causes stipe elongation. Waterlogging of the stipe tissue is another symptom of viral infection, but this can also be caused by growing uninfected mushrooms in excessive moisture. Loss of yield and bare patches (viral symptoms) may also be the result of poor compost or even the activity of mice on the beds.

In diagnosing a mushroom viral infection, there are several approaches that must be taken. First, any symptoms on the mushroom bed such as bare patches must be noted. Next, it is necessary to determine in the laboratory the growth characteristics of the putative virally infected mushroom. This can be done by making a tissue culture of that mushroom and growing the mycelium on a mycological nutrient agar. A comparison is then made between this mycelium and that from a healthy, uninfected mushroom. Differences in growth, color, amount of aerial mycelium, texture, and presence of hyphal strands (rhizomorphs) might indicate infected tissue, but these criteria are all qualitative and involve subjective evaluations. Small differences might indicate slight infection or absence of infection, while large differences would suggest the presence of an infection. This test takes time and may not be useful for practical reasons. Electron microscopic demonstration of virus particles is essential for proof of a viral infection. Because mushroom farmers do not have electron microscopes, this cannot be used as a routine diagnostic tool. However, some techniques have been developed that are useful. Hollings et al.[39] have demonstrated that, when diseased mushrooms were subjected to ultrasound treatment and the juice examined by the electron microscope, the virus particles could be identified more rapidly. With higher concentrations of viral particles present, the test was more effective. A modification of this method was to express the juice of the mushroom through a very fine cloth. Consequently, there was less cellular material in the juice, which was then prepared for electron microscopy by staining and drying on a support grid. This procedure took only 5 minutes from the time the specimen was received to the examination by electron microscopy, but there was still some difficulty if the concentration of viral particles was low.

A further advancement in electron microscopic determination of viral infections in mushrooms was the technique known as serum-specific electron microscopy (SSEM), which had been developed by Derrick.[13] In this method antibodies are prepared that bind to the virus, which acts as an antigen. Mushroom viruslike particles of *Agaricus bisporus* were used by DelVecchio et al.[12] as antigens for serum-specific electron microscopy. A specimen support grid was coated with an experimentally determined dilution of the antibody. The antibody on the grid acted as an adhesive, which specifically attracted the mushroom viruses when the grid was floated on mushroom sap. The result was that the virus particle became attached to the specimen grid. Extraneous debris from the mushroom sap could then be washed away, as it was not attached to the grid by an antigen–antibody reaction. This is a much more efficient procedure for obtaining virus particles for electron microscopic examination than the previously described method of expressing the mushroom juice through fine cloths. Atkey[2] indicates a 1000-fold improvement. He also reported that as many as 80 specimens

could be prepared in a day for electron microscopic examination. This makes it a practical procedure for the rapid determination of a viral infection in the mushroom beds.

D. Virus Morphology

By 1962, Hollings[38] had described three morphologically distinct mushroom virus types from electron microscopic studies:

1. Isodiametric particles of 25 nm diameter
2. Isodiametric particles of 29 nm diameter
3. Bacilliform particles with diameter of 18 mm and a length of 50 nm

Subsequently, additional types of viral particles were found: one that was isodiametric with a diameter of 35 nm; another that was club shaped, 120 to 170 nm long with a spherical body of 40 to 50 nm and a cylindrical tail 20 to 30 nm in diameter. It is evident that viruses of various sizes and morphologies can be present in mushrooms, but some types are more frequently encountered than others.

E. Epidemiology

Proper sanitation methods in cultivation can help prevent the spread of viral diseases. Evidence for the spread of virus infections by insects, fungi, or nematodes is inconclusive, but the knives used by pickers have been known to carry virus infections from one house to another. It is also known that infected mycelium can infect healthy mycelium through hyphal anastomosis. Following fusion (hyphal anastomosis), there occurs an exchange of cytoplasm in which the virus particles are located. This leads to viral infection of the healthy mycelium. Here again, better hygiene or stricter adherence to sanitary procedures in the growing houses can help prevent the spread of the viral infection. The routine practice of sterilization of the mushroom beds at the conclusion of cropping has been shown to decrease the number of infections. Atkey[2] reported that "the widespread adoption of end of crop sterilization has virtually eradicated this [the 29 nm] particle."

Other mushroom particles were not eliminated by crop sterilization, however, indicating that there were some other methods by which the viral particles were transmitted. The basidiospores were soon implicated in this role. Schisler et al.[59] had already demonstrated that the spores from fruiting bodies growing on the diseased trays were capable of transmitting the virus to healthy, non-infected trays. It is well known that a mushroom can produce millions of spores. This means that a single diseased mushroom can spread infected spores very widely. Moreover, the presence of viral particles in basidiospores has been demonstrated by electron microscopy. The virally infected spores germinate when they land on the mycelium. (Although the spores of *A. bisporus* are notoriously poor germinators, there is good experimental evidence that germination is greatly increased in the presence of growing mycelium.) Germination of the spore leads to the development of mycelium that bears the infected viral particle. As previously stated, the infected hyphae can anastomose with healthy hyphae and contaminate the previously healthy mycelium with the virus. Infection by virus-bearing basidiospores is especially serious when the spores land on the bed prior to casing.

Virus-infected basidiospores may be responsible for the sudden outbreaks of infection on mushroom farms. Basidiospores of many species, including *A. bisporus*, may remain viable for years. This is true of spores with a virus. Such spores may become airborne and spread to healthy mushroom beds following disturbances on the mushroom farm, such as a removal of a spent compost heap that has been kept on the farm for too long, or by construction or repair activities in the mushroom house. Any activity that stirs up spores that had lain dormant in undisturbed areas could lead to an outbreak of infection.

There have sometimes been accusations by farmers that an outbreak of viral infection on the beds was due to virus-contaminated spawn. It is unlikely that such claims have merit because the spawn maker requires strong, rapid mycelial growth in the grains used in spawn production, and this would not occur in the virus-infected material. Good spawn makers adhere rigorously to sterile techniques in the production of spawn, and they would be well aware of the consequences of the breakdown of such procedures in their production procedures. Transmission of viral infection by spawn from a spawn grower would be immediately evident by the occurrence of the disease on various farms that had used spawn from that manufacturer.

There are some mushroom strains which will not anastomose with other strains. Failure of anastomosis means that a virus-infected strain cannot transmit the virus to the strain with which it will not fuse. This feature has permitted spawn growers to produce a spawn that will not be subject to virus attack. A farm that has been infected by a virus can be freed of this virus by the use of such a "virus-breaking" spawn. However, the cultivar that had become diseased may have had certain attributes that made its use by the farmers very desirable, and the virus-breaking spawn may be deficient in these desired features. Thus, a return to the original cultivar must be delayed until there has been a complete cropping cycle with the virus-breaking spawn. Van Zaayen[65] has shown that *A. bitorquis* is not infected by the viruses of *A. bisporus*. Therefore, the infection cycle of *A. bisporus* can be interrupted by growing *A. bitorquis*, a mushroom with good marketable features. Our previous discussion of viral transmission by basidiospores should make us keep in mind that virus-contaminated basidiospores may be present in the mushroom houses. This problem must be dealt with by careful hygiene and sanitation methods before introducing a susceptible spawn into the cultivation beds.

F. Patch Disease

Although adherence to strict hygiene has curtailed the losses that occurred when LaFrance disease was first discovered, a disease with symptoms similar to LaFrance disease appeared in Britain in 1996. This became known as "patch" because noncropping patches of nonproductive casing appeared in the production beds. Initially, after spawning, the development appears normal and the mycelial running into the casing is vigorous. Pinning may even be normal, but in the patch the pins do not develop further. At the margins of the patch regions there may be an overproduction of normal mushrooms. The development of these mushrooms may be delayed a day or two. Sometimes the patches are not present until the second or third flush.[29] The distribution of the patches is random but variable in appearance. Fletcher[23] describes the patches appearing as swirls, stripes, and more or less circular areas. The symptoms of patch disease have some similarities to the LaFrance virus infection, but the LaFrance virus has not been found in the edge of the patches, nor have new virus particles been found.[23] The bacterial disease known as "mummy disease" also has similar symptoms to patch, such as slow growing of the development of the crop, making the identification of an etiologic agent more difficult in that there do not appear to be any factors that are common to all cases of patch. Gaze[29] indicates that there is an exception to this statement about lack of common factors in patch disease**,** and this is the presence of a novel double-stranded RNA in diseased mushrooms, but its absence in healthy ones. Studies by Romaine and Schlagnhaufer[53] involving polymerase chain reaction analysis of RNA genetic elements in *A. bisporus* suggest that the isolated viruslike particles are extrachromosomally replicating viral elements. The absence in the genomic DNA of sequences corresponding to the RNA elements studied (LaFrance isometric virus or LIV, mushroom bacilliform virus or MBV, and vesicle-associated double-stranded RNA or VA-2.4) is the basis for suggesting an extrachromosomal mode of replication. The significance of the presence of double-stranded RNA is that double-stranded RNA is believed to be exclusively of viral origin.[29]

Although there have been several outbreaks of patch disease in Britain, its presence in the United States had not been reported as of the end of 2000. Patch disease remains an area of vigorous

research, especially in Britain. In the absence of definitive information about the cause of patch, the best course of action for farmers to minimize losses is to exercise strict hygiene practices on their farms.

III. BACTERIAL DISEASES

A. Various Mushroom Diseases

Bacteria play an important role in the composting process and are ubiquitous in nature. It is thus not surprising to find bacteria present in various aspects of mushroom farming, and it is reasonable to anticipate that under certain conditions they might behave as pathogens in mushroom cultivation.

1. Blotch Disease

A disease known as "bacterial blotch" was first described in 1915.[61] The symptoms of this disease are the appearance of irregularly shaped spots and blotches on pilei and sometimes on stipes. The blotches are dark brown and sunken. The responsible organism was first identified as *Pseudomonas fluorescens*, but some studies have suggested that blotch is caused by a different species, which has been named *P. tolaasii*.

Excessive moisture and the presence of water on the fruiting bodies promote the development of blotch symptoms. Management of the humidity and watering of the beds in the mushroom houses are crucial factors in controlling blotch. Sporophores may carry the bacteria and be free of blotch symptoms until environmental conditions bring about condensation of water on the mushroom, which may then lead to a sudden outbreak of the disease. The damage to the mushrooms by blotch is superficial with the bacteria being located extracellularly between hyphae and covered by a film formed from bacterial mucilage and the cell contents from damaged mushroom cells. The sunken spots result from the collapse of the surface hyphae following their plasmolysis. The bacteria are clustered in the interhyphal spaces, and their presence there has been demonstrated by electron microscopic scanning.[27]

Studies by Murata and Magae[45] have shown that *P. tolaasii* produces extracellular toxins that induce typical blotch disease symptoms. It was found that trehalose, an abundant sugar in *Pleurotus ostreatus*, stimulates toxin production in the strain of *Pseudomonas tolaasii* which they researched. They suggest that signals from the bacterial cells and substances in *Pleurotus ostreatus* activate the toxin production that is required for pathogenicity.[45]

2. Mummy Disease

A mushroom disease named "mummy disease" has bacteria that are regularly associated with the diseased mushrooms and with the rhizomorphs associated with them.[64] The symptoms displayed include an area on the bed in which the mushrooms are smaller than normal and, more distinctly, pilei that are tilted at an acute angle. The veil will break prematurely and then the mushrooms become pale brown and mummify — hence the name mummy disease. There are some claims that the disease has been produced from a bacterial inoculum, but Royse[56] was unable to produce the disease with the "mummy" bacterium. However, mummy disease can be transmitted by transferring compost and mushroom mycelium from a diseased area to a healthy bed. Such a procedure is a means of diagnosing mummy disease.

3. Drippy Gill Disease

Another disease of *Agaricus bisporus* is known as "drippy gill." In this uncommon disease, caused by *Pseudomonas cichorii,*[4] masses of bacteria develop on the gills, making them become gelatinous, after which they collapse.

4. Brown Center Rot Disease of Shiitake

With increased production of specialty mushrooms (e.g., *Lentinula*, *Pleurotus*, and *Flammulina*), it is not surprising that ever-present bacteria have been found to cause damage to these mushrooms. A disease called "brown center rot" has appeared on shiitake (*L. edodes*) mushrooms grown on sawdust-containing blocks after removal of the plastic bag that contained the sawdust substrate. The name brown center rot is derived from a principal symptom of the disease. This symptom is a brown sunken lesion in the center of the cap. The lesion may develop into a pit that extends into the stipe. Other symptoms include an enlarged hollow stipe that turns brown near the cap. Frequently, the attachment of the cap to the stipe is so weakened that the cap becomes easily detached when it is touched lightly. When the cap, which matures early and is small, is examined on the underside, it can be seen that the gills turn brown from the stipe outward. Donoghue et al.[14] have identified the causal organism of brown center rot as the bacterium *Burkholderia gladioli* pv. *agaricicola*, for convenience referred to as *Bga*, and they demonstrated that the disease could be caused in healthy tissue by inoculation with a pure culture of the bacterium.

Substrate blocks of shiitake become diseased only when the bag has been removed and the block placed in an infected room, or in the tank in which the blocks are water-soaked. The purpose of the water soaking is to lower the temperature and hydrate the blocks, a procedure that stimulates pin formation and fruiting. The bacteria grow on the block surface, and the mushroom becomes infected when the pin breaks through the mycelial coat or skin formed on the surface of the block. The cells of *Bga* increase in number when there is a film of water on the block. The bacterial cells can swim in this film and thereby spread the infection from place to place. When an infected block is subjected to drops or a stream of water, the water splashed off the block can carry with it the *Bga* bacterial cells. The bacterial cells in this splash water are capable of infecting fresh, uninfected blocks.

5. Mushroom Soft Rots

Bga also has been found to be responsible for bacterial soft rot in *Agaricus bitorquis* and to do damage to several cultivated mushroom species in Japan, including species of: *Lentinula*, *Pleurotus*, *Flammulina*, *Grifola,* and *Hypsizygus.*[31] *Bga* does not produce resistant spores and consequently the bacterium does not survive the usual methods of sterilization of the substrate.

Fermor and Lincoln[18] have described bacterial "soft rot diseases" of mushrooms caused by both *Bga* and *Janthinobacterium agaricidamnosum*, which may attack *Agaricus* spp. and specialty mushrooms. They describe these soft rot diseases as constituting "an uncommon but worrying phenomenon." On this basis we now give a brief account of these soft rot diseases.

The desire to have mushrooms that fruit at higher temperatures than the customary 15 to 17°C used for fruiting of *Agaricus bisporus* created interest in *A. bitorquis*, which fruits at 22 to 24°C and has good mycelial running at 28 to 30°C. A soft rotting bacterium was isolated from *A. bitorquis* and was identified as *Bga*. Mushrooms intentionally infected with this organism displayed extensive browning and soft rot of sporophore tissue and stipe. Subsequently, there was the development of oozing lesions from the deeper tissues. It has been shown experimentally that tissue degradation of *A. bitorquis* by this organism is temperature sensitive, with degradation beginning after 18 hours and greatest at 28 hours at 28°C. Outbreaks of mushroom soft rot of *A. bitorquis* in the United Kingdom and in New Zealand showed similar symptoms and were due to the same causal organism. Infection is accelerated once the outer layer (pileipellis) or "skin" of the mushroom cap is broken and the bacteria are free to enter the inner tissue of the cap.

Devastating losses of a brown strain of *A. bisporus* due to the soft rot have occurred on mushroom farms in the United Kingdom and in France. The symptoms are similar to those described for *A. bitorquis* soft rot but may be more severe for *A. bisporus*, in that the mushrooms may dissolve completely within 2 days after the appearance of the symptoms. A different bacterium, *Janthinobacterium agaricidamnosum*, is the causal agent of this soft rot.

The two soft rots just discussed involved species of *Agaricus*, but with increasing growth of specialty mushroom species the occurrence of bacterial soft rot on *Pleurotus ostreatus* and *Lentinula edodes* has been demonstrated,[63] as well as on other specialty mushrooms, as noted previously. These soft rots were caused by *Bga*, but *J. agaricidamnosum*, as mentioned previously, has also been shown to cause soft rot on some of these species.

B. Management for Control of *Burkholderia gladioli* pv. *agaricola*

Donoghue et al.[14] have discussed at some length management strategies that may be useful in preventing or minimizing the incidence of infection by *Bga*. Much of what follows is based on this account.

First and foremost good farm hygiene must be maintained. Very important in regard to hygiene is the prompt and careful removal of infected blocks and diseased mushrooms from the fruiting room. A routine procedure that surface-disinfects floors, walls, racks, carts, and air-handling equipment must be adhered to rigorously. Avoidance of contamination of new blocks can be accomplished by use of separate cropping rooms, or, at the very least, separating new blocks from old ones. The fruiting rooms should be kept as dry as possible, and the blocks should be dried prior to soaking to reduce the bacterial population on the block. When the blocks require watering, efforts should be made to establish conditions that allow the block surfaces to dry quickly. Useful in accomplishing this are procedures to increase air circulation, ventilation, heating, or the use of dehumidifiers.

The soak tank is a very likely place for the spread of infection because the bacteria from infected blocks will be washed into the soak water. These motile bacteria can then readily swim to uninfected blocks. Therefore, it is a useful management procedure to soak new blocks separately from old blocks and to adhere to scrupulous sanitation procedures in cleaning the tanks and preparing them for the next blocks to be soaked. Sometimes disinfectants such as sodium hypochlorite (the active component in household bleach) are added to the soak water. Research has shown that, interestingly, there was a marked increase in the number of viable bacteria after the first addition of chlorine to the water, but, when additional chlorine was added at 20-minute intervals, after the third addition of chlorine there was a significant decrease in both bacteria and fungi. Within 15 minutes after the third application of chlorine there was an initial active chlorine level of 20 to 50 ppm, which is thus the level required for bactericidal activity.[14] No reduction in mushroom yield occurred at this level of chlorine, but other workers have indicated that there is a production decrease when concentrations of chlorine higher than the 20 to 50 ppm were employed. Studies also demonstrated that the pH of the solution has an effect on the amount of hypochlorous acid (the active form of chlorine in disinfection). Lower amounts of hypochlorous acid are present at alkaline values than at neutral or acid pH values. For the grower to obtain benefits from using a chlorine soak, it is important that the active chlorine concentration of the soak tanks and the pH of the water be monitored carefully. This information must be correlated with counts of bacteria, crop productivity, and the incidence of brown center rot for effective use of this control method.

The vectors of *Bga*, other than the previously discussed water vector, include flies, mites, and the mushroom workers. Flies can carry bacteria from block to block, and the number of flies increases when diseased blocks are present. Therefore, some procedures, including the use of insecticides and fly traps are useful in reducing the number of mobile adult flies, which function as vectors for the transmission of bacteria and fungi.

Populations of mites should also be controlled to prevent the spread of bacteria. Mycologists doing laboratory culture work with fungal cultures are familiar with the havoc that mites can raise by carrying bacteria from culture to subculture. Mites have trouble moving through mineral oil so one method of cutting down on bacterial contamination caused by mites in the laboratory is to stack the newly inoculated plates on top of a plate containing mineral oil.

The workers and the materials they use probably constitute the vector that is most responsible for spread of bacteria. Again, hygiene is essential for control. Equipment used should be sanitized

before workers enter a different room or when the workers turn their activities from old infected blocks to new blocks. When moving from one fruiting room to another, the worker should step into a disinfecting foot dip. Washing hands and changing disposable gloves between tasks are other obvious hygienic procedures to follow.

Attempts to bring about biological control of brown center rot of shiitake are in the early stages, but, since some growers have lost as much as 60% of their yield to this disease, we can expect continued research efforts along these lines. In the meantime, changes in management practices and attention to hygiene are the means by which brown center rot of shiitake can be kept under control.

IV. FUNGAL DISEASES

A. Introduction

The occurrence of fungi associated with mushrooms was probably observed as soon as people became interested in their cultivation. After all, fungi are ubiquitous and have relatively simple requirements for their growth — materials readily available in nature provide the carbon, nitrogen, and mineral needs of the fungi. Water and oxygen availability is not a problem in most environments, and the fungi are renowned for their ability to produce asexual spores in absolutely countless quantities. So, like the bacteria, the fungi are present to take advantage of the same substrates that are available for bacteria. Some fungi are also able to use the mycelium and sporophores of the mushroom for their own needs, often to the detriment of the mushroom by causing various diseases.

B. Mycoparasites

The fungi causing diseases affecting the sporophores (fruiting bodies) are referred to as mycoparasites (literally, a fungus that parasitizes another fungus). The mycoparasites are divided into two forms based on how they affect their hosts. The **biotrophic** form is dependent on the host for its nutrition, but the host cells are not killed, and it causes little apparent damage to the host. The **necrotrophic** forms are the ones that are important in diseases of mushrooms.

1. Necrotrophic Parasitism

The necrotrophic mycoparasites are facultative parasites in that they can grow saprophytically on a variety of substrates, but as parasites they kill host cells bringing about the death of the host or a portion of the host.[10] Two necrotrophic mycoparasitic diseases of *Agaricus bisporus* have in the past caused serious economic losses.[27] The causal organisms are *Mycogone perniciosa,* which is responsible for the disease called wet bubble and *Verticillium fungicola*, which is responsible for the disease called dry bubble.

a. Wet Bubble Disease Caused by Mycogone perniciosa

In wet bubble disease the vegetative mycelium of *A. bisporus* is not infected by *M. perniciosa. In vitro* studies have borne out this conclusion. The infection takes place only after formation of the rhizophores, which are the forerunners of the fruiting body. In "wet bubble" the growth of *M. perniciosa* on the fruiting body is thick, has a velvety texture, and is white. The sporophore (fruiting body) when examined internally shows wetness and has an offensive stench with the tissues becoming invaded by bacteria. Drops of an amber-colored exudate are present on these mushrooms. It has been reported by Gandy[27] that the expression of disease symptoms is related to the stage of fruit body development and the time of infection. When infection is early, the expression of disease symptoms is severe. This may involve failure of differentiation of stipes, pilei, or lamellae (gills). What does form is a spherical mass of false tissue, which has been called sclerodermoid tissue, and this mass may be equal to or greater than 5 cm. When sectioned, three zones are revealed in the sporophore beneath the hyphal layer of *M. perniciosa.* The zone closest

to the sporophore surface contains brown dead tissue. Beneath this is a zone of extensive enzyme secretion, and then a zone in which the mycelium of *M. perniciosa* is present between the hyphae of the mushroom.[60] Other workers demonstrated a significant difference in the hyphae of *M. perniciosa* growing on the surface (circa 5 μm in diameter) and those growing internally (circa 25 μm in diameter). On penetration of the host cells, the contents form the amber-colored droplets of exudate previously mentioned.

When infection occurs on already developed sporophores, *M. perniciosa* grows over sectors of the gills and the pilei. The gills undergo hypertrophy and they eventually become covered by the mycelium of the wet bubble organism. In view of the symptoms displayed in this disease, it is not surprising that *M. perniciosa* has been found to produce enzymes that degrade the cell wall polymers of *A. bisporus* as reported by Gandy.[27]

Wet bubble disease caused by *M. perniciosa* has been reported by Chang[8] to occur on the cotton waste compost used for the growth of *Volvariella volvacea*, the straw mushroom, in studies at the Chinese University of Hong Kong.

b. Dry Bubble Disease Caused by Verticillium fungicola

In "dry bubble disease" caused by *V. fungicola*, the mycelium of *A. bisporus* is killed as has been made evident by a number of studies. Many investigations of the interactions between the mushroom mycelium and *V. fungicola* have been conducted. With *V. fungicola* there was a severe necrosis of the mushroom mycelium, which was viewed as similar to the necrosis of dry bubble occurring on fruiting bodies.[33] However, as reported by Gandy,[27] it was learned experimentally that exudates from rhizomorphs and volatile substances from *A. bisporus* had no effect on germination of the conidia of *V. fungicola*. A main generalization from comparative studies of *M. perniciosa* and *V. fungicola* interactions with *A. bisporus* is that *V. fungicola* is responsible for more mushroom necrosis than is *M. perniciosa*.

The growth of *V. fungicola* on sporophores is thin, has a felty texture, and is grayish white. The sporophore is dry at first, but this may change if it undergoes bacterial rot. Internal examination of the sporophore reveals a gray-brown zone of variable thickness just below the surface. In some cases, there is complete discoloration of the internal tissues. Cavities lined with spore-bearing conidiophores of *V. fungicola* are present in the sporophores. This necrotrophic mycoparasite is responsible for developmental deformities of the fruiting body, which may take the form of bulbous stipes on which vestigial pilei are located, or there may be localized lesions on the stipe, which then cause the fruiting body to bend and tissues to peel. When well-developed sporophores are infected, some superficial lesions, pinpoint in size and pale brown in color, may appear within a few days. As is the case with wet bubble caused by *M. perniciosa*, early infection caused by *V. fungicola* causes the deformity known as sclerodermoid, but in the case of *Verticillium* these sclerodermoid masses are less than 1 cm in diameter.

2. Economic Importance

Serious crop losses due to wet bubble have been reported from earlier days of *Agaricus* cultivation in Pennsylvania. In 1930, annual losses were estimated at 15% of the mushrooms; in 1974 the loss was 5.2 million pounds. In the United Kingdom in 1957, wet bubble was the most common disease; *M. perniciosa* caused more losses than *V. fungicola*. However, the 1974 statistics for Pennsylvania showed a greater loss for *Verticillium*. Suggestions regarding the reason for this change involve resistance of *V. fungicola* to the fungicide benomyl, which was being used widely. Other contributing factors include a lowering of hygiene standards with greater reliance on benomyl as well as changes in cultivation procedures, especially the introduction of picking lines, which involved more movement of equipment (mainly trays) into and out of the mushroom house, increasing the chances for introduction of pathogens.[25]

C. Competitor Weed Fungi

A number of fungi grow as weeds on the casing or compost prepared for the growth of edible mushrooms. This contamination may be serious enough to prevent mushroom fruiting. The extensive growth of these weed fungi may also be an indicator that the compost has not been properly prepared to give a selective advantage to the mushroom.

1. False Truffle Disease Caused by *Diehliomyces microsporus*

Although the first report of "false truffle disease" was from losses due to the growth of *D. microsporus* in mushroom beds in the United States, this disease has also occurred in the United Kingdom, Taiwan, and the Netherlands.

Diehliomyces microsporus is a soil-borne fungus known to inhabit mushroom compost.[37] The disease occurs more commonly in beds in which *Agaricus bitorquis* is grown than in beds of *A. bisporus.* The higher temperature used for the growth of *A. bitorquis* is undoubtedly responsible for the emergence of *D. microsporus* in the compost of the mushroom bed or in the casing soil where the spores are naturally present. One consequence to the presence of the growth of *D. microsporus* is that the fruiting of the mushroom stops in the areas in which *D. microsporus* has grown. There is actually a disappearance of the mushroom mycelium and the compost becomes wetter and darker. The surface of the compost on which *D. microsporus* has grown produces ascocarps, the false truffles.[66]

Studies have also shown that *D. microsporus* grows strongly in sterilized compost in which the mushroom mycelium had been grown prior to sterilization. Thus, it is possible that a mushroom metabolite is essential for, or at least benefits, the growth of *D. microsporus.* Gandy[27] points out that another possibility is that the mushroom mycelium removes a factor that normally inhibits the growth of *D. microsporus.*

One reason that *D. microsporus* does better at higher temperatures is that the spores require a temperature above 27 to 28°C for germination. Initially, this fungus grows deep in the compost, but it later emerges into the casing soil. In appearance the mycelium is yellowish white and may form rhizomorphs. The fruit bodies are easily recognized by this appearance and color. They look like shelled nuts or calves' brains and are reddish brown when ripe.[66]

Control of false truffle disease requires pasteurization of the compost for at least 12 hours at 56 to 60°C, with maintenance of air temperature at 58°C for several hours for surface pasteurization. During spawn run, temperatures above 28°C should be avoided. When young truffle fruit bodies form, they should be gathered and burned before they turn brown. Once the crop of mushrooms has been gathered, the room should be subjected to steam and a temperature of 70°C maintained for 12 hours. Trays, woodwork, and other structures in the mushroom house should be treated with a 2% solution of sodium pentachlorophenolate if false truffle has been present.[66] Kim[43] recommends for general hygiene in mushroom houses that, after an empty house has been cleaned with water, the interior surfaces of the house, including bed structure, should be sprayed with a 1% solution of sodium pentachlorophenate at a concentration of 300 ml/m^2. The differences in the concentrations of pentachlorophenolate (also called pentachlorophenate) recommended by Vedder and Kim are probably not significant. In some situations chemical fumigants (e.g., methyl bromide) are used in place of steam pasteurization treatment, but these may be highly toxic to the workers and must be used with extreme caution. The reader is referred to an excellent concise article by Beyer and Poppiti,[5] which points out the principles in the use of disinfectants and sanitizers on mushroom farms for prevention of disease and the rationale for regulations regarding their use.

Some losses due to false truffle have been serious, but perhaps the greatest costs to growers reside in the constant care that must be taken to prevent reinfestation of new crops once false truffle disease has occurred on the farm. With modern systems of continuous cultivation, susceptible crops are always subject to contamination by workers and by flies.

2. Cobweb Disease Caused by *Dactylium*

Although *Dactylium* can parasitize fleshy, agaric mushrooms and thus could be considered a necrotrophic fungus, *D. dendroides* does not cause deformities of the sporophores, and its most prominent feature on the mushroom beds is displayed by the mycelium, which spreads over the surface of the bed and mushrooms giving the appearance of a cobweb. When fruiting bodies are overgrown by the spreading mycelium, spots can form on mature mushrooms under moist conditions.

The genus name *Cladobotryum* is used almost interchangeably with *Dactylium*, and *Hypomyces rosellus* also appears in the literature as a synonym for *C. dendroides*. Gandy[27] reported that studies of *Cladobotryum* spp. initially were concentrated on taxonomic and host range questions, not on the mode of parasitism or effect on the host.

a. Occurrence of Cobweb Disease

Fletcher[24] reported that in 1950 *Dactylium* was "not a common parasite of mushrooms." In 1982, "cobweb disease" was found occasionally on all farms in Pennsylvania, but it was not a major disease. In England in 1986, cobweb was only rarely responsible for large crop losses. Since the late 1980s, however, cobweb disease has become a problem in Europe, Australia, and the United States. It is consequently worthy of consideration here.

Although many fungal species have been named as the causal organism for cobweb disease, including species of the aforementioned genera *Cladobotryum*, *Dactylium*, and *Hypomyces*, most frequently cobweb disease is said to be the result of infection by *D. dendroides*. This organism forms spores that are easily disseminated by air currents and are also spread by flies.

Delmas[11] reported that cobweb disease caused by *Dactylium* is a dangerous disease in *Agaricus* cultivation in caves in France, but that it can be well controlled by systemic fungicides such as benomyl. Kim[43] includes *D. dendroides* cobweb disease as a common occurrence on most farms in Korea. Thus, cobweb disease has been reported in Europe, America, Asia, and Australia and might be expected anywhere fleshy mushrooms are produced in mushroom beds under moist environmental conditions.

b. Symptoms

Cobweb disease displays a range of symptoms from the previously mentioned spreading growth of the mycelium over the surface of the bed, which then forms the cobwebbing on the mushroom, to the subsequent spotting on the mature mushrooms. It is this spotting that may be primarily responsible for the crop loss. Under wet or humid conditions, spots form in a few days after germination of airborne spores that land on the sporophore. At 20 to 25°C, the germination of the spore is very rapid with the consequence that the mushroom can become damaged on harvesting or even when in the market. Spores landing on the casing do not induce disease quickly, but, when the white mycelium grows over the casing, it has a frosting-like appearance that is quite characteristic of the disease.

c. Conditions Favoring Disease

Water is responsible for the development of cobweb disease. It does this by affecting the rate of evaporation from the surface of the mushrooms and by also affecting the moisture content of the casing layer. Genetic factors affecting susceptibility to cobweb disease are probably not involved since the same spawn has been used in many countries and the disease has not become a problem in all of them. If the spawn had genetic factors for susceptibility to cobweb disease, the expression of the disease would have been expected in all countries, as the organism is widespread geographically. It appears that susceptibility to and incidence of cobweb disease may be directly affected by certain cultivation techniques. For example, if, in the attempt to obtain more open caps and flats for marketing reasons, the mushrooms are left on the beds for a longer time before picking, there is an increased likelihood of cobweb disease. Also, with increased use of the bag system in mushroom cultivation, it is not uncommon for mature mushrooms to drop off. These mushrooms

may then remain in the moist conditions of the mushroom house and become nutritionally supportive for the growth of seed fungi such as *Dactylium*.[24]

There are thus several kinds of changes that may influence the incidence of cobweb disease:

1. Changes in the ventilation system, which might affect spore dispersal
2. Alteration in the environment to conditions that are more favorable to disease by changes in the type of casing, the watering procedures, or the environmental control system
3. Production of more open mushrooms in response to market demands, thereby lengthening the time of exposure of the mushrooms to airborne spores of *Dactylium*
4. Deposits of mature mushrooms left on the floor or bed of the growing house as a consequence of poor hygiene supporting the growth and spore development of *Dactylium*
5. Resistance to fungicides as a consequence of extensive use of fungicides for the control of some disease

d. Disease Control

Recognition of the symptoms (cobwebbing, white, frosting-like mycelial covering of the casing layer, and spotting on mature mushrooms) and a careful analysis of the farming techniques are essential before the initiation of control measures. As mentioned earlier, spores landing on the casing do not induce the disease quickly. Symptoms may not appear until the third flush; however, contaminated casing is probably the source of most cobweb disease on the farm with contamination of compost less of a problem.

Of course, fungicides can be employed in control of cobweb disease, but there are only a few registered fungicides for use on mushrooms in the United States. These are benomyl, thiabendazole, and chlorothalonil. Although thiabendazole is very effective, there is evidence that *D. dendroides* becomes resistant to it quickly. Therefore, it is recommended that thiabendazole be used sparingly and in combination with chlorothalonil to which no resistance has yet been found. Even though chlorothalonil is slightly myotoxic, the decrease in yield is small and may justify its use to prevent cobweb disease.[24]

While removal of diseased or infected materials from the growing room is important, this activity may lead to the spread of the infection. Simply covering patches of cobweb may result in spore dispersal. Such spore dispersal is also brought about by watering. Reduction of spore dispersal and spread of cobweb disease can be brought about by covering the affected bed area very carefully with a damp paper towel before treating the patch with salt or alcohol to curb its spread, or before watering. The diseased patches and spots should be treated as soon as they appear with inspections made twice daily when there is a serious infection. Air used in the house should be filtered to remove spores; the exhaust air should be filtered as well to prevent further dissemination of spores elsewhere on the farm. Wetness on the casing surface and on the surfaces of the mushrooms should be avoided because this condition promotes the growth of *Dactylium* and the symptoms of cobwebbing and spotting. Cobweb epidemics often develop when heavier and wetter casing is used if the environmental control system does not provide the greater evaporative capacity that is required under these conditions of increased water. Once the cobweb disease has reached the stage where the spore density is so high that it cannot be managed by hygiene alone, the house must be subjected to a steam cookout for an effective crop termination. Actually, cobweb disease can be easily controlled, but prompt action at the first sign of the disease is necessary.

3. Mushroom Green Mold

A number of imperfect fungi form on undercomposted substrates, which still have high levels of easily available carbohydrates. This is very common on composts that have been incompletely pasteurized. Species of *Trichoderma, Aspergillus,* and *Penicillium* are most frequently encountered and are sometimes referred to as the "green molds."[19]

a. History

Early studies reported that *T. viride* was the common species of *Trichoderma* that was found as a colonizer of composts and a subsequent inhibitor of mushroom mycelial growth. Hayes[36] stated that its occurrence was associated with excessive wetness and inadequate pasteurization, which led to the incomplete transformation of nutrient materials required for mushroom growth. *Trichoderma koningii* has been cited as the causal organism for a disorder in the beds of *Agaricus bisporus* in which there was an infestation of the mold on dead mushroom tissue left in the beds. A spread of the mold over 15- to 20-cm-diameter areas of the bed followed within 2 or 3 days. Although green mold disease is primarily found in mushroom houses using a compost made up of such things as straw, manure, cotton seed hulls, rice hulls, sawdust, etc., some of the mushrooms that are produced on wood may often be damaged by *Trichoderma* green mold. For example, *Kuehneromyces mutabilis*, now known as *Pholiota mutabilis*, has been grown in Germany since the end of World War II and is a popular local mushroom where it is produced. This fungus is grown on wood, and better and longer production is obtained when the wood on which the fungus has been inoculated and is already growing is placed for two thirds of its length into bed soil. A few months later the mycelium develops into the soil, from which it receives water and nutrients. The bed soil is frequently covered with mycelial mats of lower fungi including *T. viride*. The growth of these weed fungi may have no effect, or they may impair growth of the mushroom by utilizing the substrate. The bed soil technique is used in the cultivation of other wood-rotting mushrooms such as *Lentinula* in the early days of its cultivation[67] and *Dictyophora.* Gramms[32] reported that wood covered by bed soil gave a threefold greater yield of *K. mutabilis* than wood not inserted into soil. He suggested that the functions of the bed soil are to provide humus and minerals for the mycelium, to provide greater temperature and moisture constancy, and to absorb toxic metabolites produced by the mycelium. These advantages apparently can affect the potential damage produced by *Trichoderma*.

Ospino-Giraldo et al.[46] gave a brief review of the early history of mushroom green mold including the description of green mold given by Sinden and Hauser in 1953. In the past, green mold disease occurred only periodically, generally on farms using low-quality compost or employing inadequate sanitation procedures. Thus, green mold was not a serious problem when growers used good hygiene, and proper preparation and pasteurization of the compost. In 1985, more severe outbreaks of *Trichoderma* green mold occurred in Ireland mainly in bagged compost. The disease was also found in England and Scotland with yield reductions as great as 30%. In Canada and the United States equally serious outbreaks of green mold occurred, and in 1994–1995 epidemics of *Trichoderma* green mold took place with crop losses in Pennsylvania exceeding $20 million during a 3-year period. *Trichoderma* green mold disease had become a major disease problem in the mushroom industry and extensive research was directed to studies of the causal organism and control measures.

b. Research

The research reported here deals with studies of the role of spawn in the disease, the expression of green mold following supplementation of the compost, the use of molecular technologies to establish identity of *Trichoderma* strains involved in the disease, and, finally, measures useful in control of the disease.

1. Role of Spawn in Disease

A number of significant findings were reported by Fletcher as the result of his studies of mushroom spawn and the development of *Trichoderma* compost mold.[22] Fletcher uses the term *Trichoderma* compost mold in preference to green mold to distinguish between problems in the compost and green mold occurring elsewhere such as (1) the wood of trays or (2) the remains of mushrooms left on the casing or (3) the mold causing spots on the caps of mushrooms. The initial study of Fletcher was designed to determine if Phase II compost (i.e., compost that has been peak heated or pasteurized) can serve as a medium for *Trichoderma*. It was found that Phase II compost *did*

not support the growth of *T. harzianum* even when high inoculum levels were used, but that unspawned Phase II compost did contain viable cells of *T. harzianum* as revealed by the recovery of colony-forming *T. harzianum* even when there was no visible evidence of mycelial growth or sporulation in the compost. When the compost was spawned, and subsequently colonized by *Agaricus bisporus,* the population of *T. harzianum* was reduced to undetectable levels. In the absence of *Agaricus* but with autoclaved wheat grains present in the compost, there was an increase in the *Trichoderma* population. Thus, it was concluded that cereal grains probably play an important role in the development of *Trichoderma* compost mold, a finding that led to further experimentation with the following results:

- *Trichoderma* did develop in compost when the inoculum was placed on a food base such as autoclaved wheat grain, but not when placed on inert materials such as a perlite or vermiculite.
- When spawn and compost were inoculated with germinated spores of *Trichoderma,* the germinated spores were unable to sustain growth on Phase II compost away from the grain. However, it was found that small pieces of grain were able to sustain growth of *T. harzianum* on Phase II compost.
- When various concentrations of spores were applied to grain spawn, *T. harzianum* developed on only the highest concentration.
- Temperature studies revealed that at 15°C, *T. harzianum* can colonize grains before *A. bisporus* has formed a protective barrier, and also that *T. harzianum* will grow at temperature equal to or greater than 30°C. This is the result of a rapid decrease in growth of *A. bisporus* above 28°C, and death of the mycelium is reported to occur at 32°C, thereby eliminating or diminishing the protective barrier.
- Different isolates of *T. harzianum* were tested as to their ability to inhibit growth of *A. bisporus* and there were marked differences among the isolates listed.

When control of *Trichoderma* was investigated, it was found that inoculated grain spawn treated with a 20-ppm solution of carbendazim prevented the development of *T. harzianum* with no adverse effects on the growth of *A. bisporus.*[22]

A study was performed to determine whether there was a requirement for spawn grains for the establishment of *Trichoderma* infection in compost because earlier studies had implicated cereal grains in the development of *Trichoderma* compost mold.[22] Results of experiments performed by Rinker and Alm[49] revealed that grain spawn, and compost spawn, to which autoclaved grains were added, all supported green mold colonies and drastically decreased mushroom yield. These experiments were done with biotype Th4 of *Trichoderma,* whereas the experiments of Fletcher previously mentioned used biotype Th2. The conclusion from the Rinker and Alm study is that *T. harzianum* (biotype Th4) does not need cereal grain to initiate green mold growth.

These studies led to a further evaluation of the role of spawn in green mold disease in which *T. harzianum* biotype Th4 was used to infect the spawns. The spawn was kept under refrigeration for up to 12 months before use. In some experiments the spawn was allowed to warm up to room temperature before spawning, and in other treatments the refrigerated spawn was used before spawning. The results of this experimentation were that neither the length of storage nor the warm-up period prior to use produced differences in number of colonies of green mold or their size. Thus, these treatments had no tangible effect on the incidence or severity or *Trichoderma* green mold disease. Next tested were the type of grain (rye or millet) used to make the *Agaricus* spawn and the amount of spawn used. The results here were that there was no difference in expression of the disease between rye spawn and millet spawn, and an increase in the amount of spawn up to twice the rate also had no effect on the expression or severity of the disease.[47]

2. Effect of Supplementation of Compost

Gandy[27] reported that, when the composition of the mushroom compost was amended after Phase II by the addition of sugars, the compost was rapidly colonized by *Trichoderma.* In the British Isles it is biotype Th2 of *T. harzianum* that has been responsible for the great crop losses, whereas in North America biotype Th4 is responsible for losses in yield incurred by the mushroom growers. Consequently, Rinker and Alm[48] used biotype Th4 in their studies on the effect of supplementation of the compost on *Trichoderma* infection on commercial mushroom farms. It was known that supplements added to the compost at spawning or casing can increase mushroom yield up to 25%, but it was also known that these supplements can serve as a food source for competitor molds such as *Trichoderma*. In their studies Rinker and Alm amended the compost with various commercial supplements either at spawning or before casing and then inoculated the compost with a spore suspension of 1 to 2 billion spores of biotype Th4. The spore suspension was pipetted into the bottom of a 5-cm-deep hole beneath the compost surface in the center of each tray. Supplements, applied either at spawning or casing, increased the mushroom nonproductive area due to the growth of green mold. It was observed that inoculation with biotype Th4 at the time of casing was less damaging to the crop than inoculation at spawning. A particular commercial supplement, Campbell's S-41, produced smaller green mold colonies than either the nonsupplemented compost or the other composts amended with four different commercial supplements. The compost supplemented with S-41 had increased mushroom yield, but S-41 contained the mold-inhibitory compound thiabendazole. With the further addition of benomyl to S-41, there was an additional reduction of disease symptoms on the compost. With supplementation by S-41 and also the commercial supplement called Feather Meal, the total mushroom yield was significantly greater than the nonsupplemented biotype Th4-infested control. Rinker and Alm state that care must be taken in the choice of supplement and the timing of supplementation. These factors will be dependent on the overall infection pressure on the specific mushroom farm. Although increased yield can be derived from a supplement, this increase can be negated by an expanded size of nonproductive areas resulting from green mold.

3. Molecular Technologies

Molecular technologies have been very important in obtaining knowledge about green mold disease. In particular, they have been used to distinguish aggressive from nonaggressive forms of *T. harzianum* that are morphologically similar, as well as to determine the prevailing type of green mold on commercial mushroom farms. A clinical test for the rapid determination of the aggressive biotype of *T. harzianum* (Th2 in Europe and Th4 in North America) has resulted from these molecular studies.[46] Analysis by restriction fragment length polymorphism (RFLP) has been successfully used to separate various isolated strains into groups. In one study, 81 isolates of *Trichoderma* from mushroom compost were studied by RFLP analysis of ribosomal DNA and mitochondrial DNA. These isolates could be placed in three major groups. An additional molecular technique called random amplified polymorphic DNA analysis (RAPD) confirmed this grouping.

Because morphological characteristics can vary greatly from one isolate to another, and because these traits are subject to environmentally induced changes, the identification of *Trichoderma* species and their relationships are now studied by molecular techniques and computerized analysis. For example, with green mold occurring on different continents, it would be useful to know the relationships of the various types that have been isolated as well as the origin of the biotypes. For such phylogenetic studies the molecular analysis of specimens in which specific regions of their DNA are analyzed by RFLP or RAPD methods. The degree of similarity of DNA sequences of the strains correlates with their relatedness. Organisms that are distantly related have similarity only in regions of DNA that cannot change without having a deleterious or at least significant impact in function. Closely related organisms show differences in regions where variability does not produce major effects. The results in the study of green mold–causing isolates, when the sequences

of six isolates were compared with other sequences from data banks, indicated that there were two distinct groups:

- *Group I* contained biotype Th1 and the aggressive biotypes Th2 (European) and Th4 (North American)
- *Group II* contained biotype Th3

The phylogenetic tree obtained by using the molecular data indicated that Th1 is the most recent ancestor for the aggressive types Th2 and Th4.[46]

An extensive research project on the cause, edaphic factors, and control of mushroom green mold has been carried out at Pennsylvania State University and sponsored by the Pennsylvania Department of Agriculture, using the facilities of the Penn State University Mushroom Research Center. The results of the research were presented in a series of reports.[51,52,54] Some of the important contributions of these studies are as follows:

- Demonstration that biotype Th4 of *Trichoderma harzianum* was the cause of green mold disease on Pennsylvania mushroom farms
- Development of a clinical test involving DNA genetic fingerprinting techniques for the rapid detection of biotype Th4, thus permitting early detection and control of the disease
- Evidence that a recent introduction of Th4 into mushrooms under cultivation was responsible for the green mold epidemic in Pennsylvania
- Demonstration that deployment of biocontrol *Trichoderma* on crop plants was not responsible for green mold epidemics
- Demonstration that the effectiveness of benomyl-treated spawn in control of Th4 biotype green mold disease
- Determination that the use of grain spawn exacerbates green mold disease, as does nitrogen supplementation at spawning and high casing moisture
- Demonstration that the severity of green mold was increased under low oxygen conditions during Phase II composting and that biotype Th4 cannot survive a properly managed Phase II pasteurization
- Demonstration that it is not possible to manipulate the temperature at the time of spawn run to give a selective advantage to the growth of *Agaricus* mushroom mycelium over the *Trichoderma* green mold fungus
- Demonstration that there was greater loss by the green mold fungus with infestation of the compost at spawning than with introduction of *Trichoderma* at casing
- Demonstration of a 24°C registration for the use of Terraclo as a sanitizing agent for *Trichoderma* on wood surfaces and floors
- Demonstration that other compost molds can alter the expression of *Trichoderma* green mold disease

c. Methods of Control

Information on the control of green mold has been given previously, but now we direct our attention to the results of some studies in which control was the primary concern. The first study to be examined is that of Grogan et al.,[35] which used biotype Th2, the most aggressive strain of *T. harzianum* in the United Kingdom. Th2 is responsible for serious reductions in the yield of *A. bisporus*. The study made a number of observations that are worthy of mention, even though some of these have been presented previously in reports of green mold disease in North America where biotype Th4 is the strain responsible for epidemics. Some of their observations and experimental results include the following:

- Th2 colonizes the mushroom compost during spawn run and prevents the mycelium of *A. bisporus* from becoming established.
- In the absence of cereal grains the green mold did not become established in the compost.
- Once the green mold has become apparent, it is too late to prevent substantial yield losses.
- All plots that had received spawn inoculated with biotype Th2 experienced a reduction in yield due to compost green mold.
- When spore suspensions of biotype Th2 were sprayed onto spawned compost, there was no reduction in yield of mushrooms at a spore density of 10^2 spores per kg of compost, but there was a 90% reduction in yield at a spore density of 10^5 spores per kg of compost.

Additional studies have been made on the use of fungicides to control green mold. In some cases, the spawn was treated with the fungicide; in other cases the compost was treated. Grogan et al.[35] used the fungicides carbendazim, thiabendazole, and benomyl. When the fungicide was used on the spawn, carbendazim gave the best results. With carbendazim the mushroom yield was 84% compared with 100% for uninoculated (no *Trichoderma* biotype Th2) compost and 38% for uninoculated compost with no fungicide treatment. The mushroom yield with thiabendazole treatment was 77% and with benomyl 58%. When the fungicide was applied to the compost, the mushroom yields compared to the control were 69% with benomyl, 71% with carbendazim, and 44% with thiabendazole. In these experiments, none of the treatments with fungicide had significant phytotoxic effects on mushroom yield.

From these studies,[35] generalizations can be drawn as follows:

1. Hygiene on the mushroom farm is extremely important for it was found that with poor hygiene conditions a small green mold outbreak can rapidly escalate out of control.
2. Low levels of *Trichoderma*, Th2 biotype, did not produce a significant effect on mushroom yield.
3. Treatment of spawn with fungicide was more effective than treatment of compost. The evidence for this was that only 1.15 g of fungicide per ton of compost was required when the fungicide was applied to the spawn, whereas 70 g of fungicide per ton of compost was required when the compost was treated.
4. From the standpoint of government regulation of fungicides, these studies showed that the fungicide residues in mushrooms harvested from fungicide-treated crops were less than the maximum residue level permitted in the United Kingdom for carbendazim in fungi.

In the United States, approval from the Pennsylvania State Department of Agriculture has been given for the use of Benlate (benomyl) on spawn to control *Trichoderma* green mold. The approval label allows for Benlate to be mixed with a carrier such as gypsum, lime, or chalk, and this mixture is then used to coat the spawn grains prior to their use in spawning the compost.[1]

An important generalization about control of *Trichoderma* green mold is that if the spawn grain is protected with fungicide, thus preventing colonization of the compost by *T. harzianum* green mold, the mushroom mycelium will grow vigorously from the spawn grain into the compost. Unfortunately, there may be an adverse side effect in that both *Verticillium* and *Dactylium* have shown resistance to benzimidazole fungicides, and thus these weed fungi may become a problem. Care in the use of fungicides and hygiene practices in the mushroom houses are absolutely essential.

Although the foregoing accounts about *Trichoderma* green mold disease have dealt primarily with problems associated with the production of *Agaricus* mushrooms, this disease can be a problem in the production of other edible mushrooms. An initial experimental study at the Mushroom Research Center at Pennsylvania State University demonstrated that *T. harzianum* biotype Th4 produced a loss greater than 75% in two outbreaks of the oyster mushroom, *Pleurotus ostreatus*. Because oyster mushroom production has skyrocketed in the past decade, green mold disease has

the potential of becoming a serious threat.[57] Cultivation of the oyster mushroom is commonly by bag culture techniques in which the pasteurized straw-based substrate is spawned prior to placement in the bags. The bags are then placed in a spawn run room with controlled environmental condition of moistures, temperature, and light. There are ample opportunities for *Trichoderma* to become established in the growing procedure, especially if *A. bisporus* is grown on the same farm. Therefore, the authors of the study emphasize the importance of hygiene programs.

In an article describing *Pleurotus* production in Hungary, South Africa, and the United States, Germl et al.[30] consider the common problems caused by pests including the fungi. They point out that the fungi found on the substrate are rarely parasitic, but that many species of imperfect fungi may be encountered. Control by application of fungicides, such as benomyl, during substrate preparation is generally effective in inhibiting these competitor fungi. These competitors are a greater problem at substrate temperatures above 35°C. Also, higher substrate temperatures may injure the mushroom spawn and reduce the mycelial growth rates, thereby making the substrate vulnerable to competitors such as the green mold *Trichoderma* spp. and *Coprinus*.

V. NEMATODE DISEASES

A. Types of Nematodes

Nematodes can be found anywhere that mushrooms are grown because they are ubiquitous and feed on decaying organic matter with both mushroom compost and casing materials furnishing a favorable environment for these organisms, commonly known as eelworms or roundworms. Nematodes are small, almost microscopic, about 1 mm in length, with a cylindrical body, tapered tail, and a blunt head region. The body is covered by a cuticle that is tough, nonliving, and skinlike. The cuticle is transparent and the eelworm's body is essentially colorless.

In connection with mushroom cultivation in the past, the nematodes were commonly placed in two groups: (1) the saprophagous or microphagous nematodes and (2) the mycophagous nematodes. As the name implies, the saprophagous eelworms feed on decaying organic matter, and the mycophagous eelworms are fungal feeders using fungal mycelium for food. As presented shortly, we can also include a third group of eelworms that are important in mushroom cultivation, namely, the insect parasitic or entopathogenic nematodes.

1. Saprophagous Nematodes

The saprophagous nematodes take up liquid and bacteria and are the larger type of eelworms found in mushroom beds. It is pointed out by White[68] that it is difficult to prove pathogenicity of these nematodes to *Agaricus* mushrooms, because to do so would require sterile cultures of both the eelworm and the mushroom living together. This is difficult to achieve because some species of eelworms are dependent on living bacteria for their food, and the production of *Agaricus* fruiting bodies involves the metabolic activity of the bacterium *Pseudomonas putida* in the casing layer. It is suggested by Flegg and Wood[20] that, because activated charcoal, which absorbs low-molecular-weight organic compounds, replaces the positive effect on fruiting of *P. putida* in the casing layer, the mechanisms may involve the removal by the bacterium of a self-inhibitor of fruiting that is produced by the mushroom mycelium. Of course, the removal of the inhibitor permits fruiting initials to form and develop into mushrooms, but the identity of the self-inhibitory compound or compounds has not been determined. Thus, although pathogenicity of nematodes to mushrooms has not been proved, it is known that, when mycelial growth is restricted by mushroom competitors, pests, or disease, the eelworm populations may increase very rapidly. This means that the presence of eelworms in abundance is an indicator of bad conditions for mushroom growth.

2. Mycophagous Nematodes

The situation is different with the mycophagous nematodes because these eelworms have a mouth structure called a stylet, which can puncture a hyphal cell and permit the eelworm to suck out the contents of the hypha. Although there are different mechanisms by which different species of eelworms feed on the hypha, the ultimate result is that the hyphal cytoplasm leaks from the punctures made by the nematodes and brings about the death of the hypha. With increasing numbers of eelworms, there is a great destruction of the mycelium, which is also accompanied by bacterial decomposition, followed by further migration of the eelworms on the mushroom bed.

The time of infestation by eelworms is very important. Infestation at spawning may result in a complete failure of the spawn run and thus no mushrooms are produced; however, infestation at the end of cropping may produce little loss in mushroom yield.

3. Entopathogenic Nematodes

There is always interest in biological control of organisms harmful to agriculture. We have just seen that nematodes may be injurious to mushroom farming, but there is an interesting twist to the role of nematodes on the mushroom farm. This involves the use of entopathogenic nematodes as biocontrol agents for insect pests in the mushroom house. The nematode *Steinernema feltiae* is used in the United States, Canada, and Europe to control mushroom sciarid flies.[50]

a. Treatment Methods

The effect of *S. feltiae* on mushroom mycelium of *Agaricus bisporus* and the control of the mushroom fly, *Lycoriella mali*, were studied using different methods of application of the nematode and different times and places of these applications. It was found that in mycelial growth that there was no significant effect on the surface of the casing at pinning when the nematodes were incorporated directly into the compost, but mycelial growth was reduced when nematodes were applied in the irrigation water for the casing surface. There was no difference in mycelial growth between application of the nematode directly into the casing or by irrigation onto the casing surface. These tests were made at commercial farms in which some farms used the tray and some used the shelf method of cultivation. The studies on fly control used fly-infested mushroom crops on a commercial farm. The application of these nematodes was made after the emergence of the first generation of adult flies and before the emergence of the second generation. The flies were captured in specially prepared and located plastic cups, counted, and then analyzed by statistical methods to determine significant differences between treatments. In this study,[50] it was found that nematodes irrigated into the casing layer in the amount of 81 million per 100 m^2 resulted in a 66% reduction in the emergence of flies. The researchers suggest that under conditions of heavy fly infestation the use of nematodes as biocontrol agents may reduce the yield loss due to fly damage up to 25%.

b. Mode of Action

The mode of action of the nematode in controlling insects in the mushroom house is a consequence of the nematodes harboring in their guts bacteria that are lethal to insects.[34] The nematode locates its host through sensory mechanisms, e.g., chemical cues, and enters the insect through openings in its body (anus, mouth, etc.). When established in the insect's body, the nematode releases the bacteria, causing a septicemia in the insect that brings about its death within 48 hours.

c. Compatibility with Chemicals Used on Mushroom Farms

The mushroom farm requires the use of chemical agents in other aspects of production of *A. bisporus*. Therefore, it was important to determine if such chemicals had an impact on the viability and virulence of *S. feltiae* used as a biocontrol method for sciarid flies.[34] A series of laboratory experiments were conducted to determine the compatibility of *S. feltiae* with various chemicals (Apex 5E, Azatin EC, Benlate, Bravo 500, Margo-San, Sporgon, calcium hypochlorite, Formalin, and Ventigo). In the first series of experiments the nematodes were mixed in a tank with the

chemicals to determine if the nematodes could be applied along with the chemicals. It was learned that some of the chemicals (Apex 5E, Azatin EC, Benlate, Bravo 500, Margo-San, and Sporgon) had no adverse effect on nematode viability and virulence when they were maintained in an aqueous phase for up to 24 hours. Thus, these could be applied as a tank mix. The compound Martect 340-F (thiabendazole) had no effect on viability but reduced the virulence of the nematode. Calcium hypochlorite, Formalin, and Ventigo (cinnamaldehyde) killed all nematodes within 2 hours.

The next series of experiments was designed to determine if those chemicals that did not lend themselves to use in a tank mix could be applied to the casing material prior to application of the nematodes. The results indicated that at least a 3-day interval should pass after application of Formalin to the casing before *S. feltiae* was applied. In the case of calcium hypochlorite, Ventigo, and Martect 340-F, a period of 24 hours should elapse before applying *S. feltiae*. Grewal et al.[34] mention that there is a report in the literature by Rovesti and Deseo[55] that *S. feltiae* can be tank-mixed with the insect growth regulator diflubenzuron (Dimilin), which is commonly used in mushroom cultivation for the control of the larvae of the insect pest, *Lycoriella mali*. From these studies it can be stated that by choosing appropriate time intervals between nematode and chemical applications any undesirable impact of the chemicals can be managed. Biocontrol by *S. feltiae* is a good method for the control of insect pests.

VI. INSECT DISEASES

A. Introduction

The presence of insects during mushroom cultivation has been observed from the early days of mushroom farming. With the construction of improved growing facilities in which care is taken to prevent openings through which insects can enter the mushroom house and by careful composting measures that destroy the insects and various other organisms that are present in the compost heap, the population of insects at the time of spawning can be kept to a manageable level. Thus, much of the damage that occurs in the beds is brought about by introduction into the casing or by flies that infiltrate the growing room through passageways or cracks or openings in the framework of the building. This means that growers must be cautious in the maintenance of their buildings, in the management of the houses including screening to exclude insects, and in the Phase II preparation of the compost. Even with great vigilance in these matters, insects become a problem, and pesticides are routinely employed to reduce damage by flies. This adds a significant expense to production, but, because the losses in production may amount to as much as $100 million by the sciarid fly, *Lycoriella mali*, alone, the use of insecticides is economically essential.[3]

The organisms with which we are concerned in this section are members of the phylum Arthropoda, the order Diptera, and the families Phoridae, Sciaridae, and Cecidomyiidae.

B. Insects That Serve as Agents of Disease in Mushroom Houses

1. Family Phoridae

Members of the family Phoridae are commonly referred to as phorids. They are small with inconspicuous antennae and have the general appearance of small houseflies. It is reported that species of the phorid genus *Megaselia* are among the most important of the insect pests of *Agaricus* cultivation.[36] *Megaselia halterata* is probably the species most commonly encountered in mushroom houses in Europe, Australia, and the United States,[68] although there is also a long history of infestation by *M. nigra.*

a. Mode of Infestation

The phorid species *M. halterata* and *M. nigra* differ in their methods of infestation. Although phorid female adults in general are attracted to mushrooms to lay their eggs, *M. halterata* females are

attracted to the growing mycelium by volatile chemicals produced by the mycelium. They lay their eggs close to the tips of the growing hyphae. On the other hand, the females of *M. nigra* lay their eggs on the developing fruiting body when natural light is present. In the case of *M. nigra*, these flies will not be attracted and become a cause of potential harm unless the fruiting bodies (sporophores) have already formed; whereas *M. halterata* constitutes a potential problem on the mushroom beds as soon as spawning has taken place.

b. Mode of Feeding

The larvae of some species of *Megaselia*, such as *M. halterata*, feed only on the mushroom mycelium of the bed material because they do not possess the structural mouth-hooks of *M. nigra* that are adapted for burrowing into the cap and stipe of *Agaricus* mushrooms. It does not take too many larvae to make the tunnels in the sporophore that are responsible for making the mushroom unmarketable. With *M. halterata*, a heavy infestation of the larvae after the compost has been spawned can decrease the amount of growth of the mycelium into the compost. This would be the consequence of larval consumption of the finer hyphae, which are necessary for proper spawn running.

c. Control and Economic Effects

Fly traps are essential in research studies to determine the effectiveness of various control methods, but they may also be important, along with careful hygienic practice, in keeping adult populations below threshold levels, which would be economically damaging.[30]

The most common method of controlling flies is by the incorporation of pesticides in the compost or casing, but aerosols of diazinon, malathion, and pyrethin can kill adult phorid flies.[36] Incorporation of the pesticides into the compost or casing requires much care, because diazinon, malathion, and thionazin may affect the growth and the number and size of mushrooms. Mushroom house management practices (e.g., watering and the nature of the casing layer) may also influence the pesticides' effects on the various growth phases. It is pointed out by White[68] that the pesticide may have different effects during various flushes, possibly as a result of changing concentrations of the pesticide. It has also been shown that different spawns may give different yields in regard to susceptibility to a particular pesticide. Vigilance in monitoring these variables is necessary for successful management of the mushroom farm.

The addition of pesticides to the casing layer may affect cropping in ways different from simply preventing attacks by pests. The size of mushrooms may be increased, but this may be associated with a delay in fruiting, and a decrease in the number of mushrooms and yield. On the other hand, with certain chemicals, some concentrations may lead to an increase in yield.[58] It is generally felt that treatment of the casing with pesticides provides benefits beyond those of pest control.[68]

The economic losses by phorid flies were substantial in the time period when manure spawn was commonly used. Although larvae of *M. halterata* are known to affect yield, the use of different spawns and techniques of spawning gave a greater number of inoculation points for mycelial growth for colonization of the substrate, even in the presence of a large number of larvae, and there was less loss in yield.

2. Family Sciaridae

Members of the family Sciaridae are commonly referred to as sciarids. These flies are small and have long, threadlike antennae. Their natural habitats include manure piles, rotting wood, leaf mold, and fungi. The genus of greatest concern to mushroom growers is *Lycoriella: L. auripila* is a pest in the mushroom houses of Europe and Russia, *L. agaraci* in Australia, and *L. mali* in the United States. *Lycoriella solani* occurs in Europe, Australia, and Russia.

a. Mode of Infestation

The larvae of sciarids are killed during Phase II of the composting process. Thus, it is only flies that lay their eggs after the compost has been pasteurized that can cause trouble. Sciarids are

attracted by odors present in the gases exhausted during the process of cooling the compost. The addition of nitrogenous supplements, such as soya bean, may also serve as attractants to the compost.

b. Mode of Feeding

Sciarids have the ability to damage the mushroom crop directly because their larvae have large, well-developed mouthparts capable of chewing mycelium and mushrooms. Unlike the phorids, they do not require mushroom mycelium for the growth and development of their larvae. However, the number of larvae developing to maturation and the rate at which they develop have been found to increase when composts are amended with nitrogenous substances, which are commonly added during composting to increase *Agaricus* growth and yield. Likewise, it has been found that small amounts of mycelium encourage larvae to feed on the hyphae at the advanced edges of the mycelium, thus speeding larval development and the number of sciarids emerging.[6] Although small amounts of mycelium have the effect just described (increasing larval development and the number of mature sciarids), large amounts of mycelium on the compost have the opposite effect. That is, sciarid development and larval growth are inhibited — possibly as the consequence of the accumulation of fungal metabolites, such as calcium oxalate.[7] White[68] suggests that the antagonism between mycelial growth and sciarids may be the result of a vigorous spawn run taking the available nitrogen out of circulation. This is consistent with the finding that nitrogen increases the number and speed of development of sciarid larvae.

Although sciarid larvae are not supported by casing materials such as peat and calcium carbonate alone, food from the mycelium apparently supports the sciarids that infect the casing layers.[68] The result of this infestation may be damage or destruction of the mushroom primordia (pinheads) and tunnels or burrows in the mushrooms that do develop.

c. Symptoms

Symptoms of sciarid infestation may occur at different stages of *Agaricus* cultivation. The most serious injury occurs when there is heavy infestation during pinning. The sciarid larvae use their large mouthparts to chew on and sever the rhizomorphs or mycelial attachments to the pins. The pins may turn brown and develop a leathery texture, which stops further development of the mushroom. The pinheads may be completely consumed or hollowed out by the larvae. This is more difficult to observe, but its consequences can be serious.

At an earlier stage of cultivation, around the time of spawning, large numbers of sciarid larvae may be produced by feeding on the compost. The fecal matter produced by these larvae can cause a soil-like wet mass that cannot be colonized by the *Agaricus* mycelium. Mature sporophores may display tunnels in their stipes or pilei that are produced when sciarid larvae have heavily infected the bed. These symptoms can be easily recognized but come too late for any treatment to be successful.

d. Control and Economic Effects

In the United States, *L. mali* is the major pest encountered by the growers of commercial mushrooms, mainly *A. bisporus*. Estimates are that the damage to mushroom crops is around $100 million. Thus, control measures for these pests is a high priority to the mushroom farmer.

As is the case in all situations involving pathogenic organisms, sanitation and initiating cultivation with a pathogen-free growing area are essential. The carryover of pests in the growing facility from one crop to the next must be avoided. Edwards[16] has described general methods for achieving protection. He recommends air filtration to control airborne pests. It is pointed out that the danger to new crops comes from larvae carried over from the previous crop in the shelves, trays, and other structures of the growing area. While pasteurization (Phase II) is important in controlling such infection, it is frequently not absolutely effective. Thus, a postcrop fumigation of the cropping house with methyl bromide is recommended. This must be performed with utmost safety precautions because methyl bromide is very poisonous and almost odorless. The mushroom house must be carefully constructed and insulated to avoid damage by the rapid changes in temperature and moisture during the treatment with live steam that is performed at the end of cropping and before

the removal of the trays, bed materials, etc. This cookout treatment requires a 70°C temperature in the compost in the region of the lower shelves most distant from the injection point of the steam. This temperature must be maintained for a minimum of 12 hours. Alternative to these treatments is the spraying or wetting of all surfaces with chemical disinfectants. Delmas[11] states that during the summer the caves in which mushrooms are grown are less subject to parasitic infections than mushroom houses, but local infection may spread more rapidly in caves due to absence of separating walls. He describes carefully a protocol of principles for sanitation methods for protection against infection, including:

1. Preventive disinfection of structures, tools, walkways, picking baskets, and trays
2. Protection of cropping area from noxious organisms by disinfection of workers' clothing, tools, and other equipment, keeping in mind that pathogens may be introduced into the growing area by air, compost, and casing
3. Disinfection of casing soil by pasteurization or by watering shallow layers of the soil used for casing with a solution of Formalin
4. Elimination of trash (discarded mushrooms, spent compost) only after spraying with chemical disinfectants
5. Elimination of external pollution sources, such as wastes which should be removed from the growing area, covered with lime and located at a considerable distance from the entrances; composting areas should be treated similarly
6. Isolation of contaminated zones, which must be drastically treated to kill the pests even to the extent of losing the crops

Sanitation methods are certainly important and essential, but they are not sufficient for the control of the sciarid fly. Pesticides are employed in large amounts. Through the years a large number of pesticides have been used. These include diazinon, malathion, and thionazin.[36] More recently, permethrin and diflubenzuron have become the commonly used chemicals to control infestation of the sciarid fly, *L. mali*. **Permethrin** was first used to control *L. mali* in 1981 at a time when damage to mushroom crops by this sciarid fly was estimated to be approximately $100 million annually. Unfortunately, resistance to permethrin developed rapidly. The resistance problem has been extensively studied by Bartlett and Keil,[3] who found that resistance evolved through oxidative metabolism of the toxin. In their studies they found that there was variation in resistance between farms in different geographical regions, which may have been due to the patterns of permethrin application. The farms on which the greatest resistance to the pesticide were found were those that involved a nerve insensitivity resistance in addition to the oxidative metabolism resistance.

Diflubenzuron is another insect growth regulator that has become prominent in control of the sciarid fly. It affects oviposition and larval development. It is a chitin synthesis inhibitor that, by disrupting chitin synthesis, prevents the molting or shredding of an outer cuticular layer, which is essential in the conversion from the larval (immature, feeding) stage to the pupal (nonfeeding, nonmoving) stage. In the cultivation of *Agaricus,* diflubenzuron is applied as a drench to the compost or casing. The growth and yield of *Agaricus* mushrooms is not affected by this drenching treatment of diflubenzuron. The situation is somewhat different with the specialty mushrooms shiitake and *Pleurotus*, however. When diflubenzuron was incorporated during log preparation for shiitake cultivation, there was little effect on biological efficiency, number or weight of mushrooms per log, or the size of mushrooms. When diflubenzuron was added to the soak water used to stimulate mushroom formation on the logs, there was a reduction in production of mushrooms. With *Pleurotus*, the incorporation of diflubenzuron resulted in a significant reduction in mushroom production. Fleischer et al.[21] concluded that there are important biological interactions between the growth and development of shiitake and *Pleurotus* and the populations of the sciarid fly *L. mali*, and that the use of diflubenzuron for control of *L. mali* is feasible with careful consideration of the management practices that are employed for the different mushroom species. It should be remembered, however,

that high levels of insecticides may fail to control flies after a relatively short time. Kurtzman and Zadrazil[44] suggest that the minimum recommended dosage be used and that, when control is no longer attained by this dosage, the use of the insecticide be stopped.

For mushroom growers the main problem of the adult flies is that they spread the fungal diseases of mushrooms. Control of adult flies is commonly accomplished by the use of pyrethrin-type compounds, such as the synthetic compound permethrin, which is a nerve poison that acts by interfering with the passage of impulses down the nerve axon. But, as discussed previously, *L. mali* has developed methods to impart resistance to these insecticides. For example, resistance may be by the breakdown of the toxin by oxidative metabolism or by a nerve insensitivity resistance. To make the insecticide useful by retarding the development of resistance to the insecticide, piperonyl butoxide, a botanical extract, is mixed with the pesticide. When piperonyl butoxide is applied to the insect, the metabolism involved in the breakdown of the pesticide is retarded long enough for the insect to be poisoned. Thus, piperonyl butoxide enhances the toxicity of the pesticides. Keil recommends that insecticides only be used in emergencies when adult fly population must be reduced quickly.[41]

Currently (2004), there is great interest in biological insecticides for use in mushroom houses. We have previously (Section V.A.3 of this chapter) discussed the use of the nematode, *Steinernema feltiae*, to control the sciarid fly. Foremost at the present time among the biological insecticides are the toxins produced by the bacterium *Bacillus thuringiensis israelensis* (BTI).[41] When this bacterium is grown in liquid medium, spores are produced as key nutrients become depleted. At this time, the bacterium produces a crystal that contains four different proteins. It has been demonstrated that these proteins are only toxic to the larvae of the phorid, *Megaselia halterata,* and the sciarid *L. mali.* The protein crystal must dissolve to release the protein toxins. This occurs at a basic pH of 10 to 11. This pH value is found in insect larval midguts, whereas the pH of the guts of humans and other vertebrates is quite acid, having a pH of 2 to 4. The protein crystal is dissolved after it has been eaten by a larva. Following dissolution, three of the protein toxins have specific receptor sites on the insect gut wall, which binds them to the gut wall. The result of dissolution and binding is a loss of water regulation in the gut of the larva, which then stops feeding, the gut breaks down, and the gut contents mix with other body fluids. The larva then dies. The toxin crystal, produced by the bacterium BTI, must be eaten to be effective, and there is no human or vertebrate toxicity associated with it. Laboratory trials as well as field trials involving application of a liquid formulation of BTI to the compost and casing controlled *L. mali* without adversely affecting mushroom yield or quality. However, it was expensive, and another drawback was that to achieve good mixing of the formulation into newly spawned compost a fairly large amount of water had to be used. Excessive water at spawning retards growth and may promote development of the bacterial disease known as mummy disease. The development of a less expensive dry formulation, known as **gnatrol WDG** (water dispersible granule), can be mixed into the substrate dry or diluted in water. Extensive laboratory and field trials with gnatrol WDG indicated that gnatrol WDG reduced emergence of *L. mali* from the casing to economically acceptable levels. It is reported by Keil[40] that gnatrol WDG gave better results than Amazin or Dimilin (diflubenzuron).

Azadiractins constitute a class of pesticide compounds obtained as extracts from the East Indian neem tree. Azadiractins are effective in controlling larvae and eggs of the sciarid fly, *L. mali*, in the casing of *A. bisporus* and also in cultivation of the specialty mushrooms *Pleurotus* and *Lentinula edodes.*[42] The mode of action of azadiractin compounds is as an insect growth regulator that interferes with larval development by disrupting the insects' hormonal balance. They have the advantage that they are relatively inexpensive and can be applied many times to a crop. A drawback is that azadiractin has an odor that is objectionable to the workers in the mushroom houses. It was felt that the objectionable features of azadiractin were present in the inert ingredients in the formulations and that these could be removed. Amazin 3% EC was tested for odor, taste, growth inhibition of *Agaricus* mycelium, as well as the ability to control sciarid larvae in casing in a field trial.[42] Compared with controls, the Amazin 3% EC showed no significant differences in odor, taste

(before or after cooking), or mycelial growth in concentrations that would be present during mushroom production. However, adult flies captured in traps in the control house were significantly greater in number than in the house treated with Amazin.

3. Family Cecidomyiidae

The Cecidomyiidae are different in their life cycle from most insects in that the larvae of the mushroom cecids, with the exception of two species, produce additional young larvae within themselves, and thus reproduction can be continued indefinitely with just an occasional production of an adult.

a. Species Found during Mushroom Cultivation

According to White,[68] there are six species of mushroom cecids that have been reported from cultivated mushrooms worldwide. The most common of these is *Heteropeza pygmaea* found in the United Kingdom, United States, Australia, Taiwan, and Korea. Also common are *Mycophila speyeri* reported from the United Kingdom and United States, and *M. barnesi* found in the United Kingdom, Australia, and Korea. *Henria psalliotae* occurs frequently in Taiwan and is also found occasionally in the United Kingdom. *Lestremia cinerea* and *L. leucophaea* also occur occasionally in the United Kingdom.

b. Mode of Feeding

The larvae of cecids are obligate mycelial feeders. A larva begins feeding by holding a hypha with its small mouthparts. The hypha is then pierced and the protoplasmic contents of the hypha sucked out. The larva may then move to find more actively growing hyphae. As the size of the larva increases, it engages in violent movements of its head and this tears up bundles of hyphae.

Cecids feed on a wide variety of fungi, including members of all classes. It is reported[68] that cecids reproduce more rapidly on some strains of *Agaricus bisporus* than on others. Thus, a change of spawn might alleviate the loss from cecid infestation. Under unfavorable conditions, for example, in the increasing age of mycelium, some of the larvae develop a thickened brown cuticle, which imparts longevity to the larvae. The presence of these long-lived larvae in the mushroom house or in spent compost can lead to reinfestation.

c. Economic Effects

Infestations by *Heteropeza* and *Mycophila* cause economic loss in *Agaricus* cultivation. The obvious loss is by spoilage as a result of the larvae climbing up the mushroom stem and collecting at the stem and gill junction. The larvae of *Mycophila* are bright orange and visible. The larvae of *Heteropeza* are white and less readily seen, but bacteria are carried with them, which bring about a breakdown of the stem surface and gills. These activities of both *Mycophila* and *Heteropeza* make the mushrooms unmarketable. Reduction in yield is also substantial with infestations by cecids. A reduction in yield by an early infestation by *Heteropeza* of 14% is mentioned by Hayes.[36] Experimental studies with the introduction of larvae after spawning and at casing showed substantial losses due to both spoilage and loss of yield reaching as high as 55%.[68]

VII. ACTIVITY OF MITES IN MUSHROOM CULTIVATION

A. Genera Found during Mushroom Cultivation

Mites are arachnids, a group in the phylum Arthropoda. They are very small (0.2 to 0.5 mm) and are ubiquitous. Although many species of mites are found in mushroom houses the world over, most of these do not have adverse effects on the mushroom crop by initiating decay. Their presence, however, may suggest that the compost is not sufficiently selective for *Agaricus bisporus*, as is the case of certain species of the mite genus *Pygmephorus,* which feeds solely on weed molds, such

as *Trichoderma, Monilia,* and *Humicola.*[36] On the other hand, there is a species of this same genus that does not attack weed molds but attacks the mushroom mycelium. Mites of the genus *Tarsonemus* can affect mushrooms by eating the mycelium and also by eating pits in the fruiting body and inhibiting the development of the button stage (*T. floriculus*). They also may feed on the hyphae, breaking the mycelial strands attached to the base of the fruiting body with the presence of the mites resulting in the stipe taking on a reddish brown coloration (*T. myceliophagus*). The increase in the number of mites is slow and only an infestation at spawning will result in damaging levels in the early stages of cropping. The members of the genus *Tycophagus* are saprophagous. If pasteurization of the compost is effective, these mites are uncommon. *Tarsonemus putrescentiae*, once it becomes established on the bed, may produce large numbers, which eat the mycelium and damage the fruiting bodies by pitting.[68]

In studies of compost green mold disease, there was an experiment in which green mold was observed on the compost at spawning, and a large population of red pepper mites (*Pygmephorus* sp.) swarmed on the casing surface during the first flush. The red pepper mites are known to feed on *Trichoderma* spores.[35]

B. Economic Importance

The direct loss due to infestation of mites in the mushroom house is relatively minor. The presence of mites is indicative, however, of improperly treated compost or failure to protect against green mold or other fungal diseases. Because some of the species of mites feed on bacteria, they may be secondary invaders that do not initiate decay when they are present on mushrooms or in the compost.

REFERENCES

1. Anon., AMI registers Benlate for use against green mold, *Mushroom News*, 45(7), 4–5, 1997.
2. Atkey, P.T., Viruses, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, C.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 14, 1985.
3. Bartlett, G.R. and Keil, C.B.O., Permethrin resistance in *Lycoriella mali* (Diptera: Sciaridae) populations from *Agaricus* mushroom farms, in Royse, D.J., Ed., *Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products*, Pennsylvania State University, University Park, 459–482, 1996.
4. Bateson, M., Baker, L.A.E., and Lelliott, R.A., Oozing gills and brown blotch in mushrooms caused by a *Pseudomonas* sp. resembling *P. cichorii*, *Plant Pathol.*, 21, 146, 1972 [cited in Gandy[27]].
5. Beyer, D.M. and Poppiti, J., The safe and proper use of disinfectants and sanitizers on mushroom farms, *Mushroom News*, 50(4), 4–10, 2002.
6. Binns, E.S., Mushroom mycelium and compost substrates in relation to the survival of the sciarid *Lycoriella auripila*, *Ann. Appl. Biol.*, 80, 1–15, 1975, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 16, 1985.
7. Binns, E.S., Field and laboratory observations on the substrates of the mushroom fungus gnat *Lycoriella auripila* (Diptera Sciaridae), *Ann. Appl. Biol.*, 96, 143–152, 1980, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 16, 1985.
8. Chang, S.T., *Volvariella volvacea*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 27, 1978.
9. Chang, S.T. and Miles, P.G., Historical record of the early cultivation of *Lentinus* in China, *Mushroom J. Tropics*, 7, 31–37, 1978.
10. Deacon, J.W., *Modern Mycology*, 3rd ed., Blackwell Science, Oxford, 211–217, 1997.
11. Delmas, J., Cultivation in Western countries: growing in caves, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 11, 1978.
12. DelVecchio, V.G., Dixon, C., and Lemke, P.A., Immune electron microscopy of virus-like particles of *Agaricus bisporus*, *Exp. Mycol.*, 2, 138–144, 1978.

13. Derrick, K.S., Quantitative assay for plant viruses using serologically specific electron microscopy, *Virology*, 56(6), 652–653, 1973.
14. Donoghue, J.D., Simonson, A.K., and Denison, W.C., Crop management strategies for controlling brown center rot of shiitake, *Mushroom News*, 46(2), 36–44, 1998.
15. Duggar, B.M., The principles of mushroom growing and mushroom spawn-making, *Bull. U.S.D.A. Bur. Plant Ind.*, 85, 1–60, 1905.
16. Edwards, R.L., Cultivation in Western Countries: Growing in houses, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 12, 1978.
17. Elliott, T.J., Spawn-making and spawns, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 8, 1985.
18. Fermor, T. and Lincoln, S., Mushroom soft rots, *Mushroom News*, 48(4), 16–24, 2000.
19. Fermor, T.R., Randle, P.E., and Smith, J.F., Compost as a substrate and its preparation, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 6, 1985.
20. Flegg, P.B. and Wood, D.A., Growth and fruiting, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 9, 1985.
21. Fleischer, S.J., Keil, C.B.D., and Royse, D.J., Diflubenzuron impacts on shiitake and *Pleuotus* production and *Lycoriella mali* oviposition and larval development, in *Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products*, Royse, D.J., Ed., Pennsylvania State University, University Park, 473–482, 1996.
22. Fletcher, J.T., Mushroom spawn and the development of *Trichoderma* compost mold, *Mushroom News*, 45(8), 6–11, 1997.
23. Fletcher, J.T., The patch syndrome — is this a new disease? *Mushroom News*, 47(10), 14–17, 1999.
24. Fletcher, J.T., Cobweb disease, a new challenge, *Mushroom News*, 50(4), 20–23, 2002.
25. Fletcher, J.T. and Yarham, D., The incidence of benomyl tolerance in *Verticillium fungicola, Mycogone perniciosa* and *Hypomyces rosellus* in mushroom crops, *Ann. Appl. Biol.*, 84, 343–353, 1976.
26. Gandy, D.G., *Report of the Glasshouse Crops Research Institute 1959*, 62–64, 1960.
27. Gandy, D.G., Bacterial and fungal diseases, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 15, 1985.
28. Gandy, D.G. and Hollings, M., Die-back of mushrooms: a disease associated with a virus, *Report of the Glasshouse Crops Research Institute 1961*, 103–108, 1962 [cited in Atkey[2]].
29. Gaze, R.H., Trials and tribulations: virus — more questions, *Mushrooms News*, 47(10), 10–13, 1999.
30. Germl, J., Labuschagne, P., and Royse, D.J., Oyster mushroom production on three continents: an overview of cultivation in Hungary, South Africa, and the United States, *Mushroom News*, 49(2), 4–13, 2001.
31. Gill, W.M. and Tsuneda, A., The interaction of the soft rot bacterium *Pseudomonas gladioli* pv. *agaricicola* with Japanese cultivated mushrooms, *Can. J. Microbiol*., 43, 639–648, 1997.
32. Gramms, G., *Kuehneromyces mutabilis*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 20, 1978.
33. Gray, D.J. and Morgan-Jones, G., Host–parasite relationships of *Agaricus brunnescens* and a number of mycoparasitic hyphomycetes, *Mycopathologia*, 75(1), 55–59, 1981 [cited in Gandy[27]].
34. Grewal, P.S., Weber, T., and Betterley, D.A., Compatibility of the insect-parasitic nematode, *Steinernema feltiae*, with chemicals used in mushroom production, *Mushroom News*, 46(4), 6–10, 1998.
35. Grogan, H.M., Noble, R., Gaze, R.H., and Fletcher, J.T., Compost inoculation and control of *Trichoderma harzianum* — a weed mold of mushroom cultivation, *Mushroom News*, 45(4), 29–36, 1997.
36. Hayes, W.A., Biological nature, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 8, 1978.
37. Hayes, W.A., Nutrition, substrates and principles of disease control, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 9, 1978.
38. Hollings, M., Viruses associated with a die-back disease of the cultivated mushroom, *Nature*, 196, 962–965, 1962.

39. Hollings, M., Stone, O.M., and Last, F.T., Detection and identification of viruses in mushroom sporophores and mycelium disrupted with ultrasound, in *Report of the Glasshouse Crops Research Institute 1964*, 151–154, 1965.
40. Keil, C., Field trials of the gnatrol WDG formulation of *Bacillus thuringiensis israelensis* for control of mushroom flies in Pennsylvania and California, *Mushroom News*, 50(4), 12–19, 2002.
41. Keil, C., The use of pesticides for control of adult mushroom flies, *Mushroom News*, 50(4), 11, 2002.
42. Keil, C. and Bartlett, G., A new low-odor formulation of Azadiraction, a botanical insecticide for mushroom fly control, *Mushroom News*, 48(4), 4–6, 2000.
43. Kim, D.S., Cultivation in Asian countries: growing in temperate zones, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap.14, 1978.
44. Kurtzman, R.H., Jr. and Zadrazil, F., Physiological and taxonomic considerations for cultivation of *Pleurotus* mushrooms, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, chap. 16, 1982.
45. Murata, H. and Magae, Y., Toxin production in a mushroom pathogenic bacterium, *Pseudomonas tolaasii* strain PT 814 is activated by signals present in a host, *Pseudomonas ostriatus*, and those accumulating in the medium in the course of bacterial growth, in *Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products*, Royse, D.J., Ed., Pennsylvania State University, University Park, 483–494, 1996.
46. Ospino-Giraldo, M.D., Royse, D.J., Chen, X., and Romaine, C.P., Application of molecular technologies for the study of mushroom green mold-associated strains of *Trichoderma*, *Mushroom News*, 45(9), 34–37, 1997.
47. Rinker, D.L. and Alm, G., Evaluation of spawn and its role in green mold disease, *Mushroom News*, 50(7), 18–19, 2002.
48. Rinker, D.L. and Alm, G., Effect of supplementation at spawning or casing on the expression of *Trichoderma harzianum*, biotype Th4, in commercial mushroom production, *Mushroom News*, 45(11), 6–11, 1997.
49. Rinker, D.L. and Alm, G., Efficacy and limitations of mushroom grain spawn treated with benomyl against green mold disease of the cultivated mushroom, *Mushroom News*, 46(11), 6–11, 1998.
50. Rinker, D.L., Alm, G., and Olthofs, T.H.A., Use of the nematode, *Steinernema feltiae*, to control the sciarid fly, *Mushroom News*, 45(4), 6–11, 1997.
51. Romaine, C.P., Royse, D.J., Wuest, P.J., and Beyer, D.M., Mushroom green mold: cause, edaphic factors and control, *Mushroom News*, 45(4), 20–28, 1997.
52. Romaine, C.P., Royse, D.J., Wuest, P.J., and Beyer, D.M., Mushroom green mold: cause, edaphic factors and control, *Mushroom News*, 46(4), 12–17, 1998.
53. Romaine, C.P. and Schlagnhaufer, B., PCR analysis of three RNA genetic elements in *Agaricus bisporus*, in *Mushroom Biology and Mushroom Products*, *Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products*, Royse, D.J., Ed., Pennsylvania State University, University Park, 449–458, 1996.
54. Romaine, C.P., Wuest, P.J., Royse, D.J., and Beyer, D.M., Mushroom green mold: cause, edaphic factors and control, *Mushroom News*, 47(7), 28–31, 1999.
55. Rovesti, L. and Deseo, K.K., Compatibility of chemical pesticides with entopathogenic nematodes, *Steinernema carpocapsae* Weiser and *S. feltiae* Filipzev (Nematoda: Steinernematidae), *Nematologica*, 36, 237–245, 1990 [cited in Grewal et al.[34]].
56. Royse, D.J., Identification and thermal sensitivity of two bacterial pathogens of *Agaricus bisporus*, *Plant Dis.*, 64(5), 455–457, 1980 [cited in Gandy[27]].
57. Royse, D.J., Ospino-Giraldo, M., Thon, M.R., Chen, X., and Romaine, C.P., Potential threat to *Pleurotus* spp. from *Trichoderma harzianum* (Th4), cause of mushroom green mold of *Agaricus bisporus*, *Mushroom News*, 46(2), 20–24, 1998.
58. San Antonio, J.P. and Lambert, E.B., Effects of pesticides in casing soil on growth and development of cultivated mushrooms, *Mushroom Sci.*, 5, 327–399, 1962, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 16, 1985.
59. Schisler, L.C., Sinden, J.W., and Sigel, E.M., Etiology, symptomatology, and epidemiology of a virus disease of cultivated mushrooms, *Phytopathology*, 57, 519–526, 1967.

60. Smith, F.E.V., Three diseases of cultivated mushrooms, *Trans. Br. Mycol. Soc.*, 10, 81–97, 1924 [cited in Gandy[27]].
61. Tolaas, A.G., A bacterial disease of cultivated mushrooms, *Phytopathology*, 5, 51–54, 1915 [cited in Gandy[27]].
62. Treshaw, C., Nutrition of the cultivated mushroom, *Dansk Bot. Arkiv.*, 11(6), 1–180, 1944.
63. Tsuneda, A. and Thorn, G., Interactions between *Lentinula edodes* and pesudomonads, *Can. J. Microbiol.*, 40, 937–943, 1994.
64. Tucker, C.M. and Routien, J.B., The mummy disease of the cultivated mushroom, *Mo. Agric. Exp. Stn. Res. Bull.*, 358, 27 pp., 1942 [cited in Gandy[27]].
65. Van Zaayen, A., Immunity of strains of *Agaricus bitorquis* to mushroom virus disease, *Neth. J. Plant Pathol.*, 82, 121–131, 1976 [cited in Atkey[2]].
66. Vedder, P.J.C., Cultivation, in *The Biology and Cultivation of Edible Mushrooms*, Shang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 17, 1978.
67. Wang Cheng, *Book of Agriculture*, 1313.
68. White, P.F., Pests and pesticides, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, chap. 16, 279–293, 1985.

10 Culture Preservation

I. INTRODUCTION

If no degenerative changes were to take place during the preparation or maintenance of mushroom cultures and of spawn, then the preservation of mushroom cultures would be a relatively simple, routine procedure. Unfortunately, this is not the case. Degeneration of the culture, or of the spawn produced by the culture, refers to the loss of desired qualities by changes that result in such things as slow development, poor rate of survival, and low level of productivity.

Although cultures of edible mushrooms may maintain their desired commercial and biological features when maintained in culture for long periods of time under proper conditions, a number of different commonly used methods for maintaining cultures may result in degenerative changes.

It is well known that phenotypic variation is due to interaction between the genotypic constitution of the organism and the existing environmental conditions. This means that degeneration may sometimes be caused by environmental changes as well as by genotypic alterations.

Cell degeneration in microorganisms is commonly attributed to factors such as lack of nutrients, reduced oxygen tension, accumulation of toxic products, alteration of pH to an unfavorable state, breakdown of cell organelles, and loss of essential cell components. Growth of the mycelium can bring some of the factors just mentioned into play. On the one hand, growth may result in depletion of the supply of substrates necessary for the formation of cellular materials and the generation of energy that is required for maintenance of existing biomass. This depletion of substrate in cases where the supply cannot be supplemented would retard the rate of metabolic activities within the cells of the mycelium and limit the formation of additional mycelial biomass. On the other hand, growth increases the amount of mycelial biomass. This means that there are increased requirements for nutrients. These demands for additional nutrients come at a time when the supply of nutrients is decreasing. Consequently, for the preservation of mushroom cultures it is not desirable to have unrestricted growth and increase in biomass.

Accumulation of mutations may occur during prolonged vegetative propagation of stock cultures. Mutations, arising either as gene mutations or chromosomal aberrations, may accumulate and create an intolerable load of mutations for the culture to handle and still maintain its desirable features. Although it is difficult to demonstrate that degeneration results from mutation or from genetic recombination (either meiotic or mitotic) and subsequent nuclear selection in the established cultures, these mechanisms are certainly plausible, and they are frequently advanced as explanations for observed undesirable changes in cultures or spawn.

Obviously, contamination can lead to the loss of a culture, and merely the presence of a contaminating microorganism in a culture can result in low levels of performance or of diminished viability of the mushroom culture. We should not overlook the fact that there is a role for mixed cultures of microorganisms in mushroom cultivation, however. For example, a role for bacteria in triggering fruiting body formation in *Agaricus* has been demonstrated,[5,8] but the microorganism (e.g., *Pseudomonas putida*) must be preserved as a pure culture for experimental studies or in the practice of mushroom growing, not as a mixed culture with *Agaricus* as the other member. Other investigations[22] have indicated that the role of these bacteria, present in or added to the casing, is to absorb a substance or substances, produced by the mycelium of *Agaricus*. Such substances inhibit formation of fruiting body primordia. That the bacteria perform the function of absorbing substances

that serve to keep *Agaricus* mycelium in the vegetative state was suggested by studies in which activated charcoal, which absorbs organic compounds of low molecular weight, was able to replace the bacteria in initiating primordia formation.

Although the exact factors affecting degeneration of mushroom cultures are still not fully understood at the subcellular level,[20] in the light of present knowledge, a good quality culture can be preserved by careful attention to growth conditions and details of the techniques used for preservation.

II. OBJECTIVES

Cultures of edible mushrooms can be preserved either as spores or as vegetative mycelia. Spores of heterothallic or secondary homothallic species that are produced through a sexual process will have genetic differences. Spores from a primary homothallic species would be expected to be genetically similar. Of the cultivated fungi, only *Volvariella volvacea* is suggested to be a primary homothallic species, however, and the details of the life cycle of *V. volvacea* remain an enigma; but we do know that variation is displayed by the basidiospores.[3] Thus, if single-spore cultures are maintained, mating tests for heterothallic species would be required routinely, as well as tests for fruiting ability; if spores are maintained for homothallic species, it would be necessary to check fertility of the culture by regular fruiting tests. Consequently, in practice, vegetative mycelia of known origin are stored.[19]

The main objectives of culture preservation are as follows:

- Maintenance of viability for long periods, thus permitting survival of the culture
- Maintenance of genetic, morphological, and physiological stability, thus increasing the preservation of strain characteristics of scientific significance and industrial importance
- Maintenance of culture purity so that the cultures are free of other microorganisms and viruses

To achieve any of these objectives, it is essential that the original culture be in good condition. If proper preservation techniques are followed, cultures displaying optimal growth and normal appearance will maintain the desired characteristics after preservation. Initially poor growing and aberrant-appearing cultures will rarely be improved by preservation.

III. METHODS

A wide variety of methods are available for the conservation of mushroom cultures, among which it is possible to select the one that is most suitable for a particular need, e.g., preservation for a relatively short period or for a long period. This chapter provides a general summary of the main features of various methods. For more detailed accounts of the general aspects of maintenance of microorganisms, including fungi, the reader is referred to the articles by Snell[19] and by Smith.[17] For particular details of the storage of mushroom strains, especially with the use of liquid nitrogen, details will be found in the articles by San Antonio and Hwang,[16] Jong,[11,] Elliott and Challen,[7] Jodon et al.,[10] and Challen and Elliott.[2] The choice of preservation method depends on many factors, but the availability of necessary equipment and funds is commonly a determining factor in the decision of the technique to be used.

A. Short-Term Storage

The traditional method for maintaining mushroom cultures is by periodic transfer. Cultures maintained in this way are readily available for subculturing, without any delay in recovering the culture — as occurs in long-term storage in liquid nitrogen, for example. In short-term storage there are

three factors that influence success in the preservation of the cultures: (1) the frequency between transfers, (2) the maintenance medium, and (3) the storage temperature.

In theory, the transfers should not be too frequent, to reduce the risks of contamination and technical errors (e.g., mislabeling, selection of variants or mutants, and actual loss of cultures through breakage or misplacement). The period between subcultures varies with species, but, in general, it is in the range of 3 to 12 months. Maintenance of duplicate cultures is a useful precaution against loss, and it is desirable to perform an abbreviated characterization periodically, to monitor any changes in desirable characteristics.

1. Culture Practices

The mycelial growth from the growing edge of the colony should be used in making subcultures. Deviations from the original cultures can be detected with mycelial transfers. The performance of the mycelium should be checked continuously, although not all degenerative symptoms can be detected in the mycelial stage. The degenerative symptoms commonly detected are sectors of slow growth, mycelium that is thin and of weak appearance, or mycelium that is matted or fluffy but has a normal growth rate. A slow-growing mycelium needs more time for colonization and tends to carry virus particles. A fluffy mycelium causes the grain to stick together and is harder to spread in the compost than normal grains. It tends to form "stroma," and it gives lower yields. Mycelia of these types should be discarded.

In our laboratory, the edible mushroom strains are subcultured onto Complete Agar Medium or Potato Dextrose Agar Medium slants (three slants for each strain). *Volvariella volvacea* is incubated at 32°C for 7 to 10 days. The other strains are incubated at 25°C for 10 to 14 days until the slants are fully grown with mycelium. Once full growth of *V. volvacea* strains has been obtained, they are kept at room temperature. *Volvariella volvacea* should be subcultured every 2 months. *Lentinula, Pleurotus*, and *Agaricus* strains can be kept in the refrigerator at 5°C, and they should be subcultured every 6 months.

2. Substratum

Laboratory media such as potato dextrose agar, malt agar, oatmeal agar, and Complete Agar Medium (0.5 g $MgSO_4 \cdot 7H_2O$; 0.46 g KH_2PO_4; 1 g K_2HPO_4; 2 g peptone; 20 g dextrose; 20 g agar; 2 g yeast extract; 0.5 mg thiamine HCl; 1 liter distilled water) are commonly used. As a general rule, a nutritionally weak medium is preferred, because it lowers the metabolic rate of the organism and thus prolongs the period between transfers. However, it should be remembered that growth of a pure culture in laboratory medium on agar is not a natural condition, and to obtain the fullest development the conditions encountered in nature should be simulated as far as possible. Strains tend to become attenuated under artificial conditions of culture. It is known that the medium can act as a selective agent, and, therefore, it can affect the frequency of appearance of some mutations — for example, the fluffy-type mycelium.

3. Small Flat-Sided Culture Bottles

In 1949, Wilson and Plunkett[21] described the use of small (15 ml) perfume bottles for culturing fungi. Since that time, such bottles have been used advantageously in a large number of laboratories in the United States. The bottles are approximately $4.5 \times 4.2 \times 1.9$ cm, with a neck that makes the height of the bottle 6 cm when the cap is on. Each bottle has a capacity of approximately 20 ml. It is possible to arrange 56 such bottles in an aluminum pan that has outer dimensions of $28.5 \times 19.0 \times 4.0$ cm, which can be obtained easily at hardware stores. The plastic cap should be made of a heat-resistant compound such as Bakelite, so that it can be autoclaved repeatedly without loss of shape — and without giving off formaldehyde vapors, which are objectionable to persons in the

vicinity and, if absorbed in any appreciable amount by the medium, may affect the growth of the microorganisms.

The advantages of these small bottles for culture work have been described by Wilson and Plunkett.[21] The advantages stem from both the small size and the parallel flat sides. The bottles arranged in a pan can be stored in a refrigerator or cold room where space is usually limited. A second advantage is that the bottles can be lined up both symmetrically and numerically so that it is easy to locate a particular stock by number. A third advantage is that only small amounts of medium are required (8 to 10 ml per bottle). If it is desired, when making inoculation, the bottle can be laid on its side without danger of rolling and without the mouth of the bottle touching the table surface. In general, the bottles can be handled more freely with less danger or damage than can test tube cultures.

These bottles have been used extensively for culturing filamentous fungi. In genetic studies where large numbers of single-spore isolates must be maintained, they are especially useful.[13] Inoculations may be made with a loop for sporulating species, or blocks of agar containing mycelium may be cut out by a scalpel with a narrow handle (#7 handle with #11 detachable blade), which has first been sterilized by dipping in alcohol and then flaming.

These bottles are also useful for maintaining cultures of yeasts and bacteria, which may be inoculated with loop or needle, either on agar slants or as stab cultures. A procedure for preparation of these bottle cultures is described below:

- Arrange the clean, empty, uncapped bottles in an aluminum pan; add 8 to 10 ml of medium, which has been heated in order to put the ingredients into solution, to each bottle. This is conveniently done by the use of a syringe-type pipettor (Figure 10.1), but any simple device for dispensing media is satisfactory.
- Next, screw the caps loosely onto the bottles.
- Place the pans containing the bottles in the autoclave for sterilization.
- On removal from the autoclave, lean the pans that contain the bottles up against a surface at an angle to give the desired slant to the agar medium in the bottles.
- After the medium has completely cooled, screw the caps on tightly to prevent desiccation of the medium. The bottles are then ready for inoculation (Figure 10.2).

FIGURE 10.1 Filling the flat-sided bottles with medium.

FIGURE 10.2 Inoculating the culture into the flat-sided bottles.

A pan containing 56 bottles requires approximately 3250 cc of space. Even a very small refrigerator of approximately 54,000 cc capacity would easily contain 10 pans (560 bottles), but, because wooden or plastic containers can be built of any dimensions for storing the bottles in a refrigerator, it would be possible to store about 1000 bottles in neat numerical arrangement in the same unit. Fewer than 400 test tubes can be stored in the same amount of space, and they cannot be arranged very satisfactorily for quick location and removal of a particular culture.

When cultures are to be discarded, it is best to first autoclave the bottles and then to pour out the contents. If much mycelium is present, it may be necessary to force this out by use of a stream of water or by other means. The bottles can then be washed by usual procedures.

4. Temperature

Tubes or culture bottles containing the subculture should have screw caps with liners and be sealed with paraffin film or aluminum foil (to avoid drying) and kept at low temperature (~ 5°C) to reduce the metabolic rate of the organism. Some mushrooms, however, are sensitive to chilling injury (e.g., *Volvariella volvacea*) and are best kept at 10 to 15°C.

B. Long-Term Storage

The rationale for the following methods involves the arrest or retardation of cellular metabolism. This can be achieved either singly or by a combination of the following methods: starvation of nutrients, limitation of oxygen, lyophilization, and freezing.

1. Starvation of Nutrients

Storage in distilled water suppresses an increase in mycelial mass and also has been credited with suppressing pleomorphic changes and inhibiting genetic changes in fungi. To the best of our knowledge, there is no report of storage of mushroom cultures by maintaining them in distilled water at room temperature, although we have observed viability of nongrowing cultures in liquid media lacking in an essential nutrient after prolonged periods of incubation. Because there are other, easier methods available for long-term storage of cultures and because experimental evidence is lacking for benefits in maintaining culture stability by preservation in distilled water or a starvation medium, this technique has not been exploited.

2. Limitation of Oxygen

An obvious way to retard metabolism of aerobic microorganisms is to deplete the supply of available oxygen. A simple and effective means for preservation of fungal cultures was developed by Buell and Weston.[1] The technique involved overlaying an agar slant surface, on which mycelium was growing, with sterile mineral oil. The mineral oil served to block the exchange of oxygen between the mycelial surface and the atmosphere in the container, thus retarding metabolism while also preventing desiccation of the agar medium. In conjunction with maintenance of the cultures in a refrigerator at 5°C, this is an effective method of preserving fungal cultures. Removal of the mycelium with its adhering mineral oil for subculturing is a rather messy operation, but not so difficult or time-consuming that it detracts appreciably from the benefits of this inexpensive procedure, which requires nothing in the way of equipment beyond that routinely available in a simple microbiological laboratory.

3. Lyophilization

Lyophilization is also known as freeze-drying. It is the method of choice for long-term storage of spore-bearing fungi, but it is not useful for preservation of mycelia — and thus mushroom cultures cannot be preserved in this way. In freeze-drying, spores are frozen and water is removed by sublimation.[12] This drying of the spores is accomplished by freezing under reduced pressure in a vacuum. The rapid removal of water from the spores results in many of them remaining viable, though dormant, and when the ampoules containing the dried spores are evacuated before sealing, the spores will remain viable for long periods of time. An obvious advantage of the freeze-drying technique in experimental studies is that a large number of ampoules can be frozen of the same spore sample. Further, by starting off with a culture derived from an ampoule, a culture that is very similar to one derived from one of that same set of ampoules years previously can be obtained. This assurance would not be possible with a culture that had been maintained by subculturing during that period of time, because through growth the opportunity for mutational changes, recombination events, and selection of physiological variants would have arisen.

4. Freezing

With the improvements in refrigeration systems and the greater availability of liquid nitrogen, storage of cultures in liquid nitrogen or at low freezing temperatures has become the method of choice for storage of a wide variety of biological materials including mushroom cultures. This method involves the storage of mycelium in liquid nitrogen[9] that is at a temperature of −196°C, with the vapor above the liquid at a temperature below −130°C. At temperatures below −130°C, the metabolism of mycelium is slowed to the extent that it can be considered to have stopped.[11] Therefore, it is not necessary to transfer the mycelium from time to time, and, in theory, mycelium can survive indefinite storage. A number of methods for handling mycelial cultures have been developed, and that of Elliott and Challen[7] for the storage of mushroom strains has been modified slightly and is now described.

a. Preparation of Cultures

The strains are subcultured onto complete agar medium plates and allowed to grow for about 10 to 14 days at 25 to 28°C. After the mycelia have fully grown on the plates, the mycelia are cut into very small inocula by a hollow stainless-steel tube 3 mm in diameter (Figure 10.3 and Figure 10.4).

b. Preparation of Ampoules

Polypropylene drinking straws are cut into short pieces, approximately 60 mm in length. They are sealed at one end with a polythene heat-sealer (Figure 10.5). Very small labels, 15 × 2 mm, are made with typing paper and labeled with India ink. Next, the labels are inserted into the drinking

FIGURE 10.3 Cutting the mycelial colony of a *Pleurotus* strain with a hollow stainless-steel tube.

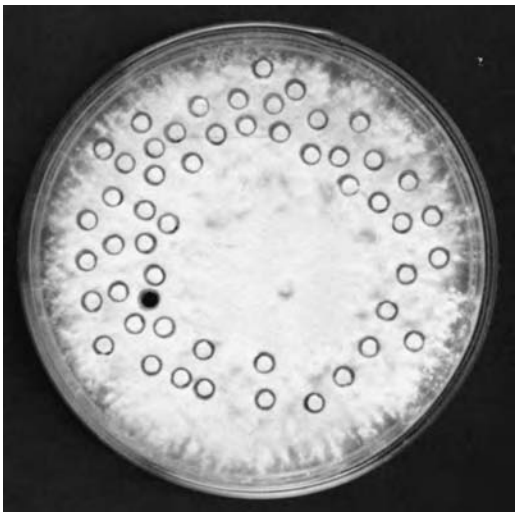


FIGURE 10.4 Plate of *Pleurotus* strain with cut inocula about 2 mm in diameter.

straws, and ten replicate ampoules are wrapped into one package with aluminum foil. The outsides of the packages are labeled with the appropriate labels (Figure 10.6). They are autoclaved at 1.2 kg/cm^2 at 121°C for 20 min.

c. Cryoprotectant

A 10% aqueous solution of glycerol or 10% aqueous DMSO (dimethylsulfoxide) can be used as a cryoprotectant. Each ampoule is filled with a 10% aqueous solution of glycerol or of DMSO to within 5 to 10 mm of the top, using a hypodermic syringe that allows the ampoule to be filled from the bottom, thus avoiding the formation of airlocks (Figure 10.7). Then, five to eight blocks of mycelium for inocula are introduced aseptically into the straw ampoule, using a sterile dissecting needle. Then, the ampoule is sealed. Next, the ampoule is tested for leakage by pressing it firmly on the table.[6]

d. Storage in Liquid Nitrogen

Ten replicate ampoules of each strain are wrapped in aluminum foil within one package. Each package measures approximately 60 × 24 × 12 mm. A canister of the nitrogen tank BT37 from

FIGURE 10.5 Sealing the end of a short drinking straw with a polythene heat-sealer.

FIGURE 10.6 The short drinking straw, the small labels, and the prepared straw ampoules wrapped in small packages with aluminum foil and labeled on the outside by marker pen.

FIGURE 10.7 Introducing the cryoprotectant, a 10% aqueous solution of glycerol, into a sterile straw ampoule with a syringe.

FIGURE 10.8 A canister of the BT37 with ampoule packages inside. Each canister can accommodate as many as 28 to 30 packages.

FIGURE 10.9 The BT37 liquid nitrogen tank of the L'Air Liquide Cryogenic Equipment division.

the L'Air Liquide Cryogenic Equipment Division, for example, can carry 28 to 30 packages (Figure 10.8). There are six canisters for the BT37 tank (Figure 10.9); therefore, the total number of strains that can be stored is about 180. After placing the packages into the canisters, the canisters are immersed directly into the liquid nitrogen for permanent storage (Figure 10.10). It should be noted that many different vessels are commercially available for storage of mushroom materials in liquid nitrogen.

e. Survival Test

One replicate of each strain is removed from the liquid nitrogen, and the ampoule is thawed by immersion in a water bath at 30°C for about 10 minutes. It is then washed in 75% alcohol to prevent contamination. Next, the ampoule is dried, and the tip is cut off at the air-space end using

FIGURE 10.10 Removing the canister from the BT37 liquid nitrogen tank.

a pair of flamed scissors. Its contents are squeezed onto a complete medium agar plate. The plate is then incubated at 25 to 28°C.

f. General Results

In 1970, San Antonio and Hwang[16] reported that liquid nitrogen preservation appears to offer a reliable means for maintaining mushroom stock cultures. San Antonio[15] further reported that culture viability and mushroom production were not affected by cryogenic storage for 9 years. Some 1012 cultures of *Agaricus bisporus* and related species were stored in liquid nitrogen for 3 to 4 years in the Glasshouse Crops Research Institute, U.K.[7] The overall recovery rate for the whole test was 95% (Table 10.1). Jodon et al.[10] also reported that eight cultivars of the commercial mushroom, *A. brunnecens* (= *A. bisporus*), were preserved in liquid nitrogen for 10 years with no apparent changes in morphological or physiological characteristics. Subsequently, Challen and Elliott[2] found that a 10% aqueous glycerol solution used as a cryoprotectant was good in preserving the cultures of *Agaricus* spp., *Coprinus* spp., *Lentinula* spp., *Pleurotus* spp., *Schizophyllum commune*, *Tremella* spp., *Polyporus* spp., and *Volvariella bombycina*, but was not suitable for *V. volvacea*. They found, however, that a 10% aqueous DMSO solution gave consistent and reliable retrieval of *V. volvacea*.

It has been reported that cultures of all 122 strains from 43 species of Basidiomycetes, including *Agaricus pratensis*, *Auricularia auricula*, *Collybia velutipes*, *Lentinula edodes*, *Pholiota nameko, Pleurotus cystidiosus*, *Pleurotus ostreatus, Pleurotus ostreatus* var. *florida*, *Pleurotus sajor-caju*, *Volvariella bombycina*, and *Volvariella volvacea*, were viable when they were thawed after storage in liquid nitrogen for 12 to 31 months.[4] Slow freezing (i.e., freezing controlled at a cooling rate of 1°C/min) gave a higher survival rate than rapid freezing (i.e., putting the culture samples directly into a liquid nitrogen refrigerator). For example, the average survival rate of cultures protected with 10% glycerol or 5% DMSO using rapid freezing was 81 and 97%, respectively; but the survival rate using slow freezing was 94 and 100%, respectively. Except for two strains of *Tremella fuciformis* (Table 10.2), all other strains tested after storage in liquid nitrogen retained the ability to form fruiting bodies.

TABLE 10.1
Numbers and Range of *Agaricus* Strains in the G.C.R.I. Culture Collection; Survival Test of Representative Strains after Storage for 3 to 4 Years

Species	Number Stored	Number Tested	Number Viable
Agaricus bisporus			
Single-spore isolates			
From two-spored basidia	172	10	10
From three-spored basidia	89	10	10
From four-spored basidia	44	10	9
Commercial spawns	41	10	9
Tissue culture strains	6	6	6
Auxotrophs	6	6	6
Aggregation strains B430/B431	2	2	2
Single spore/mutant accessions	10	10	8
Constructed heterokaryons	56	10	9
Wild *Agaricus* spp. ("W" strains)	17	17	17
Single-spore material of *Agaricus* spp.			
bitorquis	147	4	4
campestris	12	4	4
macrosporus	196	4	4
nivescens	77	4	4
silvaticus	20	4	4
silvicola	76	4	3
Two-spored spp.	41	4	4

Source: Data from Elliott, T.J. and Challen, M.P., in *The Glasshouse Crops Res. Inst. Annu. Rep.*, 194, 1979.

C. A Useful Technique in Genetic Studies

Pukkila[14] described a technique that has been successfully used for storage of genetic strains of *Coprinus cinereus*. She reported that strains can be stored for several years in 15% glycerol at –70°C. Thus, a freezer chest can be used to obtain this temperature, avoiding the necessity of the use of liquid nitrogen. A small amount (0.75 ml) of 15% glycerol is placed in a 1.2 ml cryotube. Added to the vial are small squares (0.5 cm^2) of mycelium cut out by needle from a culture that has been grown on a relatively rich mycological agar. It is reported that the vials can be frozen and thawed several times without loss of viability.

IV. CONCLUSIONS

From the above discussion it should be noted that some basic knowledge of microbiology is definitely required by the people who are interested in engaging in mushroom cultivation in general, but it is particularly important for those involved in culture collection and preservation. It should also be noted that the preparation and maintenance of mushroom cultures requires expertise in special fields for the classification and confirmation of the purity and correct identification of cultures. This is a major problem in many laboratories. The mushroom farmers, particularly those in rural areas or on small-sized farms, should not try to collect and maintain the mushroom cultures by themselves, because they generally lack the proper equipment and knowledge of the techniques required for these operations. They should purchase mushroom cultures from reliable academic institutions or from licensed spawn makers.

TABLE 10.2
The Ability of Cultures of 41 Strains from 23 Species of Basidiomycetes to Form Fruiting Bodies after Storage in Liquid Nitrogen for 12 to 31 Months

Species	Storage Period (months)			Control		After Storage	
				No. of Strains Cultivated	No. of Strains Forming Fruiting Bodies	No. of Strains Cultivated	No. of Strains Forming Fruiting Bodies
Agaricus pratensis	14	14	14	4		5	3
Auricularia auricula		29		4	1	1	1
Collybia velutipes	16	19		2	1	3	3
Ganoderma capense		17		1	1	1	1
Ganoderma japonicum		17		1	1	1	1
Ganoderma lucidum	15	13	13	8	6	4	4
Hericium erinaceus	19	20		4	4	2	2
Lactarium camphoratus	9	9	9			3	2
Lentinula edodes	16	9		2	1	2	1
Pholiota adiposa	20	20		2	1	2	2
Pholiota nameko		20				1	1
Pleurotus cornucopiae		17		1	1	1	1
Pleurotus cystidiosus		12		1	1	1	1
Pleurotus florida		16		1	1	1	1
Pleurotus ostreatus		20		1	1	1	1
Pleurotus sapidus	20	18		4	4	2	2
Pleurotus sajor-caju		19		1	1	1	1
Polyporus versicolor	22	15		2	1	2	2
Poria cocos		31		3	3	1	1
Schizophyllum commune		22		1	1	1	1
Tremella fuciformis	31	16		1	0	2	0
Volvariella bombycina		12		1	1	1	1
Volvariella volvacea	16	16				2	2

Source: Data from Chen, Y.Y., *Acta Mycol. Sin.*, 6(2), 110–117, 1987.

Companies that make mushroom spawn should be run by conscientious persons who are informed about the best available technique. Using this expert knowledge, they should collect and prepare only good, uncontaminated fruiting cultures, which have been proved, through a series of trial experiments, to be of good quality and high yield. The culture and spawn must be pure and reliable. Obviously contaminated, senescent, and degenerated cultures and spawn have no commercial value, and constant attention to details of culture maintenance and spawn production is essential to make certain that the grower is provided with a good spawn.

Because the culture and spawn contain living mycelium, they are subject to numerous changes in their status. Many conditions may cause the culture and spawn to be of poor quality. For example, if the storage conditions are unsuitable, if the spawn has degenerated, if it has become contaminated, or if it has simply become too old, it must be discarded.

Spawn manufacturers are responsible for having good-quality spawn leave their plants, and they must also consider the effect of the conditions of transport and the length of time required for the spawn to reach the grower. That is, it is not sufficient simply for the spawn to be in that good condition when it leaves the plant, but it must also be that way when it arrives at the mushroom farm. Reliable spawn growers will guarantee this.

An excellent review of culture collections and the means of preservation of edible fungi is the account of Smith.[18] Smith not only describes the methods but also discusses the scientific basis for the procedures and the research that has tested the techniques. This concise account provides excellent information for the researcher who must preserve fungal strains for genetic purposes or the maintenance of cultivars.

REFERENCES

1. Buell, C.B. and Weston, W.H., Application of the mineral oil conservation method to maintaining collections of fungus cultures, *Am. J. Bot*., 34, 555–561, 1947.
2. Challen, M.P. and Elliott, T.J., Polypropylene straw ampoules for the storage of microorganisms in liquid nitrogen, *J. Microb. Methods*, 5, 11–23, 1986.
3. Chang, S.T., Miles, P.G., and Wai, C.C., A study of monosporous isolates of *Volvariella volvacea, Mushroom Sci.,* 11(Part 2), 603–621, 1981.
4. Chen, Y.Y., The preservation of basidiomycete cultures by ultra-low temperature freezing, *Acta Mycol. Sin.*, 6(2), 110–117, 1987.
5. Eger, G., Untersuchungen zur Fruchtkörperbildung des Kulturchampignons, *Mushroom Sci*., 5, 314–320, 1962.
6. Elliott, T.J., Alternative ampoule for storing fungal cultures in liquid nitrogen, *Trans. Br. Mycol. Soc*., 67, 545–546, 1976.
7. Elliott, T.J. and Challen, M.P., The storage of mushroom strains in liquid nitrogen, in *The Glasshouse Crops Res. Inst. Annu. Rep*., 194, 1979.
8. Hayes, W.A., Randle, P.E., and Last, F.T., The nature of the microbial stimulus affecting sporophore stimulation in *Agaricus bisporus* (Lange) Sing., *Ann. Appl. Biol*., 64, 177–187, 1969.
9. Hwang, S.W. and San Antonio, J.P., Stability of spawn stocks of the cultivated mushroom after 26 months liquid nitrogen refrigeration (–160°C to –190°C), *Mushroom Sci.*, 8, 35–42, 1972.
10. Jodon, M.H., Royse, D.J., and Jong, S.C., Productivity of *Agaricus brunnescens* stock cultures following 5, 7, and 10 year storage periods in liquid nitrogen, *Cryobiology*, 19, 602–606, 1982.
11. Jong, S.C., Conservation of the cultures, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, London, chap. 5, 1978.
12. Meryman, H.T., Review of biological freezing, in *Cryobiology*, Meryman, H.T., Ed., Academic Press, London, chap. 1, 1966.
13. Miles, P.G., Takemaru, T., and Kimura, K., Incompatibility factors in the natural population of *Schizophyllum commune*. I. Analysis of the incompatibility factors present in fruit bodies collected within a small area, *Bot. Mag*. (Tokyo), 79, 693–705, 1966.
14. Pukkila, P.J., Methods of genetic manipulation in *Coprinus cinereus,* in *Genetics and Breeding of Edible Mushrooms,* Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach Science, Philadelphia, 249–264, 1993.
15. San Antonio, J.P., Stability of spawn stocks of the cultivated mushrooms stored for nine years in liquid nitrogen (–160°C to –196°C), *Mushroom Sci*., 10(Part 1), 103–113, 1979.
16. San Antonio, J.P. and Hwang, S.W., Liquid nitrogen preservation of the cultivated mushroom, *Agaricus bisporus* (Lange) Sing., *J. Am. Soc. Hortic. Sci*., 95, 565–569, 1970.
17. Smith, D., Culture collections, in *Genetics and Breeding of Edible Mushrooms,* Chang, S.T., Buswell, J.A., and Miles, P.G., Eds., Gordon & Breach Science, Philadelphia, 15–35, 1993.
18. Smith, D., Maintenance of fungi, in *Maintenance of Microorganisms*, Kirsop, B.E. and Snell, J.J.S., Eds., Academic Press, London, chap. 11, 1984.
19. Snell, J.J.S., General introduction to maintenance methods, in *Maintenance of Microorganisms*, Kirsop, B.E. and Snell, J.J.S., Eds., Academic Press, London, chap. 3, 1984.
20. Stadelmann, R., Preservation and degeneration of mushroom strains, *Mushroom J.*, 158, 41–44, 1986.
21. Wilson, J.W. and Plunkett, O.A., Practical medical mycology — the culture of fungi as an office procedure, *Arch. Derm. Syph*., 59, 414–429, 1949.
22. Wood, D.A., Primordium formation in axenic cultures of *Agaricus bisporus* (Lange) Sing., *J. Gen. Microbiol*., 95, 313–323, 1976.

11 World Production of Edible Mushrooms

I. INTRODUCTION

A variety of sources indicate that from very early times humans have used mushrooms collected in the wild as food, and it is estimated that the first intentional cultivation of mushrooms took place around A.D. 600, almost 1400 years ago.[6] The first species cultivated was *Auricularia auricula*, the next was *Flammulina velutipes,* which was first cultivated approximately 200 to 300 years later, and the third was *Lentinula edodes*, first cultivated about A.D. 1000 to 1100 (Table 11.1). All three of these species were first cultivated in China. In all, eight edible mushrooms, including five of the current most popular mushrooms, were cultivated prior to the 20th century; the remaining 31 were first successfully cultivated during the 20th century. The great increase in the number of species brought into cultivation in the 1980s and 1990s corresponds with the dramatic acceleration in total worldwide cultivated mushroom production (e.g., 0.90 million tons in 1975; 1.26 million tons in 1981; 2.18 million tons in 1986; 3.76 million tons in 1990; 4.91 million tons in 1994; and 6.16 million tons in 1997) of the ten most popular species: *Agaricus bisporus/bitorquis, L. edodes, Pleurotus* spp.*, Auricularia* spp.*, Volvariella volvacea, F. velutipes, Tremella fuciformis, Hypsizygus marmoreus, Pholiota nameko,* and *Grifola frondosa*. Production of the new species has been minimal to date, but it does reflect on the great interest of consumers in known edible mushrooms as well as in new varieties. Mushroom scientists are making a great effort to bring wild species under domestication to satisfy this need.

The extensive use of mechanized cultivated techniques for producing mushrooms in great quantities for food, as with so many other large-scale agricultural activities, is a phenomenon of the 20th century. *Agaricus bisporus*, the species that currently provides by far the greatest quantity of mushrooms, was first cultivated in France around 1600, and it has long been a favorite in Western countries, where it is variously known as the button mushroom, the white mushroom, the cultivated mushroom, or champignon. *Agaricus* mushrooms have been plentifully produced in France for a long time; and cultivation techniques were introduced from there to other European countries, to North America, and recently to countries throughout the world. Following World War II, there was a great spurt in production of *Agaricus*, and the past few decades have also seen great increases in production of *Lentinula* and *Pleurotus*, and to a lesser extent *Volvariella* and *Flammulina.* Largely responsible for this increase in production has been the development of mushroom technology, and in this book we describe many of the cultivation techniques that have been developed for different mushrooms in various parts of the world.

While the cultivation technique determines the amount of production, it is not the only factor determining success in the industry. If the mushroom produced has limited consumer appeal, if its price is too high, if the costs involved in keeping it fresh and getting it to the market in a satisfactorily fresh condition are too great, or if it does not preserve well without excessive loss of flavor, nutritional value, or a change in texture, then an increase in production will not lead to success in the industry. Modern technology making extensive use of costly equipment tends to localize production on a few large farms. Thus, the location of the farm relative to the marketplace, as well as the availability of

TABLE 11.1
Historical Record of Cultivated Mushrooms

Year First Cultivated	Species	Year First Cultivated	Species
600	*Auricularia auricula*	1982	*Dictyophora duplicata*
800	*Flammulina velutipes*	1982	*Hohenbuehelia serotina*
1000	*Lentinula edodes*	1982	*Oudemansiella radicata*
1232	*Poria cocos*	1983	*Armillaria mellea*
1600	*Agaricus bisporus*	1983	*Grifola frondosa*
1621	*Ganoderma* spp.	1983	*Pleurotus sapidus*
1700	*Volvariella volvacea*	1984	*Amanita caesarea*
1800	*Tremella fuciformis*	1984	*Coprinus comatus*
1900	*Pleurotus ostreatus*	1984	*Hericium coralloides*
1950	*Agrocybe cylindracea*	1985	*Sparassis crispa*
1958	*Pleurotus ferulae*	1985	*Tremella mesenterica*
1958	*Pleurotus florida*	1986	*Morchella* spp.
1958	*Pholiota nameko*	1987	*Lyophyllum ulmarium*
1960	*Hericium erinaceus*	1988	*Lentinus tigrinus*
1961	*Agaricus bitorquis*	1989	*Gloestereum incarnatum*
1962	*Pleurotus flabellatus*	1990	*Tricholoma lobayense*
1969	*Pleurotus cystidiosus*	1991	*Tricholoma gambosum*
1973	*Hypsizigus marmoreus*	1991	*Tricholoma mongolicum*
1974	*Pleurotus sajor-caju*	1997	*Cantharellus cibarius*
1981	*Pleurotus citrinopileatus*		

Sources: Data from Chang, S.T., in *Mushroom Biology and Mushroom Products,* Chinese University Press, Hong Kong, 3–20, 1993; Danell, E. and Camacho, F.J., *Nature,* 385, 303, 1997; Huang, N.L., *Cultivation of Eighteen Rare and Delicious Edible Mushrooms,* China Agricultural Press, Beijing, China, 164, 1997; and Chang, S.T. and Buswell, J.A., *Int. J. Med. Mushroom,* 1, 139–146,1999.

supplies of substrate materials, is obviously an important consideration. If the farm does not have access to a market large enough to accept the crop as fresh mushrooms at a financially satisfactory level, then an effective method for preservation of the mushrooms is essential. The most widely used method of mushroom preservation is by **canning**, but all species do not can well.

The length of time that a fresh mushroom will remain in good condition varies from species to species. In general, maintaining the mushrooms at lowered temperatures increases the length of time that the mushrooms can be marketed fresh, but some species may undergo rapid physiological and nutritional postharvest changes. For any species to be cultivated in large amounts, it is essential that there be a satisfactory method for preservation to take care of that part of the yield that cannot be marketed as the fresh mushroom.

Next in importance to canning as a means of preservation is **drying**. Drying can successfully preserve many mushrooms, but *Lentinula* is outstanding in this respect. *Lentinula* is readily preserved with maintenance of fine flavor by drying, and thus it can be easily shipped. The existence of good methods for preservation of *Agaricus* (canning) and *Lentinula* (drying) is one reason that these two mushrooms account for such a large proportion of the total world production of cultivated mushrooms (approximately 85% in 1983–1984). Because of the widespread familiarity of the public with the fresh mushroom as a result of its availability in many geographic areas, the preserved (canned) product of *Agaricus* is widely acceptable. The fine quality of the preserved (dried) mushroom of *Lentinula* has spread its appeal to countries outside Asia.

Pickling in brine is a common means of preservation of mushrooms to be shipped in bulk, but for the best results some treatment of the preserved product is required before they are used. **Freeze-drying** may preserve better flavor, but it is more costly.

The fresh mushroom is invariably the one with the greatest gastronomic appeal. Preservation techniques can only attempt to minimize a loss of flavor and change of texture that make a particular mushroom species popular. Even in an era when rapid transport by air permits shipment for long distances of vegetables, fruits, flowers, and even mushrooms, an extensive increase in consumption of fresh mushrooms will be difficult to achieve if the price is kept high by expensive transportation charges.

The number of cultivated species is increasing, and this will have the effect of augmenting the total world production in the future because new species are unlikely to be substituted for others by the consumer, but they will be added to the ones that are already popular. Production of the existing cultivated species has shown a steady increase for decades, and more countries are engaging in mushroom cultivation as an agricultural technology. These factors argue against total mushroom production reaching a plateau in the near future.

There is also increased interest in some of the species for which cultivation techniques have been available for some time, and this has spurred the development of modifications of the cultivation techniques. Examples of such species are *V. volvacea*, which was first cultivated in China around 1700; *T. fuciformis*, another favorite mushroom in Asia, which was first cultivated about 1800, also in China; and the oyster mushroom, various species of the genus *Pleurotus*, for which cultivation techniques were first introduced in Europe around 1900.

In this book we describe some of the techniques that have been used in the cultivation of a number of these species and illustrate with case studies the cultivation practices that have developed. It should be obvious, but it is not always recognized, that a technology that works well for a species in one country may not work well for another species, or for the same species in a different part of the world where the environmental conditions are not the same and the plentiful substrates are different. Changing mushroom technology, based on solid principles developed by mushroom science, is a trend that is accelerating and may soon bring about changes in the cultivation of edible mushrooms that we can only guess at now.

II. SPECIES CULTIVATED COMMERCIALLY

A. *Agaricus bisporus*

The cultivated mushroom produced in greatest amounts, about 2 million metric tons (MT) in terms of fresh equivalent weight in 1997, is *A. bisporus*. It is cultivated in more than 100 countries located on every continent. In 2000, the largest amount of 637,304 MT were grown in China; followed by 391,000 MT in the United States; 263,000 MT in the Netherlands; 180,000 MT in France; 105,000 MT in Poland; and 102,000 MT in Italy.

B. *Lentinula edodes*

The mushroom that is second in total worldwide production is *L. edodes*. In 1997, the production worldwide was more than 1,564,000 MT with almost 88.8% (1,397,000 MT) produced in China. As late as 1983, Japan accounted for more than 82% of the total world production of this mushroom. At that time, China represented only 9.4%. Only 8 years later, in 1991, China produced 57% and in 1997 its share had risen to 88.8%. On the other hand, the percentage of *Lentinula* production in Japan dropped from 82.8% in 1983 to 7.3% in 1997.[4] The production of this mushroom, which was initially limited to Asian countries, has now spread to the United States, Australia, Canada, Brazil, and a few European countries. It is expected that more countries will grow this mushroom, and it could surpass *A. bisporus* and rank in first place for cultivated edible mushrooms worldwide in the near future.

C. *Volvariella volvacea*

Volvariella volvacea (the straw mushroom) is a high-temperature mushroom that is grown in largest amounts in tropical and subtropical regions of Asia, although it can also be grown seasonally in temperate regions. In 1997, 180,000 MT were produced, with Vietnam, China, Taiwan, Thailand, and Indonesia the largest producers. Although Southeast Asia is the stronghold for growing *V. volvacea*, it is also grown in Madagascar, Africa, and India. In recent years, this mushroom has become the fastest expanding mushroom in Vietnam, where 20,000 MT were produced 1996, 41,000 MT in 1997, and more than 100,000 MT in 1998.

D. *Flammulina velutipes*

Another mushroom of great popularity in Asia is *F. velutipes*, the winter mushroom, which, as the name implies, is a low-temperature mushroom. The worldwide production in 1997 totaled 284,000 MT with China, Japan, Korea, and Taiwan the leading producers. In the period from 1990 (143.0 thousand MT) to 1997 (284.7 thousand MT), production of *Flammulina* in the world has doubled.

Flammulina has been used as an experimental organism in laboratories all over the world, and the requirement of low temperature for fruiting is the only significant factor that limits increased cultivation of this wood-rotting mushroom; in temperate regions this need not be a major drawback.

E. *Auricularia* spp.

Long popular in China as a food and for its medicinal properties, it is not surprising that *Auricularia* is reported to be the first mushroom cultivated by humans.[6] This jelly fungus is commonly known as *Mu Erh* (wood ear, wooden ear, or tree ear) in Chinese. It is also known as Jew's ear (a contraction of Judas' ear). It is worldwide in its distribution in temperate and tropical regions. *Auricularia polytricha* is a widely distributed tropical and subtropical species, while *A. auricula* is a temperate species that occurs occasionally in the subtropics. In 1997, 485.3 thousand MT of *Auricularia* were cultivated. Extensive cultivation takes place in China, Taiwan, Thailand, and the Philippines, and, because the mushroom can be produced on basically a sawdust substrate of a variety of woods in plastic bag culture, cultivation of *Auricularia* need not be seriously restricted by geography. Its production in 1997 ranked it as the fourth most important edible mushroom in the world.

F. *Pleurotus* spp.

Cultivated members of the genus *Pleurotus*, the oyster mushroom, is rapidly increasing, and 875.6 thousand MT were produced in 1997. Various species of this wood-rotting fungus are found the world over, and its edibility is especially appreciated in Europe and Asia. In the past decade its popularity has increased, and it is cultivated in many countries in Europe and Asia, including Italy, Germany, the Netherlands, Belgium, China, Japan, Taiwan, India, Singapore, Thailand, Pakistan, and Indonesia. To illustrate the increasing popularity of *Pleurotus* during the period from 1986 to 1997, the production in the world has increased by a factor of almost 5.2. Cultivation has begun in Nigeria, Mexico, Brazil, Colombia, Canada, the United States, Namibia, Tanzania, Zambia, and Malawi. One reason for the great interest in species of *Pleurotus*, besides their taste and nutritional appeal, is that they secrete a wide range of enzymes, which can degrade all the three key categories of polysaccharides found in the biomass of forest and agricultural crop residues: lignin, cellulose, and hemicellulose. They are therefore capable of growing on a wide range of substrates. Furthermore, there are some species that will grow and fruit at a relatively high temperature, a feature that makes for lower production costs in tropical or subtropical areas, or even in temperate regions during the summer season. It is probable that production of *Pleurotus* will greatly increase in the near future and that techniques will be developed and fruiting stocks bred that will make possible the cultivation of *Pleurotus* in geographic regions of diverse environmental conditions on a variety of available substrates.

G. *Pholiota nameko*

A wood-rotting fungus whose popularity and production are greatest in Japan, *P. nameko* (the viscid mushroom) has been produced in relatively stable amounts since 1975, with an overall annual increase amounting to about 5 or 6% in Japan between 1975 and 1985. In 1997, the worldwide total production of *Pholiota* was 55.5 thousand MT, ranking it ninth in total production. With a low-temperature requirement for fruiting, it is unlikely that this species will be extensively produced outside temperate regions, unless the mushroom breeder develops some high-temperature fruiting stocks of *P. nameko*. This may be a goal with reasonable expectation of success in light of Arita's[1] report that his earlier studies of temperature requirements for fruiting in this fungus revealed that 45 stocks separated into two groups on the basis of temperature requirement. The higher-temperature group requires temperature in the range from 8 to 20°C, not very high but sufficiently different from the lower temperature group (5 to 15°C) to indicate some promise for breeding for fruiting at more elevated temperatures. One feature of fruiting at the higher temperatures that would have to be overcome, however, is the tendency for small pilei and slender stipes.

H. *Tremella fuciformis*

Tremella fuciformis is known as the white jelly fungus or silver ear mushroom. It is a long-time favorite in China, especially for medicinal purposes, and the dried fruiting bodies of *Tremella* are conspicuous in most pharmacies specializing in Chinese medicines. It is also consumed as a food and usually as a special dessert. *Tremella* is being produced in greater amounts now than in some years past, because new techniques have been developed for its stable production using sawdust mixed with cottonseed hulls, which have removed some of the hazards in *Tremella* cultivation and the requirement for wood logs. In 1997, 130,000 MT (fresh weight) of *Tremella* were produced, mostly in China. The dried mushroom is shipped to countries in Southeast Asia, Japan, and to Europe, North America, and Australia. It will certainly be welcomed by the people of Asian origin in those areas. From the standpoint of increasing its use as a food in Western countries, the probabilities of *Tremella* being widely accepted rank below those of the other mushrooms that have been considered. Although Chen and Hou[8] consider the fact that it has no strong flavor an advantage in making it acceptable to Westerners, it is our experience that most Westerners find it tasteless although it has an interesting texture. There is room for marketing an ample increase in production in the countries where it is produced, in Hong Kong, and in various countries of Southeast Asia, however. It is interesting that in nature the distribution of this fungus is wide, but what effect this may have on local acceptance as a food is uncertain.

I. Mushroom Species Commercially Cultivated Recently

Among the 14,000 or more species of mushrooms, more than 40 have been commercially cultivated (Table 11.2). During the last decade, the following 15 species have been considered as rare and delicious species with great potential for further development*: Pleurotus nebrodensis, P. eryngii, P. citrinopileatus, Coprinus comatus, Agaricus blazei, Agrocybe aegerita, A. chaxinggu, Hypsizygus marmoreus, Grifola frondosa, Clitocybe maxima, Dictyophora indusiata, Pholiota adiposa, Lentinula giganteus, Oudemansiella radicata,* and *Tricholoma giganteum*. They are all commercially cultivated on various scales in China, Taiwan, and Japan.

J. Mycorrhizal Fungi: *Tuber, Tricholoma*

The most difficult mushrooms to cultivate are those associated with another organism in a symbiotic relationship, such as mycorrhizal fungi with the roots of plants. Because some of these mushrooms are among the most highly prized, *Tuber melanosporum* and *Tricholoma matsutake*, for example, much study has been made on methods to encourage mushroom development on acreage in which

TABLE 11.2
Species of Commercially Cultivated Edible Mushrooms

*Agaricus bisporus**	*Macrolepiota procera*
*Agaricus bitorquis**	*Oudemansiella radicata*
Agaricus blazei	*Pholiota nameko*
Agrocybe aegerita	*Pleurotus citrinopileatus*
Agrocybe chaxinggu	*Pleurotus cornucopiae*
Auricularia auricula	*Pleurotus cystidiosus*
Auricularia delicata	*Pleurotus djamor*
Auricularia fuscosuccinea	*Pleurotus eryngii*
Auricularia polytricha	*Pleurotus ferulae*
Coprinus comatus	*Pleurotus florida*
Clitocybe maxima	*Pleurotus nebrodensis*
Dictyophora duplicata	*Pleurotus ostreatus**
Dictyophora indusiata	*Pleurotus sajor-caju*
*Flammulina velutipes**	*Stropharia rugoso-annulata*
Grifola frondosa	*Tremella aurantialba*
Hericium erinaceus	*Tremella fuciformis**
*Hypsizygus marmoreus**	*Tricholoma giganteum*
*Lentinula edodes**	*Tricholoma lobayense*
Lentinula giganteus	*Volvariella diplasia*
Lepista nuda	*Volvariella esculenta*
Lyophyllum ulmarium	*Volvariella volvacea**

* Species whose production has reached an industrial scale.

a suitable ground is prepared and planted with seedlings of host species that have been inoculated with the proper fungal mycelium. Extensive ecological studies of the Perigord truffle, *Tuber melanosporum*, and the matsutake mushroom, *Tricholoma matsutake*, have been responsible for the development of techniques leading to better and more consistent yields of both of these important mushrooms. While the amounts of both of these species that become items of commerce are not great, each brings a high price.

Korea exports its total production of *Tricholoma matsutake* to Japan, and in 1984 the value of the matsutake (U.S. $24,000,000) exported to Japan exceeded the export value from Korea of all other mushrooms combined. In 1985, Japan produced matsutake mushrooms valued at approximately $82,667,000, which was almost twice the value of the production in 1975.

Production of truffles in France in 1975 was estimated at 60 to 120 MT, with a worldwide production of 150 to 300 MT. It is interesting to note that a hundred years previously, France produced 1000 to 1500 MT of truffles per year, and Italy produced 100 MT.[11] As stated above, with modern growing techniques, a consistent increase in production of *Tuber* by semicultivation methods is anticipated.

The semicultivation methods of farming these mycorrhizal mushrooms will have application to other mycorrhizal fungi whose mushrooms are esteemed for their taste appeal, such as *Boletus edulis, Cantherellus cibarius*, and *Amanita caesarea*.

K. *Termitomyces*

Another group of delicious mushrooms, termite mushrooms, has not yet been successfully cultivated. The termite mushroom species, which can, however, be "grown" or "cultivated" by termites, are grouped in the genus *Termitomyces*. Most termite species make their nests in the soil. Sometimes

the nests are practically invisible; sometimes their mounds are 10 m high. Pseudorhiza of *Termitomyces* is found in the chambers of nests or mounds, where primordial fruiting bodies are developed.

III. GENERAL INFORMATION FOR MUSHROOM GROWERS

Prospective mushroom farmers should know that whereas in typical agricultural crop production activities the farmer has to clear vast expanses of land before planting, a practice that often leads to extensive deforestation, soil erosion, and environmental degradation, mushroom farming ventures are more environmentally friendly. In fact, high-value mushroom crops can be raised in simple plastic bags, inside a low-cost mushroom house, even inside a cave. But the plastic bag must be loaded with appropriate substrates preferred by the specific mushroom being farmed, the substrates used must be appropriately treated, and the mushroom spawn ("seed stock") must be of high quality and of optimum age.

Most mushrooms require lignocellulosic substrates for growth. Fortunately, lignocellulosic substrates are very abundant in our forest ecosystems, in our woodlands, in our grasslands, and in the wide spectrum of agricultural crop residues generated by our farmers, which are often discarded as waste. Mushroom enzymes can break down lignin, cellulose, and hemicelluloses present in these organic materials into simpler molecules, which the mushrooms then use for their growth and metabolism.

Actually, in mushroom farming a very robust crop can be obtained, if the science and art of their agronomy has been mastered. This includes ensuring that the substrate in the plastic bag seeded with mushroom spawn is kept sufficiently moist and that the relative humidity in the mushroom farming microenvironment is kept at the appropriate level. Mushroom farming is both a science and an art. The science is developed through research; the art is perfected through curiosity and practical experience. Mushroom growth dynamics involve stages similar to those exhibited by common agricultural crop plants. For example, there is a vegetative growth phase, in which the mycelia grow profusely, and a reproductive (fruiting) phase, when the umbrella-like body that we call a mushroom develops. In agricultural plants when the plants switch from vegetative growth to reproductive growth, retardation of the tips for further elongation is an obvious phenomenon in nature. The same principle applies in mushroom production. After the vegetative (mycelial) phase has reached maturity, it is the time for induction of fruiting. The mycelial growth tips should be retarded by regulating the environmental factors, factors that are generally called "triggers" or "environmental shocks." Switching on the light, providing fresh air, and lowering temperatures can trigger fruiting (Figure 1.6, in Chapter 1).

Cultivation of edible mushrooms can be a kind of primitive farming or it can be a high-technology industry. In both cases, however, continuous production of successful crops requires precision (as mentioned in the introduction). Indeed, it is not as simple as some people suppose. Mushroom cultivation requires adherence to precise procedures. If one critical step is ignored, troubles can occur, which can lead to a substantially reduced mushroom crop yield. For example, if there is failure to adjust the pH of the substrate to the critical level required by the specific mushroom or if the substrate has not been properly pasteurized, the mushroom mycelium can be overgrown by unwanted, intrusive competitive microorganisms. However, by use of good-quality spawn and suitable substrates, a reasonably high and reliable yield of mushrooms can be achieved under optimum growing conditions if the ten cardinal keys to mushroom farming success, which are listed below, are closely observed. It also should be kept in mind that successful mushroom growers make liberal use of the senses of sight, smell, and touch to evaluate the progression of the composting or substrate preparation process and the quality of the final product — compost or substrate. The gross characteristics of compost result from a number of complex physical, chemical, and microbial processes that comprise composting, for example, for growth of *Agaricus bisporus*. The substrate consists of substances, such as sawdust and cereal straws, which are utilized in

growing *Lentinula edodes* and *Pleurotus* mushrooms, respectively. Some draw a distinction between "composts," which are fermented, and "substrates," which are nonfermented, both of which serve as media for the support of growth.

Ten Key Guidelines for Mushroom Farming

1. Secure a good-quality spawn from a reliable source.
2. Obtain and store nonmoldy and nonrotted raw materials.
3. Mix the ingredients of the substrate thoroughly and properly.
4. Measure and adjust the pH value of the substrate (beginning growers) or estimate the pH value (experienced farmers).
5. Maintain the moisture content of the substrate in the range of 60 to 65%. (The simplest way to measure the moisture content of the substrate is to squeeze the substrate firmly by hand. The moisture content is correct when just a little water drips from the fingers.)
6. Treat the prepared substrate to eliminate contaminating organisms according to the species of mushrooms being cultivated, e.g., *Pleurotus sajor-caju* by hot water dip method or pasteurization, *Lentinula edodes* and *Ganoderma lucidum* by sterilization method.
7. Check the treated substrate for freedom from contamination and correct moisture content before inoculating the substrate with 2 to 5% by dry weight of good-quality spawn. Hygienic conditions must be used. Spawning should be done as quickly as possible to avoid the chance of contamination.
8. Keep the inoculated/spawned substrate, usually in plastic bags, under the proper growth temperature for mycelial running of the mushroom being cultivated. The bags should be placed on the shelves rather than on the ground where the temperature is usually lower.
9. Switch the vegetative growth to fruiting. After the mycelium has matured and become established throughout the substrate, adjust the environmental factors to change from vegetative growth to fruiting.
10. Avoid putting water directly on the fruiting bags during the pinhead stage. If the growing room is too dry during hot weather, spray water on the walls or on the ground.

IV. TRENDS

A. Production Methods Breaking the Barriers of Climate and Geography

1. *Agaricus*

Agaricus production in Western countries involves the most advanced and highly mechanized technology in the mushroom industry. It has been responsible for the high levels of quality production of *Agaricus* in the United States, France, the Netherlands, and the United Kingdom. These countries were the leading producers through 1960, but in 1965 Taiwan emerged as the third leading producer of *Agaricus,* and in 1998 China became the leading producer. Production of this mushroom in 1998 was estimated to be 426,000 MT in China and 384,472 MT in the United States. This was notable for three reasons. First, both Taiwan and China did not use a highly mechanized technology for the production of *Agaricus* on a relatively few large mushroom farms, but, rather, developed the industry as a cottage enterprise on thousands of farms with small mushroom houses constructed of bamboo frames and banana leaf and/or straw and plastic for the roofs and sidings. Second, *Agaricus* had not been grown in a subtropical country previously, but it was found that this was possible on a seasonal basis with a single crop grown in the period from September through March. Last, in the absence of a plentiful supply of horse manure, a **synthetic compost** was developed with rice straw as the main ingredient.

For 15 years Taiwan remained among the top five *Agaricus*-producing countries, and Taiwan continued to produce substantial amounts in the 1980s, although much attention and effort has been devoted by Taiwan to the production of other species of edible mushrooms, such as *Lentinula* and *Pleurotus*. The result of this diversification is that mushroom growing in Taiwan is less of a seasonal activity.

While Taiwan led the way in production of *Agaricus* among the Southeast Asian nations, South Korea also produced large amounts for export; and, by 1980, China produced 100,000 MT and was the third leading producer of *Agaricus* in the world. By 1983, China was second only to the United States in total production of *Agaricus* and maintained that position through 1986. By 1998, China was the first producer and the United States became the second. From 1998 through 2000, there was a 49% increase in China. This is remarkable in that this was accomplished with limited mechanization and less efficient production methods.

The result of extensive production of *Agaricus* in Asia, much of which reaches foreign markets as canned mushrooms, is that the Asian countries are providing a greater share of the canned mushrooms, which other nations import. The reason is that the mushrooms are produced at a lower cost and can be sold at a lower price than the canned mushrooms produced by the mechanized growers in the Western countries. Consequently, a larger share of the *Agaricus* mushrooms produced by Western countries is being sold fresh.

2. *Lentinula*

Lentinula production has undergone some significant changes in recent years. Production on wood logs was, until a few years ago, the only method of cultivating *Lentinula*, and even in 1987 it remained the principal means of cultivation. The technology for wood log cultivation was highly developed in Japan, and it is also used in China, Taiwan, South Korea, and other countries; but, as described in Chapter 13, other cultivation techniques that promise to become more commonly employed in the future are being used. The major trend has been the growth of *Lentinula* in plastic bags containing a variety of substrates. The plastic bag cultures can be grown in mushroom houses in which environmental conditions can be controlled. The use of the **plastic bag culture technique** and utilization of waste materials for substrate rather than wood logs are significant factors in extending *Lentinula* production to countries where it has not previously been produced.

B. World Production of Mushrooms

Since the end of World War II, the trend in mushroom production has been one of steady increase. In an overall view, the world production of cultivated edible mushrooms was 170,000 MT in 1960 and 6,158.3 thousand MT in 1997 (Table 11.3). The annual increase in different interval periods ranges from 5.0 to 20.1%, with an annual average increase of 12.4% during the period from 1965 to 1997. Two peaks of increase developed in the early 1960s and in the late 1980s, e.g., from 1960 to 1965 and from 1986 to 1990 when mushroom production increased overall by 100.6 and 74.4%, respectively, and had an annual increase of 20.1 and 18.6%, respectively. In Western countries, mushrooms were used mainly as condiments for the flavor that they imparted to the main dish. More recently, with their greater availability and increased recognition of their nutritional value, they are widely used in various culinary dishes.[12] This change in pattern of usage has been accompanied in recent years by the availability of more species. People in Asia are accustomed to having a variety of mushrooms available for different dishes, and people in Europe have for a long time availed themselves of wild mushrooms, with many species available in the local markets. The white button mushroom still is the most preferred mushroom in Western Europe and North America; however, there has been an increase in the consumption of other mushrooms, such as the oyster and the shiitake mushrooms. Megens[20] has summarized the five main driving forces affecting the *Agaricus* mushroom industry: (1) saturation in demand of the mushroom; (2) consumer shifts toward value-added products,

TABLE 11.3
World Production of Cultivated Edible Mushrooms from 1960–1997 (fresh equivalent weight)

Year	Production (× 1000 MT)	Increase (%)	Annual Increase (%)
1960	170.0		
1965	341.0	100.6	20.1
1970	546.0	60.0	12.0
1975	916.0	67.8	13.6
1981	1257.2	37.3	6.2
1986	2176.0	73.1	14.6
1990	3794.0	74.4	18.6
1991	4273.0	12.6	12.6
1994	4909.3	14.9	5.0
1997	6158.4	25.4	8.5

Annual average increase 12.4%

Sources: Data from Delcaire, J.R., in *The Biology and Cultivation of Edible Mushrooms,* Academic Press, New York, 727–793, 1978; Sharma, S.R., in *Advances in Mushroom Biology and Production,* Mushroom Society of India, Solan, India, 193–203, 1997; and Chang, S.T., *Int. J. Med. Mushroom,* 1, 291–300, 1999.

such as new varieties and pre-cut fresh products; (3) increasing demand for organic products; (4) increasing power of supermarkets; and (5) increasing competition. Because supermarkets increasingly ask growers for higher quality and lower costs, this results in increasing competition between countries. Any country that can deliver year-round fresh mushrooms will have a greater advantage in free competition markets. The industry has to recognize that the consumers demand change rapidly, and they have increasingly higher demands in terms of quality, variety, and food safety. In the future a restructuring that can support the shift toward a demand-driven mushroom industry is necessary. As shown in Table 11.4, the world total production of *Agaricus* mushrooms as a percentage of all mushrooms produced decreased from 71.6% in 1981 to 31.8% in 1997, even though the actual production increased from 900×10^3 MT in 1981 to 1955.9×10^3 MT in 1997, a 2.2-fold increase.

World production of cultivated edible mushrooms over a number of years is shown in Table 11.3. In 1981, production totaled 1257.2 thousand MT; in 1986, 2176.0 thousand MT. During those 5 years, mushroom production increased by 73.6% and there was an annual increase of 14.7%. In 1990, 3,794 thousand MT were produced and in the 4 years between 1986 and 1990, production increased 72.5% with an annual increase of 18.1%. In 1994, 4,909.3 thousand MT were produced in those intervening 4 years, an increase of 30.5% with an annual increase of 7.6%. In 1997, production totaled 6158.4 thousand MT, and in those 3 years production increased 25.4% with an annual increase of 8.5%. Overall, the world mushroom production increased over 12% annually during the period from 1981 to 1997. However, *Agaricus* mushrooms decreased in percentage in the world total production, again, mainly due to increased demand for other alternative edible mushrooms. For example, *Lentinula* mushrooms increased both in percentage from 14.3% in 1981 to 25.2% in 1997 and in production from 180.0 thousand MT to 1564.4 thousand MT. *Pleurotus* mushrooms increased in percentage from 2.8 to 14.2% and in production from 35.0 thousand MT to 875.6 thousand MT with a 25-fold increase. *Auricularia* mushrooms increased from 0.8% in 1981 to 7.9% in 1997, and the production also increased from 10.0 thousand MT in 1981 to 485.6 thousand MT in 1997, a 48.5-fold increase.

In percentage terms, the yield of ten major cultivated mushrooms accounted for 91.6% of the total world production. The **Big Six mushrooms**: *Agaricus* (31.8%), *Lentinula* (25.4%), *Pleurotus* (14.2%), *Auricularia* (7.9%), *Flammulina* (4.6%), and *Volvariella* (3.0%) contributed 86.9%. It should be noted that among these six species, in 1994, only *Agaricus* and *Pleurotus* were cultivated

TABLE 11.4
World Production of Cultivated Edible Mushrooms in Different Years

Species	1981		1986		1990		1994		1997	
	Fresh Wt. × 1000 MT	%	Fresh Wt. × 1000 MT	%	Fresh Wt. × 1000 MT	%	Fresh Wt. × 1000 MT	%	Fresh Wt. × 1000 MT	%
Agaricus bisporus/ bitorquis	900.0	71.6	1227.0	56.2	1424.0	37.8	1846.0	37.6	1955.9	31.8
Lentinula edodes	180.0	14.3	314.0	14.4	393.0	10.4	826.2	16.8	1564.4	25.4
Pleurotus spp.	35.0	2.8	169.0	7.7	900.0	23.9	797.4	16.3	875.6	14.2
Auricularia spp.	10.0	0.8	119.0	5.5	400.0	10.6	420.1	8.5	485.3	7.9
Volvariella volvacea	54.0	4.3	178.0	8.2	207.0	5.5	298.8	6.1	180.8	3.0
Flammulina	60.0	4.8	100.0	4.6	143.0	3.8	229.8	4.7	284.7	4.6
Tremella	—	—	40.0	1.8	105.0	2.8	156.2	3.2	130.5	2.1
Hypsizigus	—	—	—	—	22.6	0.6	54.8	1.1	74.2	1.2
Pholiota	17.0	1.3	25.0	1.1	22.0	0.6	27.0	0.6	55.5	0.9
Grifola frondosa	—	—	—	—	7.0	0.2	14.2	0.3	33.1	0.5
Others	1.2	0.1	10.0	0.5	139.4	3.7	238.8	4.8	518.4	8.4
Total:	1257.2	100.0	2182.0	100.0	3763.0	100.0	4909.3	100.0	6158.4	100.0
Increasing %				73.6		72.5		30.5		25.4

TABLE 11.5
Production of Cultivated Edible Mushrooms in China as a Percentage of World Production (1000 MT)

Year	Production	Increase (%)	Annual Increase (%)	% of World Production
1978	60			
1986	585	875.0	109.4	26.8
1994	2640	351.3	43.9	53.8
1997	3918	48.4	16.1	63.6
2000	6630	69.2	23.1	—

Sources: Data from Chang, S.T., in *Mushroom Biology and Mushroom Products,* Chinese University Press, Hong Kong, 3–20,1993; Lin, C.M., *The Market of Edible Fungi* [in Chinese], 8, 5, 2002.

worldwide. In 1997, *Lentinula*, a mushroom that can be used both for edible and medicinal purposes, joined the other two species in that it was commercially cultivated in five continents. The other three of the Big Six are grown only in Asia. It is also noted that *Tremella* is cultivated mainly in China, and *Hypsizygus* and *Grifola* in Japan. *Volvariella volvacea*, the paddy straw mushroom, has become the fastest expanding mushroom species in Vietnam, from 20,200 MT in 1996 to 41,000 MT in 1997, and to 100,000 MT in 1998.[4]

One of the greatest changes in the mushroom industry worldwide must surely be the massive increase in production in China. According to figures published by Chang,[4] total production of mushrooms of all species, not just *A. bisporus*, in China in 1986 was between 550,000 and 600,000 MT. By 1997, the total annual production of mushrooms in China was estimated at 4 million MT. It is estimated that in the past decade mushroom production in China has increased at the rate of 18 to 20% per annum and is still rising. The rising rate was even faster than the predicted rate, as shown in Table 11.5. China has become a giant producer and consumer of cultivated edible mushrooms. Total mushroom production in China in 2000 was 6.63 million MT, which amounted to a very much larger share of the total world output (the exact percentage is not known as the world total production of cultivated edible mushrooms for 2000 is not now available). In 1997, production in North America and in Japan made up 7.0 and 6.0%, respectively. A comparison of 1998 and 2000 edible mushroom production in China is shown in Table 11.6. The production for 2000 took a big jump to 6,637,950 MT, increasing 52.6% during the 2 years. The largest increase was *V. volvacea* from 32,000 MT to 111,910 MT, increasing 249.7%. The next are *Hericium erinaceu*s from 2800 MT to 6407 MT, increasing 128.8%, and *Hypsizygus marmorius* from 38,000 MT to 83,832 MT, increasing 120.6%. There are many new species of mushrooms that have been cultivated in recent years in China on a commercial scale with great potential for further expansion; e.g., *Agaricus blazei* Murrill, *Agrocybe aegerita* (Brig.) Sing., *Armillariella mellea* (Vahl. Ex Fr.) Karst, *Auricularia fuscosuccinea* (Mont.) Far., *Coprinus comatus* (Mull. Ex Fr.) S. F. Gray, *Dictyophora duplicata* (Bosc) Fischer, *Dictyophora indusiata* (Vent.:Pers.) Fischer, *Lepista nuda* (Bull.:Fr.) Cooke, *Pleurotus eryngii* (DC. Ex Fr.) Quil., *Pleurotus nebrodensis* (Inz.) Quel., *Stropharia rugosa-annulata* Farlow and Murrill, *Tremella cinnabarina* (Mont.)Pat., *Tremella aurantialba* Bandoni et Zang, and *Tricholoma giganteum* Massee. The two critical success factors for further expansion in both cultivation and production will be dependent on the quality control of mushrooms and in securing markets both domestic and overseas. Good-quality products must be brought to the market at the right time and place. If there is a better response to these challenges, then larger profits will be generated for the farmers. As the production of mushrooms in China has reached about 70% of the world total production in 2000, its influence on the worldwide market cannot be ignored. Therefore, a thorough and professional study of the mushroom industry in China

TABLE 11.6
Comparison of 1998 and 2000 Edible Mushroom Production in China (MT)

Species	Production 1998	Production 2000	Increase (%)
Lentinula edodes	1,338,000	2,205,208	64.8
Pleurotus spp.	1,020,000	1,722,645	68.9
Auricularia spp.	491,000	968,567	97.2
Agaricus bisporus	426,000	637,304	49.6
Flammulina spp.	189,000	299,738	58.6
Volvariella volvacea	32,000	111,910	249.7
Tremella spp.	100,000	103,321	3.3
Hypsizygus marmoreus	38,000	83,832	120.6
Pholiota nameko	31,000	48,296	55.8
Grifola frondosa	10,000	6,234	–37.7
Hericium erinaceus	2,800	6,407	128.8
Others[a]	582,200	444,488	23.6
Total	4,350,000	6,637,950	52.6

[a] There are several new species of mushrooms that have been cultivated recently on a commercial scale with great potential for further expansion, e.g., *Agaricus blazei*, *Coprinus comatus*, *Lepista nuda*, *Pleurotus eryngii*, *Agrocybe aegerita*, *Tricholoma giganteum*, *Auricularia fuscosuccinea*, and *Tremella cinnabarina*, etc. Production of others in 2000 included also *Ganoderma ludidum* and *Boletus* spp.

Sources: Data from Huang, N.L., *Cultivation of 18 Rare and Delicious Edible Mushrooms,* China Agricultural Press, Beijing, China, 164, 1997; Lin, C.M., *The Market of Edible Fungi* [in Chinese], 8, 5, 2002.

and the existing domestic and international market scenarios should be conducted and compiled officially or privately. The results of the survey should be presented in a form that is both informative as well as attractive and provide a definitive reference regarding the global picture of the mushroom trade, the status of the mushroom industry in China and in other major producing countries, and the global demand.

The percentages of the world production of cultivated mushrooms in the major regions for the years of 1994 and 1997 are shown in Table 11.7. By comparison, between 1994 and 1997, the Asian region increased its share of percentage from 69.3 to 74.4%; Latin America, from 0.2 to 0.8%; and Africa, from 0.3 to 0.6%. The North American region decreased from 9.5 to 7.0%; Europe, from 19.5 to 6.3%; and Oceania, from 1.0 to 0.9%. Similar figures are reported[11] that, whereas in 1986 North America accounted for 15% of world production and Europe for 27%, by

TABLE 11.7
Percentage of Total World Production of Cultivated Edible Mushrooms in Different Continents for the Years 1994 and 1997

	Asia	North America	Latin America	Europe	Africa	Oceania	Total
1994	69.3	9.5	0.2	19.5	0.3	1.0	99.8
1997	74.4	7.0	0.8	16.3	0.6	0.9	100.0

1997 the corresponding figures were only 7 and 14%, respectively. By contrast, China in 1997 accounted for 63% of the world production of mushrooms. It should be noted particularly that the success of growing *Pleurotus sajor-caju* (Fr.) Sing., on water hyacinths in Africa[7] and in growing *Lentinula edodes* from coffee wastes in Colombia in 1998 (Chang, unpublished data) will enhance the mushroom cultivation and production in these two continents because both water hyacinths and coffee wastes are available in vast amounts in these two regions.

Africa is predominantly an agricultural region. Apart from agricultural and forest wastes, the region also has an overabundance of the waterweed commonly known as water hyacinth. This aquatic weed has become a serious problem because it grows too fast and, in the process, chokes waterways, blocks navigation, reduces fishing activities, and, in some cases, blocks water pumps. Many people in Africa view this as a serious problem.[21] As with agricultural and forest wastes, this undesirable waterweed is composed mainly of cellulose, hemicellulose, and lignin,[13] on which mushrooms grow. There should be no doubt that mushroom cultivation technology can be translated into thriving cottage industries, which could make important contributions to the nutrition and economic welfare of many people in Africa.

Geographically, coffee grows only between the tropic of Cancer and the tropic of Capricorn and is a very important economic crop for the tropics. Green coffee beans are obtained from coffee cherries by removal of the pulp through a dry or wet process. Green coffee beans are then transported to plants for roasting and further processing to produce instant coffee. Only 0.2% of the biomass of the coffee tree is consumed and the remaining 99.8% is considered waste material during the processing. Coffee pulp, consisting of the exocarp and the mesocarp of the cherries, has reportedly been used for the cultivation of *Pleurotus ostreatus* (Jacq.:Fr.) Quel.[14,18,19] The insoluble spent coffee ground (SCG), the major solid wastes during instant coffee production, can be utilized in two ways to cultivate *L. edodes*.[16] One way is to use it as the major component in the substrate (85% SCG plus 15% wheat bran) to replace the control substrate (65% sawdust, 20% used tea leaves, and 15% wheat bran). Another way is to use SCG as a supplementary component (20 to 35%) to replace part of the sawdust. In the former case, the yield is as good as the control substrate. In the latter case, the yield is about 40% higher than that of the sawdust substrate. The results obtained at Cenicafe in Colombia for growing *L. edodes,* the second most important cultivated mushroom, are very promising in regard to utilization of coffee wastes. This is the case for the coffee bean wastes (pulp, husk, etc.) directly related to processing, and for the leaves and wood that result from the periodically trimmed coffee trees.

C. Utilization of Various Wastes as Substrates

The cultivation of *Agaricus* has always involved the utilization of waste materials (horse manure and straw) as substrates that are composted, but the early cultivation techniques for the wood-rotting edible fungi made use of logs from trees of certain species that were cut down in the forests. This could hardly be considered a waste product, and, in fact, in ancient China special permission had to be obtained from the emperor to cut down trees for use in cultivating *Lentinula*. With the advent of the bag culture technique for growing mushrooms, it has become possible to utilize waste lignocellulosic materials for the substrate. It is general knowledge that huge quantities of lignocellulosic and other organic waste residues are generated annually worldwide through the activities of the agricultural, forest, and food processing industries. More than 3000 million MT of cereal straws were available in the world in 1999 (Table 11.8), and about half of these residues remain unused. At present, an experienced mushroom grower can obtain a biological efficiency (BE) of 60 to 75% for one crop with several flushes depending on the species cultivated. To achieve a 100% BE seems to be a reasonable goal for the mushroom industry in the years ahead. However, even at an average 67.5% BE, if only one third of the 1999 world production of cereal straws of 1190 million MT were used to grow mushrooms, about 803 million MT of fresh mushrooms could be produced. In addition, in 1999, the world produced 1057 million MT of bagasse, 6476 thousand MT of coffee pulp, 6152 thousand MT of coffee wastes, 9386 thousand MT of cottonseed hulls,

TABLE 11.8
World Production of Cereals and Their Straws during 1999 (× 1000 MT)

Cereal	Grain		Conversion Factors		Straw
Wheat	583,624	×	1.8	=	1,050,523.2
Rice	596,485	×	1.0	=	596,485.0
Maize	600,418	×	2.4	=	1,441,003.2
Other cereals	283,651	×	1.7	=	482,206.7
Total	2,064,178	×		=	350,218.1

Source: FAO Production Year Book, 1999.

TABLE 11.9
World Production of Other Major Lignocellulosic Biomass Wastes in 1999 (× 1000 MT)

Source	Production		Conversion Factors		By-Products	
Sugar cane	1,274,697	×	0.83	=	Bagasse	1,057,998
Coffee green beans	6,476	×	1.00	=	Coffee pulps	6,476
		×	0.95	=	Coffee wastes	6,152
Seed cotton	52,146	×	0.18	=	Cottonseed hulls	9,386
Sunflower seed	28,146	×	0.50	=	Seed hulls	14,073
Sisal plant	332	×	0.98	=	Sisal wastes	325

Source: FAO Production Year Book, 1999.

14,073 thousand MT of sunflower seed hulls, and 325 thousand MT of sisal wastes (Table 11.9). Furthermore, sawdust, wood chips, chopped grasses of many species, used tea leaves, banana leaves, oil palm pericarp waste, ground-up corncobs, legume stalks and pods (and almost any agricultural, industrial, or household waste that has a substantial cellulose component) — all have the potential for use as a substrate for growing mushrooms.

The sisal fiber is extracted commercially from sisal leaves, but this represents only 2% of the total plant biomass; some 98% is discarded as waste. Seed cotton produces on average 40% cotton lint and 60% cottonseed of which about 70% is kernels and 30% is cottonseed hulls. This means that about 18% is cottonseed hulls, which are usually considered waste. The waste generated in farming is also tremendous. At present, the biomass of the coffee bush (tree) used in the actual cup of coffee is a mere 0.2% (Table 11.10). This means that an international coffee economy has been built on the generation of 99.8% waste and the commercial use of only a small fraction of the tree.

Trees are logged in some countries mainly to extract the cellulose, which represents 30% in the case of hardwood and a mere 20% in the case of softwood. The rest is considered waste. The palm and coconut oils found in the tropical countries represent a mere 9% of the biomass generated; the rest is considered waste, and most of it is incinerated.[22]

With fresh mushrooms containing about 3 to 5% protein, mushrooms consumed in greater amounts could increase the amount of protein in the human diet, which is protein-deficient in one third of the world's population. This conversion of waste substances to protein in a choice food for human consumption is a property of mushrooms that merits more attention from all those interested in the food requirements of our ever-increasing human population.

TABLE 11.10
From Coffee Bush, to Coffee Cherry, to a Cup of Coffee (units: kg)

Biomass of the coffee bush 5 years	8,000
Coffee cherry	470
Parchment coffee	94
Coffee pulp	376
Coffee almond (export quality)	70.00
Coffee husks	19.85
Low-quality coffee	4.15
Toasted coffee (82% of almond)	57.40
Coffee drash	40.18
Soluble coffee (in the cup)	17.22
Percentage of coffee cherry	3.66
Percentage of coffee bush	0.20

Sources: National Federation of Coffee Farmers, Colombia, 1999; Pauli, G., *Diversification in the Tropics* [in Spanish], Una Publicacion del Instituto, Bogota, Colombia, 43–48, 1999.

V. CONCLUSION

In summary, we wish to re-emphasize that the world has an immense amount of lignocellulosic biomass resource, which like solar energy is sustainable. One of the most economically viable processes for the bioconversion of these lignocellulosic wastes is the cultivation of edible and medicinal mushrooms, which can make a great contribution to human welfare. Mushrooms arise from lignocellulosic wastes, and they are bountiful and nourishing. Mushrooms are environmentally friendly. They biosynthesize their own food from the lignocellulosic waste residues that are generated through the activities of agricultural, forest, and food processing industries. These residues would otherwise cause health hazards. After mushroom production, the spent composts/substrates can be used as animal feed, or as biofertilizers. Mushrooms can be used as food, tonics, and as medicine.[2] A regular intake of mushrooms can enhance health, and improve our lives. Mushrooms can serve as agents for promoting equitable economic growth in society. They are a unique group of fungi through which we can pilot a nongreen revolution in less developed countries and in the world at large. They demonstrate a huge potential for generating a socioeconomic impact on human welfare at local, national, and regional levels.

REFERENCES

1. Arita, I., *Pholiota nameko*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 475–496, 1978.
2. Chang, S.T., Mushrooms as human food, *BioScience,* 30, 399–401, 1980.
3. Chang, S.T., Mushroom biology: the impact on mushroom production and mushroom products, in *Mushroom Biology and Mushroom Products,* Chang, S.T., Buswell, J.A., and Chiu, S.W., Eds., Chinese University Press, Hong Kong, 3–20, 1993.
4. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China, *Int. J. Med. Mushroom*, 1, 291–300, 1999.
5. Chang, S.T. and Buswell, J.A., *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Aphyllophoromycetideae) — a mushrooming medicinal mushroom, *Int. J. Med. Mushroom*, 1, 139–146, 1999.

6. Chang, S.T. and Miles, P.G., Historical record of the early cultivation of *Lentinus* in China, *Mushroom J. Trop.,* 7, 31–37, 1987.
7. Chang, S.T., Mshigeni, K.E., Pauli, G., and Tagwira, M., The impact of mushroom cultivation on the control of the water hyacinth "weed" problem in Africa, in *A New Hope for Sustainable Development in Africa,* Mshigeni, K.E., Ed., University of Namibia Press, Windhoek, Namibia, 169–175, 1997.
8. Chen, P.C. and Hou, H.H., *Tremella fuciformis*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 629–643, 1978.
9. Danell, E. and Camacho, F.J., Successful cultivation of the golden chanterelle, *Nature*, 385, 303, 1997.
10. Delcaire, J.R., Economics of cultivated mushrooms, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 33, 727–793, 1978.
11. Delmas, J., *Tuber* spp., in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 645–681, 1978.
12. Flegg, P., The wider world of mushrooms, *Mushroom J.*, 599, 14–15, 1999.
13. Ghosh, M. and Nandi, B., Dynamics of extracellular enzymes during lignocellulose degradation of water hyacinth biomass by *Pleurotus* spp., *Mushroom. Res*., 4, 53–58, 1995.
14. Guzman, G. and Martinez, D., *Pleurotus* growing on coffee-pulp in a semi-industrial plant — a new promising mushroom cultivation technology in the subtropics of Mexico, *Mushroom Newsl. Trop.*, 6(3), 7–10, 1986.
15. Huang, N.L., *Cultivation of Eighteen Rare and Delicious Edible Mushrooms*, China Agricultural Press, Beijing, China, 164, 1997.
16. Ku, H.C., Leung, L.W., and Chang, S.T., Waste utilization of coffee spent grounds on cultivation of edible mushrooms, in *Proceedings of Symposium on Microbial and Engineering Technology in Wastes Treatment*, Chang, S.T., Wong, M.H., Wong, P.K., and Wong, Y.S., Eds., Commercial Press, Hong Kong, 85–103, 1992.
17. Lin, C.M., Special report of China Edible Fungi Association, *The Market of Edible Fungi* [in Chinese], 8, 5, 2002.
18. Martinez, D., Guzman, G., and Soto, C., The effect of fermentation of coffee pulp in the cultivation of *Pleurotus ostreatus* in Mexico, *Mushroom Newsl. Trop.*, 6(1), 21–28, 1985.
19. Martinez-Carrera, D., Morales, P., Martinez, W., Sobal, M., and Agruilar, A., Large-scale drying of coffee pulp and its potential for mushroom cultivation in Mexico, *Micol. Neotrop. Apl*., 9, 43–52, 1996.
20. Megens, H., Western fresh mushroom market, *Mushroom Int*., 81, 4–6, 2000.
21. Mshigeni, K.E., The water hyacinth "weed" in Africa: a problem or opportunity? *Discovery Innovation*, 7, 99–100, 1995.
22. Pauli, G., *Diversification in the Tropics* [in Spanish], Una Publicacion del Instituto, Bogota, Colombia, 43–48, 1999.
23. Sharma, S.R., Scope of specialty mushrooms in India, in *Advances in Mushroom Biology and Production*, Rai, R.D., Dhar, B.L., and Verma, R.N., Eds., Mushroom Society of India, Solan, India, 193–203, 1997.

12 *Agaricus* — The Leader in Production and Technology

I. INTRODUCTION

In Western countries cultivation of *Agaricus,* the most popular edible mushroom — which is variously known as the white mushroom, button mushroom, champignon, or simply the common cultivated mushroom — has developed over the past 50 to 60 years from a beginning as a risky venture to a largely predictable and controllable industrial process, particularly in Great Britain and the Netherlands. In no small measure this remarkable achievement in modern mushroom industrial development may be attributed to contributions resulting from the vigorous research activities conducted at mushroom research stations.

The world production of *Agaricus* was about 2 million metric tons (MT) in 1997 (Table 12.1). With a comparison of world production of all cultivated edible mushrooms, the percentage of world total production of *Agaricus* decreased from 73.1% in 1975 to 31.8% in 1997, even though the actual production increased from 900.0 thousand MT in 1981 to 1955.9 thousand MT in 1997, a 2.2-fold increase, and the annual increase percentage during the period 1975 to 1997 was 5.3%. This is mainly attributable to the increasing production of other cultivated edible mushrooms, such as the drastically increased production in Asia in recent years of *Lentinula, Pleurotus, Flammulina,* and *Hypsizygus,* four specialty mushrooms. According to the present proportional increasing trends for specialty mushrooms, the percentage of *Agaricus* will continue to decrease, and in the near future it will lose its position as the most popular edible mushroom.[9] *Lentinula* will likely become the new leader of cultivated edible mushrooms. In 1986, the United States was the largest producer of *Agaricus,* followed by China and France. However, in 1999–2000 China produced 637.3 thousand MT and jumped to become the largest producer of this important mushroom, followed by the United States, the Netherlands, and France (Table 12.2). Comparison of production figures between 1986 and 2000 indicates that the United Kingdom was the only producer that had a negative growth (−20.0%). It has been mentioned on several occasions that there are many difficult circumstances facing the U.K. mushroom industry. British producers have been unable to meet consumer demands in recent years, and as a result an additional 98,000 MT or more of fresh mushrooms are imported into Britain each year, more than its own production of 80,000 MT. Of these imports, approximately 63,000 MT come from Ireland, and most of the remaining 35,000 MT come from the Netherlands and Belgium. This makes Britain the second largest importer of mushrooms in Europe behind Germany.[18]

Among the top ten producers (Table 12.2), Poland's production has shown a dramatic increase of 1621.3% during the period from 1986 to 2000. In 1953, the production was only 80 MT. In 1970 it was 2000 MT, and in 1980 it was 22,000 MT and by 1990 annual production reached 105,000 MT. There have been many changes in the Polish industry over the last 10 years. Among the changes has been a development of the cooperative. This provided quite an advantageous situation for the growers since the cooperative had to buy everything that was contracted and pay a price that made it profitable for the growers. Another move was to use hybrid strains, which has enabled the Polish growers to produce the quality mushrooms necessary for expanding export sales

TABLE 12.1
Comparison of World Production of All Cultivated Edible Mushrooms with the World Production of *Agaricus* Mushrooms (fresh weight × 1000 MT)

Year	Production of All Edible Mushrooms	Production of *Agaricus*	*Agaricus* as % of All Mushrooms	Increase (%) of *Agaricus*	Annual Increase (%) of *Agaricus*
1975	916.0	670.0	73.1	—	—
1981	1257.2	900.0	71.6	34.3	5.7
1986	2182.0	1227.0	56.2	36.3	7.3
1990	3763.0	1424.0	37.8	16.0	4.0
1994	4909.3	1846.0	37.6	29.6	7.4
1997	6158.4	1955.9	31.8	6.0	2.0

Note: Annual increase % of *Agaricus* during 1975–1997 was 5.3%.

Sources: Data from Delcaire, J.R., in *The Biology and Cultivation of Edible Mushrooms,* Academic Press, New York, 727–793, 1978; Chang, S.T., *Int. J. Med. Mushroom,* 1, 291–300, 1999.

TABLE 12.2
***Agaricus* Mushroom Production in Top Ten Producing Countries in 1999–2000 (fresh weight × 1000 MT)**

Country	1986	1999–2000	% Change between 1986 and 1999–2000
China	254.6	637.3	350.3
United States	266.7	391.0	46.6
Netherlands	112.3	263.0	134.2
France	170.0	180.0	5.6
Poland	6.1	105.0	1621.3
Italy	52.0	102.0	96.2
Spain	45.0	92.0	104.4
Ireland	15.0	83.0	453.3
United Kingdom	100.0	80.0	–20.0
Germany	42.0	60.0	42.8

Sources: Data from Chang, S.T., *Int. J. Med. Mushroom,* 1, 291–300, 1999; O'Brien, A., *Mushroom J.,* 611, 17–30, 2000 and 613, 17–19, 2001; Szudyga, K., *Mushroom Int.,* 88, 3–4, 2002.

of fresh mushrooms. The export targets were the fresh markets in the Western European countries.[26] There is no doubt that the Polish industry will continue to innovate and to expand to meet the changing needs of the markets. There was a 453.3% increase in Ireland, 350.3% in China, 265.6% in Denmark, and 134.2% in the Netherlands. France increased only 5.6% and dropped in the world producer rankings from third to fourth place. In the past 10 years the French mushroom industry has undergone considerable change. Much of the industry has moved above ground and away from the traditional French system of producing mushrooms on bags in underground caves. China is now the world's largest producer of *Agaricus* as well as of all cultivated edible mushrooms although much of this production is in "exotics" such as *Lentinula* and *Pleurotu*s. However, in recent years, there has been a revival of the *Agaricus* mushroom industry for the domestic fresh market following a sharp decline in the processed mushroom industry in the 1980s.

II. DEVELOPMENT IN THE INDUSTRY

The methods of production used in Western countries require a large capital investment and considerable technically advanced (sophisticated) equipment. For example, in the Netherlands it is estimated that at least U.S. $500,000 is required to initiate and establish a modern mushroom farm with the capacity to produce 1 MT of fresh mushrooms per day. In Western countries cultivation of *Agaricus* is a professional business, and for large-scale farmers it is an industrial enterprise. However, in Asian countries, cultivation methods are mainly at the family farming level, with many if not all the people in a village producing a small quantity, although some larger modern farms have developed in recent years in China around large cities such as Shanghai.

A. France

According to the records available, *Agaricus* growing arose in France, probably in the vicinity of Paris, during the reign of Louis XIV. A French botanist, Tournefort, first described the primitive method used to grow the mushrooms in 1707. At a later date, the utilization of underground caves where the climate conditions were well suited to mushroom culture marked one of the most significant developments in mushroom history. The mushroom industry grew rapidly in France and then spread to other European countries.

France continued to lead the world as a mushroom producer until the outbreak of World War II in 1939. From that time until 2000, the United States assumed the dominant position; however, France remained in second place in production of *Agaricus* until 1985. By that year, the world production of *Agaricus* was about 1,200,000 MT. The United States produced 270,000 MT, China 200,000 MT, and France 180,000 MT. With production for 1999–2000 estimated at 180,000 MT of *Agaricus,* France is now the fourth largest producer of mushrooms in the world and the second largest producer in Europe. The French industry as a whole is very much focused on processing with more than 60% of the crop processed into cans, 25% sold as fresh, and the remaining 15% frozen.

B. Great Britain

In Great Britain the Mushroom Research Station was established in 1946 with Dr. R. Edwards as the first director. The same group set it up that had pioneered the formation of the Mushroom Growers Association in 1945. Since 1954, the main center of mushroom research has been the Glasshouse Crops Research Institute, and that has now become part of the Horticulture Research International, Warwick.

In 1985, 95,000 MT of *Agaricus* were produced, and only 80,000 MT were produced in 1999–2000. Additionally, imports from the Netherlands, Belgium, France, Ireland, and other places totaled 15,900 MT in 1985 and 98,000 MT in 1999. The number of British mushroom growers is decreasing and mushroom production figures have indicated a slight reduction over the last few years. The signs were there that only the fittest would survive (Editorial, *The Mushroom Journal*, October 1999). It was noted that the contemporary approach of the younger generation showed a slight but discernible shift away from practical/technical matters and toward business management. It should be noted that the tray system was used almost universally in Great Britain in the early years, but in recent years the Dutch mechanized shelf system has come into use. Most recently, Phase III compost has been used in increasing amounts. Of the 443,000 MT of compost produced, there are 304,000 MT as Phase I, 89,000 MT as Phase II, and 49,300 MT as Phase III. The percentage changes of composts used for production of *Agaricus* from 1999 to 2000 show that Phase I decreased by 21%, Phase II increased by 24%, and Phase III increased by 1%. The percentage changes of the average yield in kg/ton show that Phase I decreased 6% from 162 to 152, Phase II increased 4% from 217 to 226, and Phase III increased 4% from 221 to 229.

C. The Netherlands

In 1945 in the Netherlands, Dr. P.J. Bels was requested by his government to promote the cultivation of edible mushrooms (*Agaricus bisporus*). Under the difficult circumstances existing at the end and immediately after World War II, a primitive laboratory was established in the southern part of the Netherlands where mushroom growing was already in operation in the limestone caves. Dr. H.C. Bels-Koning assisted her husband in the laboratory.

It was clear that cultivation in caves was not economical. In 1947, Bels encouraged some young farmers to start growing *Agaricus* in mushroom houses with controlled temperature, humidity, and ventilation. From this, the cultivation developed into a very sophisticated industry with high yields and low costs. The strength of the Dutch mushroom industry lies in the spirit of cooperation that exists in that country.

In 1957, Dr. P.J. Bels and Dr. H.C. Bels-Koning started the Mushroom Experimental Station in Horst, supported 50% by the government and 50% by the mushroom growers. A training school for mushroom growers was established in 1962 close to the experimental station. For a number of years both institutes operated together; however, today, although cooperating closely, they operate separately. As a result of a change in philosophy of production, a new association called VPN (the Dutch Mushroom Growers Association) has recently come into existence. The treatment of spent compost, environmental issues and research regarding compost, and the cultivation of mushrooms are the priorities for promotion by the new association.[29]

The total production in 1985 was 105,000 MT. In 1999, the production was 263,000 MT and in 2002, production estimates were around 280,000 MT, an increase of approximately 10% compared with the previous year, mainly due to harvesting mushrooms mechanically. Depending on price movements, there was expected to be a production increase of around 5 to 8% in 2003.

D. The United States

The mushroom industry in the United States began about 1915, and by 1980 growers produced more than 225,000 MT of mushrooms. In 1985, the production was about 270,000 MT, an increase of 26% in comparison with production for the year 1980. Researchers in the U.S. Department of Agriculture and at Pennsylvania State University have made significant theoretical and practical contributions to the mushroom industry.[16,22] The American mushroom industry, which traditionally grew only *Agaricus bisporus,* has recently begun to include other cultivated species. The American Mushroom Institute, an association of growers, has significantly contributed to the development of the industry. The Mushroom Research Center established in the mid-1960s at Pennsylvania State University[24] annually conducts short summer courses in mushroom cultivation.

Production of *Agaricus* in the United States in 2001–2002 was 379,984 MT, 0.96% below 2000–2001 production of 390,064 MT, and down about 2% compared with the production of 389,480 MT in the 1999–2000 season.

E. Italy

Mushroom growers in Italy numbered barely 200 until 1950, with the overall production of cultivated mushrooms not exceeding 1500 to 2000 MT per year. By 1982–1983, approximately 200 growing units in the main growing regions, e.g., in Veneto and Latium, were producing approximately 45,700 MT, accounting for about 90% of the national production.[1]

Concentrated in Northern Italy, production of *Agaricus* has remained reasonably steady at or around 100,000 MT over the last 10 years, thus making it the third largest producer in Europe and sixth in the world. The market is fairly self-contained with little movement of either imported or exported mushrooms. Production of cultivated mushrooms in Italy may be subdivided as follows: approximately 30% grown in caves, predominantly in plastic bags; 50% in huts or glasshouses

cultivated in beds; and 20% in huts or glasshouses cultivated in trays. Fresh consumption absorbs about 75% of the national production, with the remainder 25% sent for processing.

F. Ireland

Mushrooms were first grown commercially in Ireland in the mid-1930s, and exports of fresh mushrooms to Great Britain commenced in 1947. Three quarters of the mushrooms produced in Ireland are now either sold fresh in the United Kingdom or processed for other markets.

Growth has been rapid in recent years, with total production rising from 4500 MT in 1974 to 13,946 MT in 1984. In the season of 1999–2000, Ireland produced in excess of 83,000 MT of fresh mushrooms, of which over 80% were exported to Great Britain. One reason for this increase has been the abundance of the necessary raw materials in Ireland, as well as the availability of relatively cheap labor.

G. Taiwan

Mushroom spawn was introduced to Taiwan in 1953 when trial cultivation and research first began. This was the first attempt to grow *Agaricus* in a subtropical or tropical climate. Research on synthetic compost was accomplished in 1960, resulting in an expansion of mushroom cultivation. The acreage expanded rapidly from around 576,000 m^2 in 1961 to 11,788,462 m^2 in 1971. The annual production rose from 2798 MT in 1961 to a high of 111,460 MT in 1978. This was a 20-fold increase of planting acreage in a decade, and a 42-fold increase in production in 17 years. The acreage of land needed for mushroom growing is small in terms of production value. In 1979, the total acreage was 10,430,000 m^2, while the land area for mushroom sheds was 420 ha. These mushroom sheds occupied only 0.046% of the total cultivated land in Taiwan, while the value of the mushrooms produced constituted 2.35% of the total value of agricultural production.

Exports, representing about 80% of production, also increased steadily from 2324 MT in 1961 to the peak of 90,117 MT in 1978 — a 38-fold increase. The value of the exports showed a 47-fold increase within two decades, rising from approximately $2.3 million to $100 million between 1961 and 1980. However, in the early 1980s, both acreage and production have shown a downward trend, with the annual production averaging about 61,000 MT. In 1997, the production dropped to only 7222 MT. Because of the great socioeconomic changes during the last two decades, it will not be surprising if the *Agaricus* mushroom industry in Taiwan experiences further decline.

H. South Korea

The cultivation of *Agaricus bisporus* in South Korea started at the beginning of 1963.[13] Since then, the growing area has increased rapidly every year, and the bed area increased tenfold from 1965 to 1967. The unit yield was impaired during the first 4 years and was quite low, only 2.0 kg/m^2. This was due to defects in composting techniques, the use of inferior spawn, and unsatisfactory mushroom growing houses. By improving the cultivation techniques and by the construction of permanent mushroom houses, the unit yield has rapidly increased since 1970.[14] However, this upward trend in Korean mushroom production has been reversed since 1979, and significant decreases have occurred. Now, however, South Korea has become one of the countries whose national average in unit yield is reasonably high. In 1997, Korea produced 13,180 MT. The main production area is located in the west-central part of the country.

I. China

Although *Agaricus bisporus* cultivation had been introduced into Shanghai, China, in the 1930s, production remained for decades at a low level. By the 1970s, China began *Agaricus* production as a family farming enterprise and even exported a small amount of canned mushrooms in the

mid-1970s. Although the level of total production was high and the total area of cultivation was large, the methods of production were rather primitive in the early years.

The concept and technique of compost pasteurization that was first introduced into China in 1978 have been responsible for the great impetus in *Agaricus* production in China. The significant increase in yield due to the use of pasteurization has been well documented. This was the principal reason for the advancement of the industry in China. *Agaricus* is now the third most important cultivated mushroom in China after *Lentinula* and *Pleurotus*, and China is now number one as the largest of the world's major producers of *Agaricus*. In recent years, there has been a sharp upward trend of *Agaricus* production due to the rapidly expanding domestic fresh market. Production was 426,000 MT in 1998 and increased to 637,304 MT in 2000. The percentage change between 1998 and 2000 was 49.6 with an average annual growth of 24.8%. In early 1997, this mushroom was produced mainly in the southern part of China, e.g., Fujian, Zhejiang, Jiangxi, Guangdong, and Sichuan Provinces. Now the production of *Agaricus* has spread to the northern part of China and some larger modern farms have been established around large cities such as Shanghai and Yantai in Shandong province.

Realizing that the potential for increase in yield is enormous, China is stressing an increase in production per square meter rather than an expansion of the area of production. Should this be successful, the repercussions on other mushroom industries worldwide would be enormous.

J. General Remarks

Rapid strides were made in the cultivation of *Agaricus bisporus* in the above countries because of the background of information from research and the well-developed technology and management practices, even though the technology and management practices had to be adapted to local situations quite different from those in their place of origin. These nearly 60 years of research experience, starting in 1946, have resulted in consideration of *Agaricus* as the leader in production and technology for other mushroom species. Although its percentage of the world production of edible mushrooms has consistently decreased since 1975 (Table 12.1), *Agaricus* is still the most popular mushroom worldwide and is grown in more than 100 countries.

The highly mechanized and even computerized techniques developed for the production of *Agaricus* in Western countries have received detailed treatment in some excellent books[4,10,28,32] that are widely available, and we do not attempt to duplicate these treatments. Instead, we emphasize the methods used for *Agaricus* production in China, because these have not been described in detail in English. These examples of a successful, low-capital, agricultural venture in a developing country will, it is hoped, be of interest to those concerned with improving the welfare of people in developing countries through modest changes in agricultural practices.

III. COMPOST MATERIALS AND COMPOSTING

A. Compost Materials

As mentioned previously, mushrooms are **heterotrophic organisms**, and as such they lack the ability to form organic compounds for their cellular growth from the carbon dioxide in the atmosphere. Thus, their total nutritional requirements must be met by materials absorbed from the substrate (in the present consideration, the compost). Those materials required for mycelial growth must be available in the compost either before or after the substrate is broken down by the composting process.

Because the ratio of nitrogen:phosphate:potassium (N:P:K) of mushroom mycelium is 6.4:2.4:4.4, Stoller[25] has suggested that each ton of compost (assumed to have a moisture content of 70%) should contain 13 lb nitrogen, 4.1 lb phosphate, and 10 lb potassium. This is roughly an N:P:K ratio of 13:4:10 and converts to 1.98% nitrogen, 0.62% phosphate, and 1.5% potassium on a dry weight basis. This ratio is still used as a guide in the preparation of compost materials.

TABLE 12.3A
Nutrient Content of Major Cereal Straws

Sample	N (%)	P (%)	K (%)	Organic Matter (%)	C (%)	C:N
Rice straw	0.63	0.11	0.85	78.60	45.59	72.30
Barley straw	0.64	0.19	1.07	81.20	47.09	73.58
Wheat straw	0.48	0.22	0.63	81.10	47.03	98.00
Sugar cane residue	0.43	0.15	0.18	—	—	—
Sawdust	0.10	0.20	0.40	84.80	49.18	49.18
Grain husk	0.64	0.19	0.49	71.80	41.64	65.00
Cornstalk	0.48	0.38	1.68	80.50	46.69	97.20

TABLE 12.3B
Carbon and Nitrogen Content of Cattle Manure

Sample	C (%)	N (%)	C:N
Cattle manure	36.80	1.78	21.70
Buffalo manure	39.78	1.27	31.30
Cow manure	31.79	1.33	24.00

TABLE 12.3C
Nutrient Content (%) of Manure and Urine of Swine and Cattle

Sample	H_2O	Organic Matter	C	N	P	K	Ca
Swine							
Manure	82.00	15.00	0.70	0.56	0.40	0.44	0.09
Urine	96.00	2.50	1.45	0.60	0.12	0.95	—
Cattle							
Manure	83.00	14.50	8.41	0.32	0.25	0.15	0.34
Urine	76.00	3.00	1.74	0.50	0.03	0.65	0.01

The most common materials used for compost are animal manures and cereal straws, the nutrient contents for which are given in Table 12.3A to D. The nitrogen content of straw is less than 1%; therefore, in the preparation of compost a supplementation of nitrogen to a certain level must be considered. Schisler and Sinden[23] have suggested that the optimum nitrogen content of compost at the stacking stage (i.e., before composting) should be 1.5 to 1.7% on a dry weight basis. In 1978, Wuest[31] further suggested that prior to composting, the nitrogen content of a manure compost should have a range of from 1.5 to 1.7% and that of a synthetic compost should not be less than 1.6% nor greater than 1.85%.

To provide an example, and for purposes of comparison, values on the nitrogen content of compost materials at the stacking stage, after Phase I composting, and after Phase II composting are given in Table 12.4.

Examples of different compost formulations and their nitrogen contents before composting are shown in Table 12.5. It can be noted that the nitrogen content of the raw materials lies in a range between 1.11 and 1.68%. It is recommended that the nitrogen content of the compost materials be

TABLE 12.3D
Nutrient Content of Major Supplementary Materials in Compost for *Agaricus*

Sample	N (%)	$P(P_2O_5)$ (%)	$K(K_2O)$ (%)	C (%)	C:N
Peanut cake	6.39	1.10	1.90	49.46	7.76
Soybean cake	6.30–7.00	1.09–1.79	1.20–1.90	45.43	6.78
Cotton seed cake	5.32	2.50	1.77		
Rice bran cake	2.33	3.01	1.76		
Vegetable seed cake	4.60	2.48	1.40		
Tea seed cake	1.11	0.37	1.23		
Tung seed cake	3.60	1.30	1.30		
Castor bean cake	5.00	2.00	1.90		
Urea	46.00	—	—		
Ammonium sulfate	21.00	—	—		
Lime nitrogen	21.00	—	—		
Calcium superphosphate	—	16.50	—		

TABLE 12.4
Nitrogen Content (%) in Compost Materials

	After Composting		
Before Composting	Phase I	Phase II	Ref.
	17.50	2.35	22
	1.68	2.18	16
	1.59	2.24	16
	1.50	2.13	16
	1.50	1.86	16
1.68	1.92	2.62	24
1.65	1.89	2.38	24
1.61	1.84	2.19	24
1.65	1.89	2.15	24
	1.6–1.8	1.8–2.0	28

neither too high nor too low. From the standpoint of yield and economic return, the nitrogen content of the substrates before composting should be around 1.6%.

B. Amount of Compost Material

The amount of compost material for bedding 100 m^2 can be 5000 kg, but it may also be 2500 kg. Certainly the former has a thicker layer of compost than the latter. More mushrooms can grow per square meter on a thick bed of compost than on a thin bed. Theoretically, the yield is directly related to the amount of dry matter contained in 1 m^2 of compost. In practice, there are two methods used for calculation of yield: one is based on the yield of fresh mushrooms per unit area, and the other is based on the weight of mushrooms produced by a unit weight of dry substrate material at the time of spawning.

The Max Planck Institute in Hamburg, Germany, conducted an experiment, described by Vedder,[28] in which eight trays, each with an area of 0.5 m^2, were peak-heated and spawned. The contents of four of the trays were placed in a vat that had a surface of 0.5 m^2; the yield from this

TABLE 12.5
Examples of Compost Formulations with Nitrogen Content before Composting

Material	Wet Wt.	Dry Wt.	N Content (%)	Total N	N (%)	Ref.
American manure compost						15
Horse manure	80.00	50.00	1.20	0.60		
Chicken manure	7.50	6.00	4.00	0.24		
Beer residues	2.50	2.50	4.00	0.10		
Gypsum	1.25	1.25	—	—	1.57	
Total		59.75		0.95		
American synthetic compost						15
Paddy straw	15.00	12.80	2.00	0.26		
Chicken manure	3.80	2.40	4.00	0.09		
Ammonium nitrate	0.30	0.30	32.00	0.10		
Potassium carbonate	0.30	0.30	—	—		
Gypsum	0.60	0.60	—	—		
Corn cob	15.00	12.80	0.30	0.04	1.68	
Total		29.20		0.49		
Netherlands manure compost						28
Horse manure	1000.00	600.00	1.30	7.80		
Chicken manure	100.00	63.00	4.00	2.52		
Gypsum	25.00	—	—	—	1.57	
Total		663.00		10.30		
Synthetic compost prepared on GCRI Mushroom Unit (U.K.) (kg)						20
Wheat straw	1000.00	850.00	0.59	5.02		
Chicken manure	200.00	140.00	3.14	4.40		
Sporavite	140.00	108.00	7.14	7.71		
Gypsum	30.00	30.00	0.00	0.00	1.52	
Total		1128.00		17.13		
Synthetic straw compost in Taiwan						17
1. Paddy straw	100.00	85.00	0.62	0.53		
Urea	1.00	1.00	46.00	0.46		
Ammonium sulfate	2.00	2.00	21.00	0.42		
Calcium superphosphate	3.00	3.00	—	—		
Potassium sulfate	0.80	0.80	—	—		
Calcium carbonate	2.50	2.50	—	—	1.50	
Total		94.30		1.41		
2. Rice straw	100.00	85.00	0.62	0.53		
Ammonium sulfate	2.00	2.00	21.00	0.42		
Urea	0.50	0.50	46.00	0.23		
Calcium superphosphate	2.00	2.00	—	—		
Calcium carbonate	3.00	3.00	—	—	1.28	
Total		92.50		1.18		
Synthetic compost in South Korea						12
Paddy straw	1000.00	850.00	0.62	5.27		
Chicken manure	100.00	63.00	4.00	2.52		

TABLE 12.5 (continued)
Examples of Compost Formulations with Nitrogen Content before Composting

Material	Wet Wt.	Dry Wt.	N Content (%)	Total N	N (%)	Ref.
Urea	12–15		12–15	46.00	6.60	
Gypsum	10–20	10–20	—	—	1.54	
Total		948.00		14.59		
Synthetic compost in Shaoxing District, Zhejiang, China						30
Paddy straw	1155.00	981.80	0.62	6.09		
Barley straw	2345.00	1993.00	0.64	12.75		
Pig manure	4200.00	1050.00	2.60	27.30		
Dry cattle manure	3150.00	2677.00	1.30	34.80		
Mustard seed cake	630.00	630.00	4.60	29.00		
Urea	28.00	28.00	46.00	12.88		
Lime nitrogen	24.50	24.50	21.00	5.20		
Gypsum	203.00	203.00	—	—		
Lime	70.00	70.00	—	—		
Calcium superphosphate	70.00	70.00	—	—	1.65	
Total		7727.30		128.02		
Synthetic compost in Yi-Wu District, Zhejiang, China						30
Paddy straw	1000.00	850.00	0.62	5.27		
Barley straw	2000.00	1700.00	0.64	10.88		
Wet cattle manure	20000.00	4400.00	1.30	57.20		
Mustard seed cake	300.00	300.00	4.60	13.80		
Pig manure	10000.00	2000.00	2.50	50.00		
Ammonium water	100.00	—	6.00	6.00		
Lime nitrogen	50.00	50.00	21.00	10.50		
Calcium superphosphate	80.00	80.00	—	—		
Lime	180.00	180.00	—	—	1.61	
Total		9560.00		153.65		
Synthetic compost in Pu-Tun District, Fujian, China						30
Paddy straw	3500.00	2975.00	0.62	18.45		
Dry cattle manure	3500.00	2975.00	1.30	38.68		
Calcium superphosphate	70.00	70.00	—	—		
Urea	30.00	30.00	46.00	13.80		
Gypsum	100.00	100.00	—	—		
Lime	50.00	50.00	—	—	1.11	
Total		6200.00		76.93		
Synthetic compost in Lung-Xi District, Fujian, China						30
Paddy straw	3333.00	2833.00	0.62	17.56		
Dry cattle manure	5555.00	4721.00	1.30	61.38		
Urea	22.20	22.20	46.00	10.21		
Calcium superphosphate	55.50	55.50	—	—		
Gypsum	111.00	111.00	—	—		
Lime	22.20	22.20	—	—-	1.15	
Total		7764.90		89.15		

TABLE 12.5 (continued)
Examples of Compost Formulations with Nitrogen Content before Composting

Material	Wet Wt.	Dry Wt.	N Content (%)	Total N	N (%)	Ref.
Synthetic compost in Fujian Research Institute of Light Industry						30
Paddy straw	2496.00	2122.00	0.62	13.20		
Dry cattle manure	4160.00	3535.00	1.30	46.00		
Barley straw	1664.00	1414.00	0.64	9.00		
Peanut cake	333.00	333.00	5.00	16.60		
Ammonium water	33.00	—	6.00	2.00		
Wood ash	70.00	70.00	—	—		
Calcium superphosphate	83.00	83.00	—	—		
Urea	42.00	42.00	46.00	19.30		
Lime nitrogen	83.00	83.00	21.00	17.40		
Gypsum	125.00	125.00	—	—		
Lime	83.00	83.00	—	—	1.56	
Total		7891.00		123.50		

0.5-m^2 surface area with a thick layer of compost was almost the same as that from the four other trays, which contained the same quantity of compost spread over a total growing surface of 2 m^2.

Based on various calculations and practical considerations, Vedder[28] suggested that the most economical method of growing mushrooms in the Netherlands is with a relatively thick layer of compost. A **thick layer** is one that is 15 to 18 cm (pressed after spawning), consisting of 100 to 120 kg of compost per square meter at filling. In Great Britain the thickness of the compost layer is 15 to 20 cm with about 100 kg of compost per square meter.[8]

In China, traditionally a **thin compost layer** is used. The layer is always less than 10 cm, and the quantity of compost used per square meter is always less than 50 kg. Some farmers have tried increasing the thickness as well as the quantity of compost, but they did not obtain a yield correspondingly higher for the increase in compost. This may have been due to improper preparation of the compost.

The factor that limits the thickness of the compost layer in practice is the increase in temperature that occurs in the bed with an increase in thickness during mycelial growth and after casing. After spawning, if the temperature is 32°C or higher, mycelial growth is retarded and the mycelium may even degenerate. During fruiting, if the temperature in the bed is over 23°C, the primordia (pinheads) of the mushrooms become yellowish and die.

After spawning, the rise in bed temperature is mainly due to the degradation of compost materials by growth of mushroom mycelium. Vedder[28] estimated that in order to grow 1 kg of mushrooms, 220 g of dry substrate material is required, of which 90 g is used for mushroom growth, and the remaining 130 g used in the energy-yielding phases of the process. During spawn running, CO_2 is released from the mycelium, and heat is liberated into the compost. The more bed materials, the more heat is created by mycelial metabolic activities. In addition, if the compost is not well fermented or has not completely matured, the compost will continue to undergo fermentation, which will generate more heat. It should also be noted that if the mushroom is not grown under controlled conditions, the thickness of the compost layer must be at a level determined as suitable for the local climate conditions.

C. Composting

The purpose of composting is to convert a rich mixture of organic materials into a satisfactory and stable medium suitable for the growth of *Agaricus bisporus*, but not so for other kinds of organisms.

The importance and mechanisms of composting have been mentioned in Chapter 5. Further information can be found in several special references.[10,11,28]

Now that the amount and nature of the compost materials have been considered, it is desirable to mention briefly at this time what happens to these materials in composting and the nature of the process. Composting is a biological process in which the activities of numerous types of microorganisms are involved. It takes place in two distinct phases. In **Phase I composting**, the raw materials are mixed and wetted. This wetting makes it possible for the microorganisms that are naturally present in these materials to begin their degradative metabolic activities. The cellulose and hemicellulose of the straw are converted in this way to sugars, which, along with the available nitrogenous substances, support the further growth of bacteria and fungi. The increase in numbers of these microorganisms thereby increases the amount of protein present in the compost. In the later stages of Phase I composting, thermophilic Actinomycetes become dominant; and in Phase II composting the thermophilic organisms play an important role. **Phase II composting** is a pasteurization process that eradicates insects and contaminating microorganisms, but the promotion of decomposition of the substrates by the thermophilic organisms is the activity that prepares the compost as a selective substrate favoring the growth of *Agaricus*. Phase III compost was made in Italy almost 30 years ago. The system was then adopted and developed by the Dutch. It is now almost exclusively the substrate used in the Dutch, Belgian, and Italian mushroom industries and is increasingly being produced through the rest of the developed world. Definition of **Phase III compost** is spawn run in a bulk tunnel, and it is ready for casing when delivered to the grower. In its simplest form, Phase II compost is taken from a bulk tunnel, as well as spawn, transferred to another bulk tunnel, and spawn is run for 2 weeks before emptying and dispatching to the growing unit. For the details of Phase III compost regarding its growth, effect, and future, reference may be made to Canna[2] and Dewhurst.[6]

IV. SPAWN AND SPAWNING

A. Definition

The word *spawn* is derived from an old French verb, *espandre*, meaning to spread out or expand,[21] which was derived from the Latin, *expandere*, meaning to spread. Spawn is also defined by *Webster's Dictionary* as "the mycelium of fungi, especially of mushrooms grown to be eaten, used for propagation." In the *Agaricus* mushroom industry, **spawn** is a substrate into which mushroom mycelium has been impregnated and developed, and which will be used as a seed in propagation for mushroom production. In addition, the verb, **to spawn**, is used to mean inoculation of a substrate with mushroom spawn.

1. Natural Virgin Spawn

Natural virgin spawn may be defined as the spawn that occurs "wherever in nature" the species germinates and produces a mycelium. Ordinarily, such "spontaneous" appearances of spawn may be anticipated in compost heaps, rich garden beds, pastures near the feeding places of animals, etc.[7] It seems reasonable that over a number of years the distribution and disposal of spent compost would be an important factor in the spontaneous appearances of many different species and strains of edible mushrooms.

2. Flake Spawn

Flake spawn is the earliest natural or wild spawn developed by the French, and it was produced by digging wild mycelium and planting it in a specially prepared small bed of composted manure. After the beds became permeated throughout with mycelium, they were broken, dried, and then used to inoculate other beds.

3. Brick Spawn

Brick spawn was originally developed in England and also is a kind of natural or wild spawn. A mixture of horse manure, cow manure, and loam was pressed into a layer 2 inches thick and then cut into bricks, which were set on edge to dry. When partly dry, the brick was inoculated with a piece of old spawn. After the mycelium had grown throughout the brick, it could be broken into pieces for use, or dried and stored, or sold to mushroom growers. Because both flake and brick spawns are natural spawns, they are not pure cultures. Therefore, neither the identity of the mushroom spawns nor the absence of pests could be assured.

4. Pure Culture Spawn

Pure culture spawn is produced by inoculating a bottle of sterilized horse manure with tissue cultures from a quality mushroom or spores germinated under sterile conditions. Production of this bottle spawn has led to the development of various types of pure culture spawn, which differ principally in the preparation of the spawn substrates and the ingredients, e.g., manure spawn, grain spawn, pellet spawn, and perlite spawn.

5. Liquid Spawn

All the mushroom spawns mentioned above have been prepared on a solid substrate. However, under certain conditions, liquid medium is inoculated with mushroom mycelium to produce a liquid mushroom spawn that is suitable for effective sowing of a bed, bottle, or bag of compost on which mushrooms can grow.

B. Preparation of Spawn

Although the principles and practices of preparation of spawn have been available for some time for the modern mushroom industry, the following information and experience dealing with spawn and spawn making in China is considered as it is relevant to the development of a mushroom business on a semi-industrial scale in other developing countries.

1. Pond Mud–Manure Spawn

The first step is to prepare the mother spawn, which will be used to inoculate the manure with pond mud spawn. The latter is called a cultural spawn and is used in spawning the mushroom compost.

a. Substrate of the Mother Spawn

The raw materials used are barley straw and swine manure in an 8:5 ratio. The straw is first moistened, and 3 days later it is mixed with the manure. The mixture is composted for 30 days with three turnings. After removing the residual clumps of manure, the composted straw is dried and cut into pieces 2.5 cm long. These pieces are then mixed with 10% composted cattle manure and 1% gypsum and moistened with limewater until the moisture content is around 60%. The pH value is about 9. After further composting for 12 hours, the substrate can be bottled, sterilized, and inoculated with pure mushroom mycelium.

b. Substrate of the Cultural Spawn

The substrate for the cultural spawn contains pond mud and dry cattle (swine) manure in a 1:1 ratio (by volume). First, the dry manure with a small quantity of paddy straw is composted for 20 days. During the composting period, it needs to be turned two to three times. It is then mixed thoroughly with pond mud and fermented for 10 to 15 days. The substrates can then be air-dried and stored. When spawn is to be made, the substrate is moistened with limewater. The final moisture

content should be around 45%. One bottle of mother spawn can inoculate about 60 bottles of cultural spawn substrate. Each bottle contains about 0.5 to 0.6% kg of the manure and pond mud spawn compost.

2. Straw–Manure Spawn

The raw materials of the substrate contain dry paddy straw, 45.5%; dry cattle manure, 45.5%; peanut bran, 1.7%; gypsum, 0.9%; ammonium sulfate, 0.5%; urea, 0.5%; calcium superphosphate, 4.5%; and lime, 0.9%. All materials are mixed thoroughly with water to give a water content of 70 to 75%, and then the mixture is composted for 16 days, with four turnings.

3. Grain–Manure Spawn

a. Materials

Wheat grain	40–42 kg
Dry cattle manure	4–5 kg
Rice hulls	1.5–2.5 kg
Calcium carbonate	1 kg
Calcium hydroxide	0.8 kg

b. Procedure

Wheat grains are first washed and then soaked for 18 to 24 hours. Before cooking, the grains should be rinsed several times and boiled for 15 minutes. The grains should be well cooked but should not be broken. After cooking, the grains are mixed with the other ingredients. The moisture content should be adjusted to around 52%, but it should not exceed 55%. Before sterilization, the pH value should be in the range of 11 to 12 and then dropped to about 7 after sterilization.

c. Applications

The grain-manure spawn is widely used in Guangdong Province. The other two kinds of manure spawn are generally used in other provinces in China. In general application, three times as much straw–manure spawn is used as grain–manure spawn per unit of compost area. In most Western countries, manure spawn has been completely replaced in the market by pure grain spawn. The advantages of grain spawn are that it contains more nutrients and more initial growing points, the spawning can be handled more quickly, and the spawn can be distributed more easily over the compost. Therefore, the mycelium from grain spawn can colonize the compost rapidly and form fruiting bodies early. In general, the yield can be from 17.3 to 33.1% greater, in comparison to the yield when straw–manure spawn is used.

C. Spawning

A simple definition of spawning is the planting of mushroom spawn in the prepared compost. Spawning was the important step in the early development of mushroom cultivation as is noted in the following quotation from Treshaw.[27] "Sometime between 1678 and 1707, someone, whose name is lost to history, must have discovered and practiced a method of spawning stable manure beds by inserting small masses of infected manure from naturally infected beds." Along with advances in spawn making, the methods of spawning have also been continuously developed and improved, making it possible for the mushroom mycelium to grow through the compost more quickly; e.g., the methods of mixed-spawning, shake-up spawning, and super spawning are summarized by Vedder.[28] In China, one of these methods is to spawn 70% of the spawn by the point method, and then to spawn the remaining 30% by the distribution or spreading method.

V. CASING

The main purpose of applying a casing layer to the surface of the spawn running compost is to stimulate and promote formation of fruiting bodies. When the mycelium grows from the spawn substrate, e.g., and the grains permeate the compost, it is called spawn running. The production of mushrooms will not occur until the mycelium is covered by a casing medium.

Before the early 1950s, sterilized soil or subsoil was used for casing, and this is still used by some farms. The characteristics of a good casing medium are that it should have an open texture, good water-holding capacity, freedom from pests and diseases, and a pH between 6.5 and 8.0. A peat moss mixture with the pH adjusted by lime, chalk, or ground limestone fulfills the requirements of a good casing soil and is now widely used in many advanced mushroom industries. With the help of modern technology, understanding the specific relationship between water tension in the casing layer and mushroom yield could add a great deal to the future success of mushroom crop management.

VI. HARVESTING

Mushrooms can be harvested at different developmental stages according to the grades used in marketing. The classification of grades depends on the mushroom's size, degree of maturity, and freedom from damage or blemishes. The Food and Agriculture Organization of the United Nations (FAO) issued in 1970 a small booklet entitled "International Standards for Edible Fungi" (Codex Alimentarius Commission No. 38). It was inspired by the English practice, and the standards are as follows:

1. Buttons — Mushrooms with membrane closed, only just forming; stem length not to exceed 2 cm (3/4 inch), cap diameter 2.5 to 6 cm (1 to 2 1/2 inches).
2. Caps — Mushrooms with membrane well developed or just opening, with cap retaining a pronounced cap shape. Stem length not to exceed 2.5 cm (1 inch) from the apex. Cap diameter 2.5 to 7 cm (1 to 2 3/4 inch).
3. Flats or Opens — Mushrooms that have advanced beyond the cap stage, the cap forming a letter "T" with the stalk. Cap diameter 2.5 to 7 cm (1 to 3 1/2 inch) and stem length not to exceed 2.5 or 3 cm, according to the class. These mushrooms are also called "opens."

These definitions are widely accepted, but the measurements may vary on a wider range.

REFERENCES

1. Borzini, G., Mushrooms in Italy, *Mushroom J.*, 151, 232–233, 1985.
2. Canna, C. M., Phase III — is it the future? *Mushroom J.*, 564, 14–15, 1997.
3. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinula edodes* (Berk.) Sing., in China, *Int. J. Med. Mushroom*, 1, 291–300, 1999.
4. Chang, S.T. and Hayes, W.A., *The Biology and Cultivation of Edible Mushrooms,* Academic Press, New York, 1978.
5. Delcaire, J.R., Economics of cultivated mushrooms, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, chap. 33, 727–793, 1978.
6. Dewhurst, M., Phase III — the future? *Mushroom J.*, 626, 17–18, 2002.
7. Duggar, B.M., The principles of mushroom growing and mushroom spawn-making, Bull. 85, Plant Industry, U.S. Department of Agriculture, 1905.
8. Edwards, R.L., Cultivation in Western countries: growing in houses, in *The Biology and Cultivation of Edible Fungi*, Chang, S.T. and Hayes, W.A., Ed., Academic Press, New York, 299–336, 1978.

9. Flegg, P., The wider world of mushrooms, *Mushroom J*., 599, 14–15, 1999.
10. Flegg, P.B., Spencer, D.M., and Wood, D.A., *The Biology and Technology of the Cultivated Mushroom*, John Wiley & Sons, New York, 1985.
11. Hayes, W.A., Biological nature, in *The Biology and Cultivation of Edible Mushroom*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 191–217, 1978.
12. Kim, D.S., An introduction to mushroom growing in Korea, *Mushroom Sci*., 9, 121–126, 1974.
13. Kim, D.S., Cultivation of edible mushroom in Korea, *Mushroom Newsl. Trop*, 1(1), 11–13, 1980.
14. Kim, D.S., Rural Development Administration, Suweon, Korea, personal communication, 1987.
15. Kinrus, A., Techniques and methods in the U.S. mushroom industry, *Mushroom Sci.,* 9, 165–173, 1974.
16. Kneebone, L.R., Strain selection, development and maintenance, *Mushroom Sci.,* 7, 531–541, 1968.
17. Lin, C.T. and Hu, J.S., The chemical composition of rice-straw composts (personally distributed printed sheets), China, 1977.
18. O'Brien, A., Update on the European mushroom industry November 2000, *Mushroom J*., 611, 17–30, 2000.
19. O'Brien, A., Update on the European mushroom industry. Part 2, *Mushroom J*., 613, 17–19, 2001.
20. Randle, P.E., Mushroom compost (printed sheets) Glasshouse Crops Research Institute, Littlehampton, England, October, 1980.
21. San Antonio, J.P., Origin and improvement of spawn of the cultivated mushroom *Agaricus brunnescens* Peck., *Hortic. Rev.*, 6, 85–118, 1984.
22. Schisler, L.C., Stimulation of yield in the cultivated mushroom by vegetable oils, *Appl. Microbiol*., 15, 844–850, 1967.
23. Schisler, L.C. and Sinden, J.W., Nutrient supplementation of mushroom compost at spawning, *Mushroom Sci.,* 5, 267–280, 1962.
24. Schisler, L.C., Schroeder, M., Barr, W.L., and Snetsinger, R., The environmental control system of the Pennsylvania State Mushroom Test Demonstration Facility, *Mushroom Sci.,* 8, 847–855, 1972.
25. Stoller, B.B., Preparation of synthetic composts for mushroom culture, *Plant Physiol*., 18, 397–414, 1943.
26. Szudyga, K., The Polish mushroom industry, *Mushroom Int.*, 88, 3–4, 2002.
27. Treshaw, C., Nutrition of the cultivated mushroom, *Dansk. Bot. Arkiv.*, 11(6), 1–180, 1944.
28. Vedder, P.J.C., *Modern Mushroom Growing*, Educaboek, Culemborg, the Netherlands, 228–254, 1978.
29. Vestjens, T., An overview of the Dutch industry, *Mushroom J*., 629, 10–12, 2002.
30. Wang, H.C., Cultivation techniques of *Agaricus bisporus,* Monograph 15, Fujian Research Institute of Light Industry, Fujian, China, 1983.
31. Wuest, P.J., Compost and the composting process, *Mushroom News*, May, 11–66, 1978.
32. Wuest, P.J., Royse, D.J., and Beelman, R.B., *Cultivating Edible Fungi*, Elsevier, Amsterdam, 1987.

13 *Lentinula* — A Mushrooming Mushroom

I. INTRODUCTION

Lentinula edodes (Berk.) Sing. (common name: black forest mushroom; Chinese name: shiang-gu; Japanese name: shiitake) is the second most important edible mushroom in the world from the standpoint of production; and it is the most popular fungus cultivated in China, Japan, and other Asian countries. In 1997, the total production was estimated to be 1,322,000 metric tons (MT) in terms of fresh equivalent weight (Table 13.1), which was 25.4% of the total world production of edible mushrooms in that year.[3]

For a long time, this mushroom has been valued for its unique taste and flavor and as a medicinal tonic. A famous Chinese doctor, Wu Shui, during the Ming Dynasty (1368–1644) wrote that the *Lentinula* mushroom was capable, expressed in modern terminology, of generating stamina, curing colds, improving the circulation, and lowering blood pressure.[16] At the present time, numerous scientific investigations have established the nutritive value and medicinal benefits of *Lentinula* in lowering serum cholesterol levels,[24] and possessing antitumor and antiviral activities as discussed in detail in Chapter 3. For all these reasons the demand for *Lentinula* has greatly increased in recent years, and with the demand there has also been an increase in production. In 2000, China alone produced 2,205,208 MT of the mushrooms, which accounted for more than 80% of world production. With new production methods it is anticipated that there will be continuous expansion of production in the future.[6] It has been predicted that within a few years world production of *Lentinula* will surpass the world production of *Agaricus,* and *Lentinula* will become the number one cultivated mushroom in the world.

II. EARLY HISTORY OF CULTIVATION*

Confusion exists in the literature in regard to the time of the first successful cultivation of *Lentinula edodes*. This is probably the consequence of reports of the early use of this mushroom as food when it was collected in the wild being mistaken for the deliberate cultivation of the mushroom, which, of course, occurred later than its first use as food.

The account of Singer[23] on the cultivation of *L. edodes* is frequently cited. In this account Singer mentions, in a section entitled, "Cultivation of the Shiitake," that historical documents in Japan recorded that the Emperor Chuai in the year A.D. 199 praised the shiitake given to him by the natives of Kyushu. It should not be construed, however, that these were cultivated shiitake mushrooms that were presented to Emperor Chuai. It is more likely that they had been collected in the wild. Singer suggests that a primitive form of cultivation was introduced into Japan by Chinese farmers, and toward the end of the 17th or beginning of the 18th centuries (275 to 300 years ago) semicultivation techniques were developed by the Japanese.

* The materials presented in this section appeared in an article written by the authors[4] that was published in the *Mushroom Journal for the Tropics*, 7, 31–37, 1987. It is published here with permission.

TABLE 13.1
World Production of *Lentinula edodes* in Different Years in Fresh Weight MT × 1000

	1983		1985		1991		1992		1993		1994		1995		1996		1997[a]	
Country	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**	**Amt.**	**%**
China	19.5	9.4	50.0	13.9	380.0	60.5	450.0	63.9	550.0	68.9	626.0	73.6	580.0	72.5	670.6	76.3	1,125.0	85.1
Japan	171.2	82.8	227.3	63.3	179.7	28.6	177.1	25.2	170.4	21.3	157.4	18.5	155.2	19.4	144.0	16.4	132.6	10.0
Taiwan	7.5	3.6	49.0	13.7	36.8	5.9	39.4	5.6	36.4	4.6	28.0	3.3	26.9	3.4	27.0	3.1	27.0	2.1
Korea	4.9	2.4	23.4	6.5	17.2	2.7	22.5	3.2	25.8	3.2	22.0	2.6	19.0	2.4	18.7	2.1	17.0	1.3
Others	3.6	1.7	9.4	2.6	14.5	2.3	15.0	2.1	16.0	2.0	17.0	2.0	18.0	2.3	19.0	2.1	20.0	1.5
Total	206.7	99.9	359.1	100	628.2	100	704.0	100	798.6	100	850.4	100	799.1	100	879.3	100	1,321.6	100

[a] The national production of *Lentinula* mushrooms in China in 1998 was 1,338,000 MT.

Sources: Data from Royse, D.J. et al., *Interdiscip. Sci. Rev.,* 10, 329–335, 1985; Chang, S.T., *Int. J. Med. Mushroom,* 1, 291–300, 1999; Yao, S.X., in *The Development Strategy of Lentinus edodes Cultivation in North China,* Association of Edible and Medicinal Mushrooms in Northeast Three Provinces in China, 19–32, 1998; Yamanaka, K., *Food Rev. Int.,* 13, 327–333, 1997.

From what follows in this section, it would be unrealistic to assume the availability of cultivation techniques for shiitake as early as A.D. 199, even though Ito[10] seems to imply that the shiitake given to Emperor Chuai in the year A.D. 199 resulted from a primitive form of cultivation. Ito states, however, that cultivation began in China about 800 years ago and was first introduced to Japan by Chinese farmers.

Furthermore, it is certain that Kendrick's account of shiitake cultivation is in error regarding the time of appearance of cultivation, and the techniques that he describes are not the primitive ones, but are techniques of the present century. In his book, entitled *The Fifth Kingdom*, Kendrick[11] wrote about the shiitake as follows: "For 2,000 years the Japanese and Chinese have cultivated it by boring holes in specially stacked oak and chestnut logs and inoculating them with plugs of infected sawdust." Actually, the use of sawdust or wood spawn in this manner is a modern development.

Accounts written in Chinese of *Lentinula* cultivation in China have presented a picture of the development of the cultivation methods which we shall attempt to put into historical perspective. In doing this, the report of Zhang Shou-Cheng[32] in which he described the story of Wu San Kwung has been especially helpful.

Wu San Kwung is known by both legend and historical account as the originator of *Lentinula* mushroom cultivation. He was born during the Sung Dynasty (960–1127) in Lung-Shyr Village in Lung-Chyuan County in southwest Zhejiang Province bordering on Fujian Province.

The area where *Lentinula* was first cultivated is mountainous, and cultivation continues today in the three counties of Lung-Chyuan, Qing-Yuan, and Jiing-Ning, which are located between 118°43′ and 120°15′ East longitude and 27°00′ and 28°21′ North latitude. The climate in this region is warm and humid with the regular occurrence of four seasons. The average annual rainfall is 1200 mm, and the average temperature is 17°C with 300 frost-free days to be expected each year. About 98% of the land area is mountainous, and the majority of trees in this area are broad-leaved, although there are some coniferous trees present as well. Here 95% of the farmers grow mushrooms.

The story about Wu San Kwung that comes down to us today is that he stayed in the deep forests of the high mountains where he hunted for and collected wild mushrooms for food. One day he discovered that broken trees, which had fallen to the ground, "produced" mushrooms. He collected these and found them to be nonpoisonous. They had a nice scent, and he called the mushroom Shiangshyuhn, "nice-smelling mushroom." Later, he discovered that when he used a knife to cut the logs, the mushrooms grew larger and more vigorous, and "the more cuts, the more mushrooms. No cuts, no mushrooms."

Occasionally, after cutting, there were no mushrooms for years. When this happened, he became angry and beat the logs vigorously; and several days after the beating, mushrooms emerged all over the log. This may be the origin of the practice of cutting and beating the logs. When the logs are beaten, the mushrooms emerge. This whole process is called the "shocking method."

Recognition for the contribution that Wu San Kwung made to the welfare of the people of this agriculturally poor area is revealed by the fact that there are two temples to Wu San Kwung — one old and one new. The older was built in 1739 during the Ch'ing Dynasty in the 30th year of the reign of Emperor Ch'ing Lung; the newer temple was built in 1875 in the first year of the rule of Kwang-Shu of the Ch'ing Dynasty. The temples are managed and supported by the three counties. The new temple is large, beautiful, and well kept. There is a statue of Wu San Kwung in the main building. Every year from July 16 to 19 on the Chinese calendar, which is approximately 5 weeks later on the Julian calendar, there is a celebration, day and night, to give thanks to Wu San Kwung. In almost every mushroom-growing village there is a small temple to Wu San Kwung.

The following is one of the tales that has been told about the mushroom growers of Lung, Qing, and Jiing Counties by Zhang Shou-Cheng:

> For several hundred years, when leaves fall from maple trees, it is time for husband and wife to separate. The husband, with quilt and cooking pans, goes to the mountains, after first worshiping Wu San Kwung,

> to cut the trees for mushroom cultivation. The people are poor in Lung, Qing, and Jiing Counties. During spring, when the buds of the maple open, and the flowers bloom, and it is warmer, the farmer returns and is reunited with his family after the long, cold, hard winter months.

After farmers had used this method for a long period, Wang Cheng in A.D. 1313 wrote the section on the cultivation of the fungi in the *Book of Agriculture*.[28] The account of the cultivation method included the selection of site, the selection of type of trees, the cutting and the covering of the logs, the opening of the cover, the beating of the logs, and so on. The completion of the sequential steps of the cutting/beating technology was already established by A.D. 1313 when Wang Cheng's book was published. The first part of the section on fungi deals with wild mushrooms. The second part deals with cultivated mushrooms, including *Lentinula*. The following is a direct translation from the *Book of Agriculture* using present-day terminology, from the section on cultivated mushrooms.

> Now, in the cultivation of the "nice-smelling mushroom" (*Lentinula)* in mountainous areas, the same method is used as described above (i.e., with other cultivated mushrooms). But for *Lentinula* cultivation, select the site on the shady side of the mountain. Select suitable trees, such as: maple (*Acer*), sweetgum (*Liquidambar*), chestnut (*Castanopsis*), and so on. Cut down the trees. Use a hatchet (or axe) to cut holes (or pits) in the logs. Then use soil to cover the log, and, after 1 year, the log will have decayed. During this year, the wood was degraded by the mycelium, which grew within the notches or pits in the logs. Cover the log again with branches, leaves, and soil. Using water left after washing rice, or kitchen wastewater, gently water the logs frequently. A few hours after watering, use a wooden club to beat the log. Later the mushrooms will grow out. This is called Jingshiang — "shocking the mushroom," and the mushrooms are called "shocking mushrooms."
>
> After rain or dew and warmer weather, the mushrooms appear. Although this complete process takes years, a profit can be made. Furthermore, this profit is sometimes quite good.
>
> After harvesting, the "seeds" (i.e., the mycelia) are left in the logs. Therefore, the mushrooms will come out the next year. Select another suitable location and repeat the process for the next year.
>
> Collect, cook, and eat the fresh mushrooms. They have a nice smell and a good taste, or they can be sun-dried to become dry Shiangshyuhn (dry *Lentinula*). Now the people living in these poor villages in the deep mountain areas cultivate mushrooms rather than cereal crops. It is fortunate that due to the natural conditions in this area these mushrooms can be cultivated for the benefit of the people.*

It is interesting to note that the section on fungi in the *Book of Agriculture* by Wang Cheng contained only 160 Chinese characters. Yet this included a brief, but accurate, description of the sequential steps of *Lentinula* cultivation including:

1. Selection of location
2. Selection of types of trees
3. Cutting of notches on the logs
4. Beating the logs with wooden clubs
5. Processing (i.e., drying of the mushrooms)

These five steps, described by Wang Cheng in A.D. 1313, are similar to the methods used by the mushroom farmers of Lung, Qing, and Jiing Counties today. Furthermore, although present-day cultivation makes use of inoculation with mycelial spawn, the woodlot is 95% the same as that

* It is noted elsewhere in this book that today mushrooms are cultivated in relatively poor agricultural areas in China and other countries using simple techniques based on modern research findings.

described in the *Book of Agriculture*, that is, consisting of maple, chestnut, and sweetgum. This similarity between the woods reported by Wang Cheng to be used for *Lentinula* cultivation and the woods of the three counties supports the belief that these methods originated in the area of Lung, Qing, and Jiing Counties. Also, the first emperor of the Ming Dynasty, Chu Yuen-Chang (1368), gave special permission to these three counties to cut trees in the forest for the growing of *Lentinula* mushrooms. In addition, we can see from the account of Wang Cheng that there was a high standard of *Lentinula* cultivation that was reported almost 675 years ago and attributed to Wu San Kwung who lived almost 900 to 1000 years ago. The beating of the logs (shocking method) is still used by some farmers in Japan.[8]

It is generally believed that cultivation of *Lentinula* was introduced into Japan from China. This may have occurred in the 15th century, some 400 to 500 years after its origin in China, but the first book about *Lentinula* (shiitake) in Japan, written by Sato,[22] was published in 1796. The title of this book is the *Record of Jingshiang*. Jingshiang, which, again, means "shocking the mushroom," is a reference to the method of beating the logs to induce mushroom formation.

Nakamura[17] in his book written in Japanese on the historical studies of shiitake cultivation, places the beginning of cultivation in Japan as the 15th century, but he does not accept information from earlier writers that shiitake cultivation was introduced into Japan from China.

Although Nakamura does not accept the accounts in the literature (e.g., Sato[22]) that indicate that the cultivation of shiitake was introduced into Japan from China, his reasoning seems to us to be contrived and contrary to a direct interpretation. It is possible that the shocking method for cultivation of *Lentinula* might have developed independently in China and Japan, but the method is so strange that the probability of this happening is extremely low. Furthermore, during the period following the development of the cultivation method for *Lentinula* in China, it is known that many people, including Buddhist monks, went to Japan from China, taking with them the practices and customs of everyday life, as well as religious philosophy.

In our judgment the most reasonable explanation of the available records indicates that the cultivation of *Lentinula* had its beginning in China in the area of Lung-Chyuan, Qing-Yuan, and Jiing-Ning Counties in Zhejiang Province between A.D. 1000 and 1100. This in no way denigrates the Japanese use of a foreign concept that they had received and accepted. In the case of shiitake cultivation, the Japanese have modified and improved methods to such an extent that in 1983–84 Japan led the world in production, producing more than two thirds of the *Lentinula* cultivated.

At the present time there is increased interest in *Lentinula* in other countries of the world, particularly in Europe and America, as well as in Asia. This interest has been inspired by the success of the Japanese shiitake growers whose techniques have been imitated and modified. This is the pattern of progress.

III. MAJOR DEVELOPMENTAL EVENTS OF CULTIVATION

The major events of cultivation of *Lentinula edodes* mushrooms can be divided into two categories, **cultivation on wood logs** and **cultivation in synthetic substrates** (Table 13.2). The primitive form of *Lentinula* cultivation, which was first developed in China between A.D. 1000 and 1100 and introduced to Japan about 500 years later, often depended too much on luck. However, from 1892 on, the cultivation methods on wood logs were consistently improved and further developed by Japanese scientists. In 1946, the mushroom industry in Japan experienced a great thrust forward due to the invention of Tanegoma spawn (wood peg spawn) by K. Mori. In 1983, Japanese production accounted for 82.2% of total world production, and the production of *Lentinula* in China was less than 10%. The innovation of the synthetic sawdust log method in China in 1986 has greatly invigorated the *Lentinula* industry in China, and in 1987 China for the first time overtook Japan as the world's leading producer of this mushroom, and China has dominated the world market ever since. The major developmental events of artificial cultivation of *Lentinula* in China can be roughly divided into the following eight phases:

TABLE 13.2
History of Cultivation of *Lentinula edodes*

A. Cultivation in Wood Logs			
Wu San Kwang	China	1000	Cutting–beating method
Genbei	Japan	1624–1643	Hatchet-notch method
Tanaka	Japan	1892	Spore-germination
Y. Nishikado	Japan	1935	Sexuality
K. Kitajima	Japan	1936	Pure culture spawn
K. Mori	Japan	1946	Tanegoma spawn
B. Cultivation in Synthetic Substrates			
In bottle	Fuzhou, China	1958	
In plastic bags	Taiwan	1967	
In bricks	Shanghai, China	1979	
In artificial logs	Fujian, China	1986	
In plastic blocks	Australia and U.S.A.	1989	

1. The bottle method for small-scale cultivation was used in Fuzhou in 1958.
2. The plastic bags referred to as the "space bags" method, in which mushrooms were cultivated on particulate, sawdust-based substrates in autoclavable small plastic bags, was developed in Taiwan around 1967. This method appears to have been practiced commercially in both Taiwan and China from about that time. The method offers an attractive alternative for the cultivation of *Lentinula* and also for certain other species. Substrate and labor costs are not substantially higher than for wood log culture. Greatly foreshortened production cycles and higher yields and productivity may more than compensate for the additional capital investment involved. As space bags are usually incubated in controlled environments, production is consistent all year round. Thus, growers using this method have a great marketing advantage over those who use logs and whose product generally comes in seasonal flushes.
3. The brick method or the pressed cake method was introduced in Shanghai in 1979. It has been used for large-scale cultivation of the mushroom for a short period.
4. The synthetic log, also called the "mushroom cylinder method," was invented and developed by Mr. Z. W. Peng, a mushroom farmer in Gutian County, Fujian Province, in 1986. This innovation greatly accelerated the *L. edodes* industry in China. Since the introduction of this method, *L. edodes* farming has increased more than 20 times in 15 years.
5. The off-seasonal cultivation of *Lentinula* was initiated in Pingnan County, Fujian. Traditionally, the mushrooms were harvested only in the autumn and winter seasons — the cooler seasons. This new method, however, allows the mushrooms to be harvested in summer, which means that the mushrooms can be cultivated and harvested all year round.
6. The synthetic bag method developed in Qingyuan County, Zhejiang Province. The fruiting bags are much shorter than the synthetic logs but much wider. The inoculated bags are laid on shelves rather than on the ground in the fields, as was the practice using the synthetic log method. This efficient production of one county represented 10% of the world shiitake production and 20% of the Chinese output in 1993. This was one of the reasons that Qingyaun City was officially named by the Chinese Government "Shiang-gu (*Lentinula edodes*) City of China" in 1994.
7. The Shouning (County) method in Fujian province introduced a new method for producing "flower mushrooms," referring to the cracked *Lentinula* in synthetic bags. This highest grade of mushrooms is usually produced on wood logs under special weather conditions in nature.

8. The small rush shed or plastic shed method was derived from the synthetic bag method but was further developed and adapted in Biyang County, Henan Province. The bags are much bigger and are laid on shelves within the shed. By this method, during dry and cold weather, the best grade of mushroom, the flower mushroom, can be consistently produced on a commercial scale.

IV. GENERAL REVIEW OF PRODUCTION

World production of *Lentinula edodes* (fresh equivalent weight) in 1976 was about 130,000 MT, in 1979 about 170,000 MT,[5] and reached 1,321,600 MT in 1997 (Table 13.1). In 1983 in Japan, 158,885 MT fresh equivalent weight of *Lentinula* with a value of U.S. $689 million, were produced by almost 167,000 growers. If we consider those engaged in spawn production, distribution, marketing, etc., it is readily recognized that the *Lentinula* industry in Japan is an important branch of agriculture. In 1983, of this total production, 84,175 MT or about 53%, were processed to give 12,025 MT of dried *Lentinula*. In the same year, Japan exported 2795 MT of dried *Lentinula,* which amounted to 23.2% of the total dried mushrooms produced. Total *Lentinula* production in 1997 was only 55.1% of the production in 1984, the record high (Table 13.3). The production of dried *Lentinula* has been decreasing since 1985. Production of fresh *Lentinula* also has been decreasing since 1989. The major reasons for the decline in fresh *Lentinula* production are the aging of *Lentinula* growers combined with a lack of new growers, a shortage of low-cost high-quality wood logs for bed-log cultivation, and the rapid rise in imports of fresh *Lentinula* from China.[30] Fresh *Lentinula* imports from China increased from 15,586 MT in 1993 to 26,028 MT in 1997. The amount imported in 1997 equals 34.7% of the domestic production of fresh *Lentinula*. In addition, the decline in domestic shiitake production is also due to the decrease in price caused by the rapid increase in imports of both dried and fresh *Lentinula* from China (Table 13.4).

Although cultivation of *Lentinula* mushrooms is said to be centuries old, it became an industry in Japan only after World War II. As late as 1983, Japan accounted for more than 82.0% of the total world production of this mushroom. At that time, China represented only 9.4%. Only 4 years later, in 1987, China overtook Japan for the first time as the leading producer of *Lentinula*, with 178,800 and 162,600 MT, respectively. Since then, China has dominated the world market of this specialty mushroom. This is mainly due to the successful use of sawdust as a cultivation medium for mass production of shiang-gu (shiitake) in the Gutian area of Fujian Province. This innovation greatly advanced the *L. edodes* industry in China. Other parts of China quickly adapted the Gutian method but with modifications to suit their respective climatic conditions. This occurred sequentially and the more significant modified methods took on new names after the counties in which they were developed, such as: the Qingyuan method, the Shouning method, and more recently the Biyang method. The year 1990 can be considered the turning point in mushroom production in China, during which for the first time China produced more than 1 million MT of cultivated edible mushrooms. Among these, 230,000 MT were *Lentinula*. In 1991, China produced 60.5% of total world production of this mushroom, and in 1997 its share had risen to 85.1%. On the other hand, the percentage of *Lentinula* production in Japan dropped from 82.8% in 1983 to 10.0% in 1997. A comparison of the *Lentinula* production in 1985 and 1995 indicates that during those 10 years China increased production by 1060%, and Japan, Taiwan, and Korea decreased their production by 46.5, 23.2, and 82.2%, respectively (Table 13.5). Production of this mushroom in 2000 in China was estimated to be 2,205,208 MT. When compared with the production of 1,125,000 MT in 1997, this is an increase of 96% with an annual increase of 32%. Now China has become the world's largest producer, exporter, and consumer of *Lentinula.* The average biological efficiency of using synthetic medium to grow this mushroom in China is about 60 to 80% over a period of 6 months. With an extended cropping period, 100% yield is not unusual.

Lentinula edodes was usually regarded as a regional variety of mushroom from Eastern Asia, but now its cultivation is rapidly increasing in other parts of the world. It is expected that this

TABLE 13.3
Production of *Lentinula* in Japan in Different Years (unit = MT, fresh weight)

Year	1970	1977	1980	1984	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998[a]	1999[a]
Dried	7,997	11,487	13,576	16,685	11,066	11,236	10,168	10,036	9,299	8,312	8,070	6,886	5,786	5,552	5,582
Fresh	38,064	67,388	79,855	73,921	82,395	79,134	78,047	76,804	77,390	74,294	74,495	75,157	74,782	74,217	70,511
Total[b]	118,034	182,258	215,615	240,771	193,055	191,514	179,727	177,154	170,380	157,414	155,195	144,017	132,642	129,737	126,331
% fresh	32.25	36.97	37.04	30.70	42.68	41.32	43.23	43.35	45.42	47.20	48.00	52.19	56.38	57.20	55.80

[a] K. Yamanaka, personal communication.

[b] In fresh weight equivalent. The conversion factor from dried to fresh weight of *Lentinula* mushrooms is × 10.

Sources: Data from Yamanaka, K., *Food Rev. Int.,* 13, 327–333, 1997; Yao, S.X., *Edible Fungi of China,* 19(Suppl.), 41–44, 2000.

TABLE 13.4
Production, Export, and Import of Dried and Fresh *Lentinula* in Japan in Different Years (unit: MT)

	Dried			Fresh	
Year	Production	Import	Export	Production	Import
1958	2,803	—	921	—	—
1968	8,193	—	1,986	34,089	—
1980	13,579	78	3,104	79,855	—
1984	16,685	47	4,087	73,921	—
1985	12,065	140	3,330	74,680	—
1990	11,238	2,404	1,568	79,134	154
1991	10,168	2,813	1,042	78,047	1,265
1992	10,036	4,799	790	76,804	4,634
1993	9,299	7,208	696	77,394	15,586
1994	8,310	7,804	959	74,290	24,320
1995	8,070	7,539	544	74,495	26,308
1996	6,886	7,206	519	76,157	24,394
1997	5,700	9,400	281	75,000	26,028

TABLE 13.5
Comparison of 1985 and 1995 World Production of *Lentinula edodes* (in fresh weight MT × 1000)

	1985		1995		Increase/Decrease
Country/Region	Volume	%	Volume	%	(%)
China	50.0	13.9	580.0	72.5	+1060.0
Japan	227.3	63.3	155.2	19.4	–46.5
Taiwan	49.0	13.7	26.9	3.4	–23.2
Korea	23.4	6.5	19.0	2.4	–82.2
Others	9.4	2.6	18.0	2.3	+91.5
Total	359.1	100.0	799.1	100.0	+122.5

fastest growing species of mushroom, which can be used for both food and medicine, will continue to expand. Over the past decade the industry has profited from applied scientific research, in particular the use of artificial logs by substituting hardwood sawdust with mixes of agro-industrial waste materials. However, to continue this positive trend, it is of paramount importance that this mushroom should be farmed in ecologically sound areas to secure good-quality mushrooms for both healthy food and medicinal nutriceuticals. Its products should not be limited to the traditional fresh and dried mushroom products. Multiple products of *Lentinula* should be emphasized for the 21st century mushroom industry, e.g., canned, pickled, tonics and extracts, or as ingredients in foods, tonics, and nutriceuticals.[2]

Although *Lentinula* is the second most common cultivated mushroom, there is potential for it to become the most common mushroom in the near future. As we know, of all the mushrooms that are cultivated in quantity, by far the greatest amount of research and development has been conducted in connection with the most common cultivated mushroom, *Agaricus bisporus*. In the last three decades, millions of dollars have been spent in Western countries to develop the basic and practical cultivation process into a highly advanced technology. By contrast, the cultivation processes of *Lentinula* are relatively less funded. However, its cultivation technology is compara-

tively much simpler and requires less sophisticated equipment. Further concerted efforts in research on this mushroom are most important to continue expansion of its cultivation and marketing. It may not be surprising then, based on the past few years' expansion rates, to see this mushroom replacing *A. bisporus* and becoming the number one cultivated mushroom in the world within the next decade. The time has come for the *Lentinula* industry to design a marketing strategy like those of the coffee and tea industries in order to secure with the same volume of production more income for the growers, the creation of more qualified jobs, and better use of the limited resources.

V. CULTIVATION IN WOOD LOGS

Lentinula grows naturally in the dead wood of many hardwood tree species under different climatic conditions. Cultivation of the mushroom on natural wood logs is an important agricultural and industrial business in China, Japan, and South Korea. The stocks used can be divided into three categories: (1) those that can fruit at or above 20°C; (2) those that fruit between 10 to 15°C; and (3) those fruiting around 10°C. Therefore, there is no single stock that can produce fruiting bodies the year round. If we wish to achieve year-round production of *Lentinula*, a combination of different stocks must be considered.

A. Preparation of Logs

The tree trunks cut for mushroom culture are called logs, and logs in which fruiting cultures are propagated are called "mushroom logs." *Lentinula* is a saprophytic wood-rotting fungus. It grows on the cambium of dried logs by absorbing necessary nutrients as the wood is broken down. The bark of the log protects the growing mycelia and plays an important role in the development of the fruiting body. Therefore, logs to be used for *Lentinula* cultivation should belong to a tree species containing sufficient nutrients for the fungus, have enough sapwood, and be well protected by bark.

Lentinula grows on logs of broad-leaf trees belonging mainly to the family *Fagaceae*, which includes the oak tree species, thereby causing this mushroom sometimes to be called the oak mushroom. Other tree species from several genera, however, are also used to grow this mushroom, as shown in Table 13.6.

Although the mushroom will grow regardless of the age or thickness of the logs, it is best to use logs of uniform size for convenience of handling as well as for economy. Generally speaking, old and thick logs are inconvenient to handle, and give rise to slow growth of the mushroom mycelia and a resulting delay in the development of mushrooms. However, once the development has begun, mushrooms of good quality are formed to give a high yield, and the logs last for many years. On the other hand, young and thin logs produce small mushrooms with thin caps, and the logs last for only 2 to 3 years. Young logs have thin bark and a small proportion of heartwood; whereas old logs have thick bark and more heartwood. It is, therefore, a common practice in mushroom cultivation to use mixed logs of young and old, or thin and thick woods, with a standard of 9 to 18 cm diameter and an age of 15 to 20 years old.

1. Felling of Logs

The best season for logging (felling) is that in which the logs contain the highest content of carbohydrates and other organic substances and their bark is not easily peeled off. Because trees store the highest content of nutrients in the trunk, and the bark is attached tightly to the woody part during its resting period, the best season for logging is necessarily that period from leaf-fall in the autumn to the time when the sap flows in the tree in the spring. This season is advantageous from two aspects: (1) reforestation occurs readily from sprouts arising from the roots of the logged trees, and (2) seasonal workers from other pursuits who are available at this time can be used to

TABLE 13.6
Common Tree Species Used for Cultivation of *Lentinula edodes*

Alniphyllum fortunei (Hemsl.) Perkins	*Elaeocarpus lancaefolius* Roxb.
Altingia chinensis Bent. & Hook.	*Engelhardtia chrysolepis* Hance
Betula dahurica Pall.	*Eriobotrya deflexa* (Hemsl.) Nakal
Carpinus fargesii Franch.	*Eurya loquiana* Dunn
Carpinus laxiflora Blume	*Garcinia multiflora* Champ.
Carpinus tschonoskii Maxim	*Lagerstroemia subcostata* Kochne
Carpinus turczaninowii Hance	*Liquidambar formosana* Hance
Castanea crenata Sieb. & Zucc.	*Lithocarpus calophylla* Schlecht & Cham.
Castanea henryi (Skan) Rehd. & Wils.	*Lithocarpus glaber* (ThuNb.) Nakai
Castanea mollissima Blume	*Lithocarpus spicatus* Rehd. & Wils.
Castanea seguinii Dode.	*Mallotus lianus* Croizat
Castanopsis cerlesii (Hemsl) Hayata	*Platycarya strobilacea* Sieb. & Zucc.
Castanopsis chinensis Hance	*Quercus acuta* Thunb.
Castanopsis cuspidata (Thunb.) Schky.	*Quercus acutissima* Carruth
Castanopsis fabri Hance	*Quercus aliena* Bl.
Castanopsis fargesii Franch.	*Quercus bella* Chun & Tsiang
Castanopsis fissa Rehd. & Wils.	*Quercus dentate* Thunb.
Castanopsis fordii Hance	*Quercus fabri* Hance
Castanopsis hickelii Camus	*Quercus glandulifera* Bl.
Castanopsis hystrix A. Dc.	*Quercus glauca* Thunb.
Castanopsis lamontii Hance	*Quercus grosseserrata* Blume
Castanopsis sclerophylla (Lindl) Schky.	*Quercus mongolica* Fisch.
Castanopsis tibetana Hance	*Quercus myrsinae folia* Blume
Cornus capitata Wall	*Quercus serrata* Thunb.
Corylus heterophylla Fisth. ex Bess.	*Quercus spinosa* David
Distylium myricoides Hemsl.	*Quercus variabilis* Bl.
Distylium racemosum Sieb. & Zucc.	*Rhus succedanea* L.
Elaeocarpus chinensis Hook.	*Sapium disolor* Muell.-Arg.
Elaeocarpus japonicus S. & Z.	*Sloanea sinensis* (Hance) Hemsl.

do the logging work. In logging, extreme care must be taken not to cause any cracks or damage to the bark and to the log itself.

2. Moisture Content

The optimum moisture content for the log is 40 to 45%. If it is lower than 40%, the growth of mycelium will be reduced; if below 20%, the mycelium cannot grow. If the moisture content is 60%, but too alkaline (the optimum pH should be 4.5 to 5.5), the log is also unsuitable for growth of *Lentinula* mycelium, and it may easily become contaminated with other harmful fungi.

After the felled trees have been put aside without cutting off their branches, the moisture in the logs evaporates, causing some cracks at the cut ends. When the cracks reach halfway from the center of the log to the bark, the trunks are cut into 1- to $1^1/_2$-m lengths for convenience in handling.

This drying period varies depending upon the location and tree species, ranging from 20 to 40 days; and it results in a lowering of the moisture content in the logs from 50 to 55% to 40 to 45%, which is suitable for the growth of *Lentinula*. The exposure of logged trees to sunlight also has the effect of killing contaminating microorganisms on the bark and causing the bark to adhere more closely to the woody part, thus preventing the tendency for the bark to peel.

If the trees are overdried, the vitality of the spawn is weakened because of insufficient moisture content, and inoculation work becomes more difficult. There is, therefore, an increasing tendency

to inoculate the logs immediately after logging and cutting. This type of wet-log inoculation has the advantage of encouraging the smooth development of fungal mycelia, because the log begins to dry from the inoculation hole, and the growth of the mycelia follows in the direction of this drying process.

B. Preparation of Spawn

1. Stock

Lentinula spawn culture is a dikaryotic mycelium, which here is called a stock or fruiting culture. The stock culture is derived either from a piece of fruiting body tissue or from dikaryotic mycelium resulting from a mating made between two compatible monokaryotic mycelia. The selection of the mushroom stock is a matter of great importance, just as is the selection of the variety of seed in crop plants. A superior stock should be one that gives to the mycelium an excellent vitality, a high yield of fruiting bodies of superior quality, and, if possible, a broad adaptability to the environment. The stock cultures can be maintained in different agar media as mentioned in Chapter 8.

2. Spawn

Because the pure culture of a stock mycelium growing on an agar plate is not suitable to be used directly as mushroom spawn, it should be transferred for growth on a specific culture medium that is easy to handle, cheap, and convenient to inoculate. In general, there are two kinds of spawn for *Lentinula* cultivation. One is made by inoculating the fungal mycelium into sawdust medium and is called **sawdust spawn**. Different countries and places may use different formulas in the preparation of the medium for sawdust spawn. Three of them are given here as examples.

1.	Sawdust	800 g
	Rice bran	200 g
	Sucrose	30 g
	Potassium nitrate	4 g
	Calcium carbonate	6 g
	Water	2000 ml
2.	Sawdust	65%
	Wheat bran	15%
	Used tea leaves	20%
	Water content adjusted to	65%
3.	Sawdust	78%
	Sucrose	1%
	Wheat bran	20%
	Calcium carbonate	1%
	Water content adjusted to	65%

Sawdust should be sieved first to remove impurities and then mixed well with the supplementary ingredients. Water in which sucrose has been dissolved is sprinkled and blended into the above mixture to the extent that water drips down slightly when the wetted mixture is squeezed by hand. Then the medium can be filled into either culture bottles or polypropylene (pp) bags. Pressing flattens the surface of the medium, and pressing a rod into the center of the medium makes one inoculation hole of 1.5 to 2 cm in diameter. The containers are plugged with cotton and covered with aluminum foil. After being autoclaved, the medium can be inoculated by a selected stock culture and then incubated at 24 to 25°C for 30 to 40 days.

The second kind of *Lentinula* spawn is called **wood plug (peg) spawn**. This is made by inoculating the fungus into wedge-shaped pieces or cylindrical pieces of wood. When the mycelium has fully grown into the pieces of wood, these wood plugs can be used as mushroom spawn.

C. Inoculation of Spawn into Logs

When the logs are ready, the spawn is inoculated into them. This process of inoculating the spawn into the log is called spawning.

1. Time of Spawning

The mycelium of *Lentinula* grows in the temperature range of 5 to 32°C, but 20 to 26°C is preferable. It should be noted that the time of spawning will be different from place to place and also from stock to stock; however, it is recommended that when the monthly average temperature is about 10°C, this is a good time for spawning. The mycelium of *Lentinula* starts to grow vigorously when the temperature rises above 10°C, and the harmful microorganisms do not grow easily at such a low temperature. When the temperature reaches 20°C or above, it is more suitable for mycelial growth, but it is also suitable for the growth of competitor microorganisms. At the same time, during spawning, the relative humidity in the air should range from 70 to 80%, and it should not be higher than 85%; otherwise the inoculated logs can easily become contaminated.

2. Method of Spawning

For small-scale culturing, inoculation holes are drilled into the log with a manually operated boring tool, but a boring machine or electric hand drill with a fixed-sized bit should be used for large-scale culturing (Figure 13.1). The suitable size of the hole is 1.0 to 1.5 cm in diameter and 1.5 to 2.0 cm in depth. It should be noted that the depth of the holes should be at least 1 cm into the wood under the bark. The holes are spaced at intervals of 20 to 30 cm along the long axis of the log with 5 to 6 cm between each row of the axis. The holes are arranged in an alternating pattern, making the spaces between every other row 12 cm apart (Figure 13.1). The number of holes in a log can also be calculated on the basis of two holes per 30 cm^2 of area as follows:

$$\text{Number of inoculation holes} = (\text{diameter of log in cm}/3) \times (\text{length of log in cm}/20)$$

For example, the number of holes for a log 9 cm in diameter and 1.2 m long is 18, as calculated from $(9/3) \times (120/20)$.

Spawn is taken out of the culture bottles or polypropylene plastic bags, divided into small pieces which are about thumb size and weigh approximately 1 g, and then one piece of spawn is placed gently into each hole. The spawn should not be ground into powder or pressed down so hard that it forms a cake. The spawn should be left as loose and spongy as possible, similar to the condition of the spawn in the bottle. After filling each hole, the spawn should be pressed lightly, like tamping tobacco into a pipe.

The holes are then sealed with the correct-sized plug made of bark or other material, e.g., profoam. A hole puncher or drill may be used to make these plugs. The plugs should be slightly larger

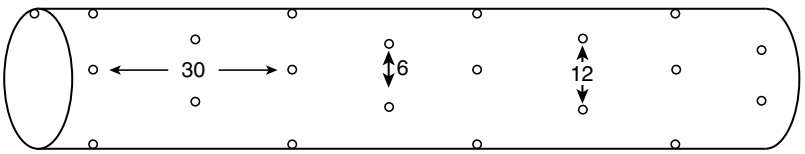


FIGURE 13.1 Method of inoculation indicating the holes arranged in alternating patterns.

than the hole to ensure a good seal. The proper thickness of the plug is 3 to 4 mm, as plugs that are too thin are apt to be lost during drying, and ones that are too thick tend to enter too deeply into the hole. Bark plugs should be obtained from the bark of young trees or branches so that some woody part is included. These plugs are tapped into place with a hammer and then sealed with a coat of paraffin to prevent contamination by fungi and also to keep the moisture level constant.

Spawn used for the initial inoculation in bag cultivation may also take the form of small wedge-shaped or rod-shaped pieces of wood impregnated with active mycelium. Wood plug spawn for log inoculation is more convenient, but it is more expensive than sawdust spawn. Both spawns can be stored at 4 to 10°C for a maximum of 6 months.

Spawning work should be done in the shade and not on rainy or snowy days. During the process of spawning, any possible causes of contamination should be avoided.

D. Laying Logs for Mycelial Running

After inoculation or spawning, logs should be placed in a situation that is favorable for the development of mycelium. The main requirements for rapid and vigorous mycelial growth are (1) correct water content within the logs and (2) a suitable temperature surrounding the logs. Under natural conditions there is no watering to be carried out during mycelial growth and, therefore, logs should be arranged so as to prevent excessive water loss by evaporation.

Selection of the laying yard is important in the success or failure of mushroom cultivation, because the place can greatly influence the dryness of the logs, the humidity of the culture site, the amount of sunshine and ventilation, etc. This means that the site selected should be located to face south or southeast with a slight inclination, receive 30% sunshine through the tree branches, have good ventilation, and a low humidity.

To lay the logs, two sticks of 50 to 60 cm length can be fixed at a distance of 1 to 1.5 m, and a cross wood bound between the two sticks. The inoculated logs are laid down along the cross wood as a pillow. A second cross wood piece is put on the logs and more logs can be laid down. These processes can be repeated to make a pile of logs. In this way, 15 to 20 logs (9 cm × 1.2 m) can be laid per square meter, including cross ways (Figure 13.2). Sometimes, 100 to 150 logs can also be arranged in flat piles to a height of 50 cm to 1 m high and covered with straw matting, vinyl netting, or with tree branches.

FIGURE 13.2 Arrangement for the laying of the inoculated mushroom logs in rows between the trees.

Under favorable conditions, a white mycelium begins to grow inside the plug about 20 days after inoculation, and it then slowly spreads into the logs. The spreading rate of the mycelium is slow in the initial stage, but it becomes more vigorous and reaches the sapwood through the inner tissues of the stem. About 40 to 50 days after spawning, white mycelium growing from the cambium layer of the log is observed at both ends of the log. This is the simplest and most convenient method to examine the extent of mycelial development, although there are various other methods that can be used to assess the advance of the mycelium after spawning.

Unless the weather is extremely dry or wet, the piles require no special care during the mycelial running period, which ranges from 6 months to 1 year, depending on the degree of compatibility between the stocks of *Lentinula* and the species of wood log used. With a good stock, the mycelium can penetrate and spread rapidly into the log. This produces robust mycelia, a good yield, and continued production. If the weather is too wet, the logs easily become contaminated by molds, which commonly grow in places that are humid and have insufficient ventilation. If this is the case, the logs should be spread out to give more space, and any stagnant water should be drained away. If it is too dry, the spawn becomes dry and shrinks. This requires immediate care. One simple way is to provide shade for any spot where there is continuous sunshine through the branches of the trees. The laying yard that receives sunshine from the side in the early morning or late afternoon should also be sheltered. For flat piles, the logs can be completely and thickly covered on the top, leaving the sides and bottom open for ventilation. If the logs have become very dried out, water can be applied, but this is seldom advisable.

E. Management of the Raising Yard for Fruiting

In general, fruiting of *Lentinula* depends on environmental conditions. The fruiting of most *Lentinula* stocks requires a drop in temperature, increased humidity, and a certain degree of light. After the mycelium has fully developed within the logs, the logs may be transferred to another location that offers different environmental conditions for stimulation for fruiting. The proper place for the development of fruiting bodies should be more humid and less ventilated than that of the laying yard.

There are two general methods for stimulation for fruiting. In one method, logs are at first arranged in an upright position by leaning them in rows, either on one side or on both sides alternately on different supporters. Then the logs are watered with sprinklers. An alternative to spraying with water is to immerse the logs in a tank of water for a length of time that depends on the season and the stock. In winter 2 to 3 days with a water temperature of 10 to 15°C and in summer 1 day with a water temperature 15 to 18°C are the conditions commonly employed. Then the logs are leaned against the supports. The method of arranging the logs should allow for the convenient management and easy harvesting of the mushrooms. The number of logs to be stacked for raising is about 10 to 12/m^2, including a crossway supporter (Figure 13.3). Because development of mushrooms at this stage requires considerably more moisture than during vegetative growth, the raising yard must be carefully shaded and the moisture must be kept high. Under natural conditions, depending on the kind of tree used, within a few days after the logs have been removed from their immersion in water, fruiting bodies will appear. The bed logs should now be protected from drying and be kept warm, especially in winter, until the mushrooms achieve a size of 1 to 2 cm.

The second general method involves the housing of the bed logs in a greenhouse (Figure 13.4). The temperature should be maintained at 15 to 20°C and the humidity be kept at 80 to 90% during winter. The mushrooms reach maturity in 10 to 15 days. In summer, the mushrooms mature in 4 days, while in spring and autumn maturity is reached in 5 to 7 days. On an average, three harvests may be obtained from one log per year. After harvesting, the logs should not be watered immediately, but should be left alone for about 40 days and then watered for a few days. Mushrooms will then appear, and approximately 1 week later, the mushrooms can be picked. It is possible to obtain

FIGURE 13.3 The mushroom logs are stacked toward the supporting brace from both sides alternately.

FIGURE 13.4 Mushroom logs arranged inside a greenhouse.

mushrooms from logs for up to 6 years, but the yield by the fifth year is generally poor. However, a 3-year period of using logs for production is a normal practice.

F. Cropping

The yield of fresh *Lentinula* through the lifetime of natural logs may be as high as 35%, and on an average it is about 15 to 20%. The harvest time depends on the nature of the product desired; e.g., for the fresh mushroom, harvesting is done when 50 to 60% of the cap is open; and for dry mushrooms, harvesting is done when the cap is 70 to 80% open. The fruiting bodies, which develop slowly in spring at a relatively low temperature, have thick caps and short stalks and are the high-quality product called donko or dong-gu. Those that develop rapidly in rainy seasons or autumn at high temperature and humidity have thin caps and long stalks and are a poorer-quality product called Hyangshin or Shiangshyuhn. Within these two general grades, mushrooms are also further divided into several subgrades for marketing purposes.

When the mushroom is harvested, the lower part of the stalk is grasped, and it is picked by pressing first and then twisting. If the mushroom is pulled up directly by force, the bark of the log may be peeled off. This damage to the log may cause infection by competing fungi and enhance the loss of water by evaporation, which could eventually shorten the lifetime of the logs for *Lentinula* production.

VI. CULTIVATION IN POLYPROPYLENE BAGS ("BAG LOG" CULTIVATION)

As mentioned in Section V, *Lentinula* has been traditionally cultivated on hardwood logs outdoors in the natural environment, but alternative, more intensive, cultivation techniques have been developed. By using sawdust mixed with various ingredients as a substrate and growing under controlled or semicontrolled environmental conditions, techniques for cultivation in synthetic wood logs in plastic bags have developed rapidly. Three special features of the plastic bag method in cultivation of this mushroom can be generalized:

1. The materials used to make the synthetic logs are mainly sawdust and other agricultural by-products or residues, such as coffee wastes, sunflower seed hulls, cotton seed hulls, and corncobs, which are abundant and some of which are generally available in every corner of the world.
2. This method shortens the production time and gives a high yield. Using natural logs, the time from spawning to harvesting the first mushroom generally requires about 8 months to 1 year. The completion of harvesting takes more than 3 years. Under natural conditions, on an average, 100 kg of natural logs can produce about 10 to 15 kg fresh mushrooms over that period. In the synthetic log, mushrooms can be harvested about 60 to 80 days after spawning. This is about 5 months earlier than those cultivated in natural logs. The completion of harvesting takes less than 8 months, which is shorter by about $1^1/_2$ to 2 years, compared with the natural log method. Under controlled or semicontrolled conditions, the biological efficiency can reach 80% on the average. This means that 100 kg of sawdust with supplements can produce 80 kg of fresh mushrooms. As high as 145% mean efficiencies in 6 months have been reported from synthetic logs by Royse et al.[7,21] This is about an eightfold increase over natural logs.
3. Plastic bag cultivation is relatively easy to manage. For example, cultivation of *Lentinula* in plastic bags can be handled by both young and old workers and operated as a business in both rural areas and urban districts. Therefore, it has already developed into a new and important way for cultivation of this increasingly important mushroom. It should also be noted that although the mycelium in synthetic logs runs faster and gives a higher yield, the quality of the mushrooms produced in synthetic logs is generally lower than that from natural logs. In practice, the technique used for growing mushrooms in synthetic logs involves sterilizing the substrate in heat-resistant containers, followed by the inoculation of the cooled substrate in each container. This process is considered labor-intensive, and it consumes a substantial amount of energy. To save labor and energy, cultivation of the mushroom on pasteurized rather than sterilized synthetic logs or composts must be developed. The sawdust with supplements could be composted, pasteurized, cooled, and inoculated in the same way as for cultivation of *Agaricus bisporus*. Certain species of thermophilic microorganisms should be encouraged to develop during the composting process, because those microorganisms could prevent the contamination by competitors and promote the growth of the mushroom.

TABLE 13.7
Main Components of Mixed Substrates and Their Proportions for *Lentinula* Cultivation in Polypropylene Bags

Material	Dry Sawdust (kg)	Compost Sawdust (H_2O about 50%) (kg)	Volume Ratio (parts)
Sawdust	100	100	100
Rice bran	8–15	4–8	4.5–9.0
Corn bran	4–8	2–4	1.5–3.0
$CaCO_3$	0.6–1.0	0.3–0.5	0.06–0.12
H_2O	180	40	(adjust to 60%)

A. Materials

The materials used for bag cultivation of *Lentinula* are polypropylene bags plus various substances used as substrates for the growth of *L. edodes*. The plastic bag must be made of polypropylene, because this polymer can withstand the temperatures required in autoclaving (121°C). The size of the bag that we commonly use is 15 to 30 cm. Glass bottles can also be used as heat-resistant containers for cultivation of the mushroom in synthetic substrate. The principal constituent of the substrate is the sawdust of various types of wood, but rice bran, corn bran, and $CaCO_3$ are also included; and other sources of cellulose, hemicellulose, and lignin may also be used in various combinations with or without sawdust, including waste tea leaves, cotton seed hulls, sunflower seed hulls, coffee wastes, and ground corncobs.

1. Sifting the Materials

The common types of sawdust usually contain small pieces of wood or other hard materials. Consequently, the sawdust should be sifted to remove these hard and unwanted pieces, which may perforate the bags when the bags are filled. Such perforations could provide entrance to the substrate for harmful, contaminating fungi. Corncobs, corn ear husks, and wood chips should be ground into small pieces. All these materials should be fresh and not be moldy.

2. Mixing the Materials

The sifted sawdust is mixed with other ingredients (supplements) in various proportions as shown in Table 13.7.

Recently, Royse[20] used a mixture of maple and birch (60:40) sawdust as the main substrate ingredient and found that the proportion of sawdust (80%), millet (10%), and spring wheat bran (10%) was the best formula for nutritional components. Actually, there is a wide range of components, and various proportions for mixed substrates may be used in preparation of synthetic medium for cultivation of the mushroom. However, it should be noted that mycelial growth, primordium formation, and fruiting body productivity can vary greatly with different stocks on synthetic substrates. One stock grows better than the other stock on Formula A. The result may be reversed on Formula B. Therefore, a good stock means simply that it gives a higher yield on a particular medium. Stocks should be tested for biological efficiency, quality, and average weight of mushrooms[1] before the selected stock is used in commercial-scale production.

B. Inoculation

The mixed substrate mentioned above can now be placed in heat-resistant containers, commonly in 30×15 cm polypropylene bags, then gently pressed into the shape of a small log, and autoclaved

at 121°C for 1 hour. When the contents of the containers have cooled, mushroom spawn is inoculated on the surface of the substrate. The inoculated substrate is then incubated at 20 to 25°C in the dark for 80 days to 6 months, depending on the substrate and stock used.

It should be particularly noted that mushroom spawn must be fresh, robust, and not degenerated. After long periods of storage or use in cultivation, highly productive stocks or strains of cultivated mushrooms may show a gradual or sudden retardation of growth or decrease in yield. This attenuation of fungal stocks or strains is a well-known phenomenon that may be attributed to genetic variation and influenced by an unfavorable environment. Degeneration may occur and be detected at various stages of mushroom cultivation.

C. Incubation

Observations of mycelial growth or spawn running of *Lentinula* in synthetic substrate in plastic bags and in liquid with and without supporting materials have led to the description of the developmental stages for fruiting cultures of *Lentinula*. The sequence of events may be altered slightly, but the usual order of events is as follows.

1. Mycelial Running Stage

This stage refers to mycelial growth that occurs immediately after inoculation (spawning) until the compost substrate in the container is completely permeated with mycelium. During mycelial running, the mycelium secretes enzymes that degrade complex substances in the compost, such as lignin, cellulose, and hemicelluloses, into smaller soluble molecules, which can be absorbed by the growing mycelium and used as nutrients for further growth of the mycelium.

2. Established Mycelial Stage

The established mycelial stage is a stage from the end of the mycelial running stage to the hardening of a mycelial coat. During this period, the mycelium absorbs and accumulates the necessary nutrients in sufficient amounts required for fruiting.

a. Mycelial Coat Formation Phase

This is a phase of the established mycelial stage in which a thick, sheetlike covering of mycelium develops on the outer surface of the substrate.

b. Bump Formation Phase

According to our experience, most stocks cultivated in plastic bags form bumps of various sizes and irregular shapes on the mycelial coat when the bag is completely permeated with mycelium. Sometimes the bumps are formed when the mycelium has grown on only half the bag, and the bumps form continuously with growth of mycelium from the upper to the lower part of the bag.

Bumps are clumps of mycelium and potential fruiting body primordia. However, most of the bumps abort prior to becoming fruiting body primordia. The time of formation of the bumps varies with the stocks, the compost, and the temperature. In general, the bumps form 10 days earlier at 25°C than at 15°C. After formation of bumps from the mycelial coat, there are air spaces formed between the mycelial coat and the plastic bag. This can help increase air circulation and may promote or enhance the formation of brown pigment in the mycelial coat.

Under the bumps, some cells continue to differentiate and eventually to form primordia. When environmental factors are favorable for further development, the primordia develop into fruiting bodies. About 60 to 75 days after spawning, bump formation usually ceases.

c. Pigmentation Phase

When bumps are formed, the plug of the bag can be loosened slightly to allow air to enter the bag. Air enhances pigmentation.

d. Coat-Hardening Phase

After pigmentation, the cotton plug should be removed to provide more fresh air for the mycelium. The coat gradually becomes hard. After 20 to 30 days, the coat will be hard and dry. At this time the moisture content of the compost inside this artificial bed log (bag log) may increase to 78%. This kind of bag log, moist inside and dry outside, is the best quality for formation of fruiting bodies. If the inside of the bag log is too dry, it indicates that the plug was removed too early.

e. Change of Moisture Content

There is a change of moisture content in the substrate during the established mycelial stage. It has been observed that the interior areas of the bag logs of *Lentinula* after mycelial coat formation are softer and apparently moister than the compost at the time of spawning. The reasons for increased moisture content from the top of the compost with longer spawn runs may include greater mycelial biomass, increased levels of enzymes present in the substrate, increased solubility of substrate components, and increased metabolic activities.

D. Fruiting

If the mycelium in the bag is not mature or adequately established as a result of poor degradation of the compost, inadequate assimilation of the breakdown products, or a shortage of nutrients in the mycelium, the bag log will produce a great number of malformed or abnormal fruiting bodies (e.g., without pileus, pileus with irregular shape, with stipes that are short, stunted, and swollen, etc.). Therefore, before removing the plastic bag completely for treatment to encourage fruiting, the top of the bag should first be opened completely. Following this, some fruiting bodies will be formed on the top (Figure 13.5), and, if they are normal, the bag can be completely removed for further fruiting (Figure 13.6). If, however, some abnormal fruiting bodies are formed, the bag should be tied loosely again for further maturation and development of the mycelium.

If initially the log is considered ready for fruiting, the plastic bag may be removed completely and the bag log inverted and placed on moist ground or a wet bed surface. After 2 to 3 days, the log should be reinverted and placed on shelves or the ground. At this time some dark-colored, pinhead mushrooms will appear at the top of the bag. After further development for 3 to 4 days, the mushrooms can be harvested (Figure 13.7 and Figure 13.8). Sometimes, after removal of the plastic bag, the log is soaked in cold water for 1 day. As a result, fruiting bodies form more evenly.

When is the inoculated mycelium in a synthetic bag log established? How can the established mycelial bag log be treated or stimulated for fruiting? There are many different ideas from different investigators. For example, one, as mentioned above, considers that the formation of the mycelial coat and the hardening of the coat are reasonable indicators that the mycelium is established and mature and thus ready for fruiting. The function of the mycelial coat may be like the bark of natural wood logs and may serve as protection from water loss, for holding CO_2 in the compost inside the

FIGURE 13.5 Shiang-gu cultivation on sawdust blocks. Because the plastic bags were removed at a later stage of mycelial development, most of the mushrooms appeared at the top. (Courtesy of Dr. Myra Chu-Chou.)

FIGURE 13.6 Shiang-gu mushrooms grown on synthetic compost logs. Because the plastic bags were removed at an early stage, mushrooms appeared on the sides of the logs as well as the top.

FIGURE 13.7 Harvesting stage of *Lentinula* fruiting bodies cultivated in sawdust and used tea leaves substrate in a plastic bag.

coat for improved vegetative growth, and for preventing contamination. After the inoculated mycelium is established, the plastic bag can be opened at the top or removed completely. Then the logs can be treated for fruiting, e.g., by lowering the temperature or by soaking in water. In another method, the inoculated substrate is simply incubated at 20 to 25°C in the dark for 60 to 70 days. Once the mycelium has developed into the substrate of the entire bag, the plastic is completely removed and the synthetic logs are placed in a room with the temperature about 16 to 18°C and a relative humidity of 85 to 90%. On a suitable sawdust medium, the primordia appear in 3 to 4 weeks, and the mushrooms develop 7 to 10 days later.

In some places in China, when the inoculated mycelium has grown throughout the container (usually glass bottles are used), the mycelial running sawdust medium is removed from the bottles and replanted on a wooden frame, $30 \times 30 \times 4$ cm. If the mycelial coat is already formed in the

FIGURE 13.8 *Lentinula* mushroom bags arranged on shelves in the cultivation house.

bottle, the coat should be removed and should not be replanted in the frame, because mycelia in different development stages mixed in the frame will affect the uniformity of colonization and fruiting. Next, the frame with the replanted medium is wrapped up with a thin plastic sheet. The purpose of this is to protect against water loss, maintain temperature, and avoid contamination. When placed at 22 to 25°C, the mycelium develops rapidly. The coat is formed with or without brown pigmentation. Some strains can have fruiting bodies after 7 days of replanting the substrate, and others may be delayed for 3 more weeks.

E. Proper Care of the Bag Log

The following precautions should be noted for proper care of the bag log:

1. **Watering:** Avoid overwatering. Under normal conditions the bag log does not need to be watered before the initiation of mushroom pinheads because the moisture content of the compost is usually about 70%. However, if the moisture content drops below 60%, and it drops even lower than 50% sometimes, the bag must be watered, but the yield will be affected.
2. **Proper Treatment for Fruiting:** The length of time between spawning and fruiting varies greatly, depending on the stocks used and the growing conditions. In general, at least 60 days and, at most, 180 days are required for fruiting. If treatment for fruiting is too early or too late, the log may produce abnormal mushrooms and give a low yield. If the bag log is treated for fruiting within 120 to 150 days, mushrooms of suitable size and shape, and a good yield will be produced
3. **Maintenance of Suitable Temperature:** If the difference between day and night temperatures is about 3 to 9°C and the average temperature is 20°C, ranging from 15 to 25°C, good-quality mushrooms can be produced.
4. **Maintenance of Suitable Moisture:** When small pinhead mushrooms appear, the relative humidity should be kept between 85 and 90%, but in the earlier stages it is better to keep the moisture in the culture room below 60% to avoid the chance of contamination from the cotton plugs.
5. **Proper Treatment for Refruiting:** The first flush of mushrooms usually lasts about 7 days. When a mushroom is harvested, a scar is left on the log. After a period of about 3 days, only a gentle sprinkle of water should be applied to the log, or else the scar may

become moldy or rotten due to becoming too wet. The log should be slightly watered twice daily for about 2 weeks.

VII. SPECIAL CULTIVATION PRACTICES

A. Taiwan

Cultivation of *Lentinula* in Taiwan is either by wood logs or by plastic bags. In 1987, the two methods were approximately of equal importance. Careful consideration has been given to the matter of spawn. Because there are many companies producing spawn, it has been suggested that spawn should be certified by government standards to eliminate poor-quality spawn that has sometimes been sold to mushroom growers and to recognize the spawn that respond differently to various local conditions and seasonal changes, as well as those differing in yield and quality. Of particular interest in Taiwan is the temperature at which the variety produces fruiting bodies. High-temperature varieties are those that can fruit at 20°C, and mid-temperature varieties fruit at 10°C. For cultivation on hillsides, these varieties are most suitable.

Log cultivation in Taiwan is not significantly different from the procedures that have already been described. With a single spawning, the so-called log mushrooms can be harvested for 3 years with the greatest number and best-quality mushrooms produced by the first harvest. Fewer and lower-quality mushrooms are produced in the second harvest, and the third harvest is low in both yield and quality.

Plastic bag cultivation makes use of sawdust or small pieces of wood that are ground into powder. This is mixed with rice bran and sufficient water to reach a water content of 60%. The bags and contents are then sterilized and, when cool, inoculated with spawn. This method is used at low levels of the hillsides and is commonly organized as a large-scale operation. Plant residues other than sawdust can be used, but a commonly used substrate for the plastic bag cultivation consists of the following materials:

Sawdust	90%
Rice bran or wheat bran	5%
Cornmeal	4%
Calcium carbonate	1%
Water adjusted to (pH 4.5 to 5.5)	60%

B. China

The following account is based on articles in Chinese by Ting Hu-Guang.[25] It describes a method of cultivation of *Lentinula* in the field as currently practiced in Gutian County, Fujian Province. The method is interesting from several standpoints, the first of which is that it is a bag culture technique in which the bags are cultivated outdoors, not indoors as was the case when bag culture was first introduced. This is thus a reversal of the trend elsewhere, in which cultivation outdoors with wood logs has moved to indoor cultivation in bags. In Gutian County the new method involves cultivation in the paddy fields. This is presented here with some specific details and constitutes what some might consider a case study in 1984 of the method of cultivation of shiang-gu in the field in Gutian County.

After the harvesting of rice in the autumn, paddy fields were used for the cultivation of shiang-gu. The yards in the front and behind houses were also used. In Gutian County 12 million bags were cultivated in the field, and the harvest that year was generally good. It is possible, on the average, to cultivate between 8000 and 10,000 bags on a Chinese acre (12 to 15 bags/m^2). From those bags 350 to 400 kg (dry weight) of mushrooms were harvested. It was calculated that 100 kg of sawdust or cottonseed hulls produced, on the average, 5 to 6 kg of dry mushrooms, a biological

FIGURE 13.9 *Lentinula* mushrooms being harvested in one of the cultivation houses in Shanghai, China. Note that here the brick method of cultivation has been used. (Courtesy of Mrs. Y.S. Ho.)

efficiency of about 40%. This yield is ten times higher than the log method, and it is 20% higher than the indoor brick method that is used in Shanghai (Figure 13.9). Furthermore, the shape, color, and smell of the mushrooms were all reported to be better than those of the mushrooms produced by the indoor brick method. The quality is said to be almost the same as that of the mushrooms produced from wood logs. The following is a description of the field cultivation method.

1. Seasonal Development

The mycelium of *Lentinula* does not tolerate high temperatures. Fruiting requires both a low temperature and a range of fluctuation of temperature. In Gutian County the season for mycelial running in bag culture is from late August to the end of September when the temperature is around 25°C. Small fruiting bodies appear in November or the first part of December. The first crop of winter mushrooms of shiang-gu can be harvested during the Chinese New Year, and 1 week later the spring mushroom of shiang-gu will appear. The spring mushroom will have a thinner cap due to temperature changes during the period of mushroom development.

2. General Formulas for Substrate

1.	Sawdust	50 kg
	Wheat bran	1.5 kg
	Cornmeal or cassava powder	1.0 kg
	Cane sugar	600 g
	Gypsum (calcium sulfate)	1.5 kg
	Ammonium sulfate	20 g
	Calcium superphosphate	30 g
2.	Cottonseed hulls	50 kg
	Wheat bran	20 kg
	Gypsum (calcium sulfate)	1.5 kg
	Lime (calcium carbonate)	30 kg

3.	Cottonseed hulls	25 kg
	Sawdust	25 kg
	Wheat bran	10 kg
	Cornmeal	1 kg
	Gypsum (calcium sulfate)	1.5 kg
	Cane sugar	500 g
4.	Bagasse	50 kg
	Rice bran	12.5 kg
	Gypsum (calcium sulfate)	1.5 kg
	Potassium sulfate	15 g
	Urea	15 g
	Magnesium sulfate	10 g
5.	Corncobs	40 kg
	Sawdust	10 kg
	Wheat bran	12.5 kg
	Cane sugar	1 kg
	Pectin	15 g
	Urea	20 g

To each of the above formulas (1 to 5) add 65 to 72 kg of water to make the water content of the mixture about 60%. The pH should be about 5.5 to 6.0.

3. Method for Filling the Bags

The bags used are either 500 × 160 × 0.09 mm in size made of low-pressure resistant plastic or 500 × 150 × 0.04 mm in size made of high-pressure-resistant plastic. After mixing the substrate ingredients thoroughly, the bags should be filled immediately to avoid the onset of fermentation and contamination of the substrate. The bags are first filled loosely with the substrate, and then a pressure of 20 kg is applied which results in the filled bag having a cylindrical shape.

4. Sterilization

Two holes are punched per side on opposite sides of the filled bags. The holes are 15 mm in diameter and 20 mm deep. They are closed with 35-mm squares of an adhesive medical tape. The openings covered by the squares of tape will be used for inoculation after the bags have been sterilized.

Sterilization is accomplished by steam generated by a homemade boiler. The steam is at normal pressure; that is, the temperature is 100°C. For sterilization this temperature is maintained for 12 hours.

At the conclusion of the sterilization period, the bags are allowed to cool for about 2 hours. They are then removed and dried by means of ventilation. When the temperature of the bag drops to 20°C, the bags are transferred to a special room for inoculation.

5. Inoculation

Under aseptic conditions the tape squares are removed, and then forceps are used to put sawdust spawn into each hole. The amount of spawn inserted into each hole is about the size of a broad bean. Light pressure is exerted where the spawn has been placed and the holes are resealed with the squares of adhesive medical tape. From 25 to 30 bags can be inoculated with one 750-g bottle of spawn.

6. Indoor Mycelial Running

Immediately after inoculation the bags should be placed in a room for incubation with the cylindrically shaped bags in a crisscross pattern with a space in the middle of the layer that consists of three or four bags. One stack is made up of ten layers.

The temperature during the first 4 days should be controlled at 26 to 29°C. After 4 to 5 days some white mycelium should show around the inoculation sites. Then the temperature should be adjusted to 25 to 27°C and the windows opened slightly for ventilation. This is particularly important in rooms heated by coal, so that CO_2 will be removed and to assure the presence of fresh air in the room. Generally, incubation requires a period of 15 to 20 days. When the mycelium grows to a distance of 10 cm from the inoculation site, a corner of the square of medical tape covering the inoculation site should be lifted to provide a means for some air to enter the bag. At this time the room temperature should be decreased to 23 to 25°C, and the relative humidity should become 80%. Sprinkling the area twice daily with water should maintain this relative humidity. In the event that the room temperature rises to more than 30°C, the door should be opened for ventilation and the frequency of sprinkling should be increased.

7. Shift to Outdoor Cultivation

When the mycelium covers 50% of the bag (approximately 50 days after inoculation), it is time to move the bag from the incubation room to the field. This is the time that the plastic bag should be cut with a sharp knife for removal of the cylindrically shaped "log" (Figure 13.10).

The time of removal of the bag is critical. In cultivation by the bag method, inoculation should generally be completed before the end of September, and following mycelial running the time for removal of the bag will be in October–November. Some growers depend only on the number of days following inoculation to determine the time for removing the plastic bag, but there are other criteria that should be taken into consideration, including the characteristics of the spawn and the degree of vigor of mycelial growth. If these other criteria are not considered and the bag is removed too early, then the mycelium may not have reached physiological maturity, or the mycelial coat may not have changed color, and the mycelium may easily become contaminated. On the other hand, if the bag is removed too late, the mycelium will be overly mature, and, as a result of this, large quantities of a yellow fluid will accumulate in the bag, which can lead to contamination and decay of the substrate. Thus, the time of bag removal should be determined by (1) characteristics of the spawn, (2) the ratio of spawn to substrate, (3) the age of the mycelium, and (4) the condition of mycelial growth (physiological maturity), which is a reflection of the environmental conditions during growth.

FIGURE 13.10 Synthetic logs with mushrooms are laid in rows against bamboo supporting sticks. This arrangement of synthetic logs is then covered with a plastic sheet. (Courtesy of Mr. Y. C. Kong.)

Examples of spawn characteristics are early-, middle-, and late-maturing varieties. Under normal conditions when early-maturing varieties of spawn have been used, the removal of the bags should be considered after 50 to 60 days. For example, with one variety (CR-O2) it was reported that the mycelium was fully grown 50 days after spawning, and the bags were removed. On the other hand, the middle and late maturing strains require 80 to 100 days for the mycelium to reach physiological maturity. Physiological maturity is determined by the appearance of bumps on the surface of the mycelium. If bumps have formed on the mycelial coat in two thirds of the bags, and some of these already have a brownish color, it is indicative of the best time to remove the bag.

After removing the bag, the log is then placed on end in a bed, which is 1.2 to 1.3 m wide and 15 cm high. Strips of bamboo are bent in a bow shape over the bed to support a plastic covering, thus forming a kind of simple mushroom shed. The distance between the cylindrical logs as they stand on end is 2 to 3 cm. The temperature of the mushroom bed should be 20°C and not exceed 23°C. The relative humidity of the air surrounding the logs should be controlled between 85 and 90%. Once the plastic bags have been removed, thin plastic sheets should cover the synthetic logs.

8. Formation of Mycelial Coats

To promote the formation of the white, dense mycelial coats, the plastic sheets should be opened for 20 to 30 minutes two to three times per day to give more oxygen and light to the mushroom bed. For 3 days the ventilation must be carefully controlled to maintain a constant temperature. During this period the aerial mycelium on the surface of the synthetic log grows faster and reaches maturity. A white, woolly mycelium will cover the log completely. This is the time to increase moisture as well as to ventilate. Ventilation should be increased gradually from gentle to strong and for a longer period of time. A relative humidity of 85 to 90% should be maintained by sprinkling once or twice daily. If the air temperature becomes too high, the plastic sheets should be opened a little bit.

At 7 to 10 days after removal of the bag, the mycelium usually begins to change in color. The exposure of the surface of the log to alternating periods of moisture and drying retards the growth of the surface mycelium and promotes pigment formation (this is sometimes called the stage of pigment formation).

If the mycelium grows too rapidly, the covering plastic sheet should be opened for 3 to 4 hours around noon when the air is dry and the light is strong, so that the surface of the log becomes dry and the aerial mycelium collapses. Then the logs are again covered with the plastic sheet, and in another 3 to 4 days the mycelium will begin to secrete some pink pigments that develop further into a red color. Finally, a barklike layer, the mycelial coat, is formed, which is dark brown.

The mycelial coat functions in the protection of the mycelium within the cylinder to permit subsequent normal development and to prevent water loss and contamination.

9. Stimulation of Fruiting by Temperature Fluctuation

Under ordinary conditions, the primordium of the fruiting body starts to appear 7 to 10 days after the change of color of the mycelial coat. Indication that the primordium will begin to form is signaled by secretion by the mycelium of white water droplets, which changes to the secretion of yellow-reddish droplets, and finally to that of dark brown droplets that are sticky rather than watery.

The time required from the formation of mycelial aggregate to a primordium is 3 to 6 days, but this occurs only under the condition of fluctuating temperature. To bring this fluctuation about, the temperature in the bed can be increased during the daytime by completely covering the bed with a plastic sheet, and then opening the sheet at midnight for ventilation. This procedure will decrease the temperature suddenly by as much as 10°C. If this operation is continued for 3 to 4 days, the mycelium will become intertwined and knotted together, and the surface of the log will have an irregular appearance because of the presence of white cracks in the mycelial coat. After a

short time, pinheads of mushrooms will emerge from the inoculation holes and from cracks in the mycelium. In general, mushrooms appear 64 to 80 days after inoculation.

10. Management of Fruiting

There are two general management methods for fruiting of *Lentinula* in China.

a. Bag Removal Method

First, when the mycelium covers the whole bag (approximately 60 days after inoculation) and forms a thick, sheetlike covering of the outer surface of the substrate, it is time to move the log-bag from the incubation room to the pre-emptied paddy field. For this transfer, the plastic bag should be cut with a sharp knife and removed from the cylindrically shaped log. This is often referred to in China as the point when "the first half of the cultivation life of log-bag method of *Lentinula* has ended and the second half of the new life living outdoors has begun," usually in a plastic house. The timing for the removal of the bag is critical. If the bag is removed too early, then the mycelium may not have reached physiological maturity, and the mycelium may easily become contaminated. On the other hand, if the bag is removed too late, the mycelium will be overly mature and, as a result, large quantities of yellow fluid will accumulate in the bag, which can lead to contamination and decay of the substrate. When the bag is removed from the log at the proper time, the color on the mycelial coat will quickly change. The "artificial log" can form fruiting bodies according to the biological conversion principles. If the bag is removed too early, the mycelium may not have reached physiological maturity, and the mycelial coat usually will not change its color. Even if it does change color, the coat will be thin and the color pale. The "artificial bark" will not form, so the yield and quality of the fruiting bodies will be affected. In addition, the early removal of the bag can lead to loss of water from the log and easily cause the log to be dry and have fruiting difficulties. If the bag is removed too late, and the mycelium is over matured, a large amount of accumulated yellow fluid can penetrate into the substrate, inducing contamination. In addition, it could cause a thickening of the coat, which may affect the normal formation of primordia. If the bag is not instantly removed when the pinheads of the mushroom have formed in the bag, the young mushrooms may die due to the lack of oxygen. According to practical experience, the maturity of mycelium is determined by these four aspects:

1. **Mycelium Age:** A temperature of 22 to 25°C for 60 days depending on the strains used for incubation is good enough for removal of the bag for general cultivated strains.
2. **Morphology:** Various sizes and irregular shapes of bumps on the surface of the mycelial coat extend to about two thirds of the log. This indicates that the mycelium has accumulated sufficient nutrition, and it is ready to switch from the vegetative growth to the reproductive phase.
3. **Pigmentation:** The mycelium with white color grows vigorously in the bag and starts to exude a yellow fluid, which could induce pigment formation.
4. **The Nature of the Substrate:** During spawn running, the substrate is firm and hard. When the mycelium has reached maturity, the substrate softens and has a degree of sponginess.

After the mycelial coat has thickened and has bumps and a brownish color, the log can be triggered for fruiting by fluctuation of temperature, moisture, and light. In addition, good aeration is also very important for the healthy development of the fruiting bodies. Second, the development of fruiting bodies also depends on nutrition and water. The synthetic log may be submerged or soaked in water for certain periods depending on the nature of the log. Alternatively, the water can be injected into the log by a multiheaded syringe.

b. Stationary Bag Method

Approximately 50 to 60 days after spawning, the bumps are usually formed on the mycelial coat and yellow fluids may also be exuded and accumulate in the space between bumps. The bags should

then be punctured with a needle to release the fluids, which otherwise may cause the decay or contamination of the substrate. Bumps are clumps of mycelium and potential fruiting body primordia. However, most of the bumps are aborted prior to becoming fruiting body primordia. The time of formation of the bumps varies with the stock, the compost, and the temperature. When bumps are formed, the plug of the bag can be loosened slightly to allow air to enter into the bag to increase pigmentation. Under suitable conditions, the mycelial coat should be relatively thick with a shiny brownish color. Then the log can be soaked in cold water for 12 to 24 hours for enhancing fructification. After 2 months of spawn running indoors, the logs of high-temperature strains can be transferred to the field, and the plastic bag can be removed. Then the logs should be watered heavily in order to enhance the formation of pigmentation. In general, these high-temperature strains do not require cold shock treatment for fruiting. The fruiting bodies can be formed and will develop normally under natural conditions with regular fluctuation of temperature.

c. Three Harvest Seasons

With the method of cultivation of *Lentinula* in the field, there can be three seasons of harvesting — autumn, winter, and spring.

Autumn Mushrooms: Most autumn mushrooms are harvested before December 12. With robust mycelia and logs that have a high moisture content, management to obtain a good harvest involves the maintenance of a suitable temperature and good moisture conditions. When the first flush of mushrooms has been harvested, the mushroom beds should be ventilated for 3 to 4 hours. A week after harvesting, a white mycelium appears in the scars left by the picking of the mushrooms. The appearance of this mycelium indicates that the mycelium has recovered, and the range of relative humidity can be increased, which stimulates the formation of the second flush. It is common for autumn mushrooms to be harvested in three to five flushes with an interval of about 7 days between flushes.

Winter Mushrooms: The season for winter mushrooms occurs during January and February, a period when the temperature is comparatively low, and consequently the mycelium grows slowly. The main requirement in management is to raise the temperature of the mushroom bed and to decrease the watering, which should be done to just keep the log moist. If a suitable temperature is maintained, two to three flushes can be produced with an interval of 10 to 15 days for each flush.

Spring Mushrooms: The season for spring mushrooms occurs between March and June. This is the most productive season for the plastic bag–field cultivation method, with 70% of the total yield produced during this season. As the log has already produced two crops (autumn and winter), the water content of the log is reduced, and it should be soaked in water for 18 to 20 hours. To facilitate the uptake of water to restore the logs to their original weight, the mycelial coats of the logs are perforated with a metal punch. After soaking, the logs are stood on end on the mushroom beds and then covered with plastic. This raises the temperature and consequently increases the activity of the mycelium. Careful management of ventilation is required for the formation of fruiting body primordia and management of the fruiting process. On average, the spring mushroom has three to five flushes, with an interval of 9 to 13 days for each flush.

11. Reasons for Abnormal Mushrooms

Although production of shiang-gu in Fujian Province by the bag method was very good in 1985 and 1986, there were individual farmers who were not successful. Although there are several reasons for failure, the main reasons were lack of good fruiting and the production of abnormal fruiting bodies that were of low commercial value. This subject has been considered by Huang,[9] who has observed that sometimes even though the mycelium grows well in the bag, there is not good fruiting, and sometimes the fruiting does not occur at a suitable time. He suggests that the main causes for abnormal mushrooms are (1) the use of an unsuitable variety as spawn and (2) the removal of the plastic bag before the mycelium has reached physiological maturity. Three types of spawn are used in China:

- Low temperature: A temperature less than 10°C is required for fruiting.
- Mid-temperature: The fruiting temperature is 10 to 20°C.
- High temperature: The fruiting temperature is greater than 20°C.

This varies slightly from classification of spawn as used in Taiwan. (In Taiwan the mid-temperature varieties fruit at 10°C, and the high-temperature varieties fruit at 20°C.) If a low-temperature spawn is used under high-temperature cultivation conditions, good results will not be obtained. Similarly, if a spawn selected to give good results with wood log cultivation is used for sawdust cultivation in plastic bags, the results may not be good. That is, it is essential to select a spawn variety that is suitable for the local conditions and the method of cultivation to be used.

Sometimes poor fruiting occurs even though a suitable spawn has been selected. A common reason for this is the premature removal of the plastic bag, i.e., the removal of the bag before the mycelium has grown sufficiently and reached physiological maturity. Even with a good spawn and careful management after the bag has been removed, there will not be good fruiting if the bag is removed too early. Even if the log is soaked in water, the quantity of mushrooms will be small and usually of an abnormal type. It stands to reason that, if the mycelium has not grown well and matured, there will be a shortage of nutrients for the formation of fruiting bodies. Thus, if a fruiting body is formed, there may only be enough nutrients to form the stalk but insufficient nutrients to form the pileus. This would explain the frequent observation of abnormal mushrooms, which have a stalk but not a pileus, or just a small pileus.

The mycelium grows well at a temperature of 24 to 25°C, and it breaks down the sawdust substrate well at 27 to 30°C. Therefore, if incubation has been at 25°C or lower, the mycelium may grow well throughout the bag, but there may be very limited accumulation of nutrients by degradation of the substrate. Under these circumstances, in which mycelial growth has taken place at a suitable range of temperatures, it is important to place the synthetic log at the temperature of the upper limit (27 to 30°C) for a period of time so that nutrients necessary for fruiting can be accumulated from substrate breakdown.

It is to be noted that in these case studies of cultivation practices, many explanations are given without supporting experimental evidence. It is the successful practice that we wish to report at this time, however. The scientific explanations will be forthcoming from the future studies of mushroom scientists, and, for the present, the explanations presented by these workers in China provide reasonable working hypotheses.

12. Case Studies: Qingyuan and Biyang

a. Qingyuan

As mentioned in the section on historical background, Qingyuan is the birthplace of the artificial cultivation of *Lentinula edodes* dating to about A.D. 1000. The County of Qingyuan is located in a tropical monsoon climate, which is considered ideal for the production of *L. edodes*. The production of this mushroom in Qingyuan has grown from a mere 2765 MT of fresh weight in 1986 to 48,202 MT in 1993 and to 106,500 MT in 1997. At present, only 20% of the production comes from cultivation on wood logs, the remaining 80% is obtained by using the synthetic sawdust log technique. The overharvesting of wood has impelled the government to encourage farmers to abandon the traditional log technique. The imminent environmental damage of logging wood for mushroom cultivation has spurred new technological breakthroughs including improving the average biological efficiency of approximately 100%. This efficient production of one county represented 10% of the world production and one fifth of the Chinese output in 1993. This was one of the reasons the city was officially named by the Chinese Government as "Shiang-Gu (*Lentinula edodes*) City of China" in 1994 (Figure 13.11).

It is interesting to note that the total population of the county is just less that 200,000 people, of whom 120,000 are directly engaged in mushroom cultivation. This means that 60% of the

FIGURE 13.11 (Color figure follows p. 232.) The authors at the entrance to the "*Lentinula* Mushroom City of China," Qingyuan City, Zhejiang Province, China, in 1994.

population is engaged in mushroom production and management. In terms of jobs, in 1997, the mushroom industry employed an additional 4000 persons in the trading and marketing of mushrooms, and about 2000 people are engaged in the manufacturing of plastics for bagging substrates, sales, production and maintenance of machinery, printing of labels and packaging, and related businesses. The total value of mushroom production in 1997 was U.S. $46.3 million. It is the main source of revenue of the local government, and in recent years, the economic status of the population of Qingyuan is among the 100 richest counties of some 3000 counties in China. This improvement is solely due to the cultivation and marketing of *Lentinula.*

Prior to 1991, trading in *L. edodes* was conducted at numerous stores, but the regional government decided to invest in a trading floor that has been expanded in the years since. Today, some 280 traders are active each day, except during the Chinese New Year Festival. Each trader employs as many as eight persons (most of whom are women). The success of the trading floor required a new expansion in 1999 with an additional 137 trading stalls. The market system and support services such as banking, hotels, and restaurants now employ 15,000 persons, of whom 3000 are paid directly by the traders. There are 60 traders who export as many as 50 MT dry weight of mushrooms per annum.

The county also produces medicinal extracts from *L. edodes* and *Grifola frondosa* for sale to the Chinese communities worldwide. The spent substrate is now under study for its use as a medium for the cultivation of earthworms, which are a source of natural enzymes. In this way, the county expects to continue to increase the level of well-being of its population.

Qingyuan County was the first county to hold an International Symposium sponsored by UNESCO on *Lentinula* in China. In 1994, more than 300 people including 80 foreigners as well as the authors of this book attended this memorable event.

FIGURE 13.12 Flower (cracked) *Lentinula* mushrooms grown from sawdust substrate in bags.

b. Biyang

Biyang in Henan Province is the home of the flower (cracked) mushroom (Figure 13.12). Biyang County is located about 400 km to the southwest of Zhengzhou, the provincial capital of inland Henan. Zhengzhou is the cradle of Chinese civilization, which dawned at the start of the Shang Dynasty nearly 4000 years ago. Today, Zhengzhou is best known as an inland transport hub, a crossroad for trains and highways. The county is surrounded by two mountain ranges that are rich in oak trees. Forests cover about 50% of the county, and only 40% is farmland. The county has a population of 910,000 of whom 800,000 are engaged in farming. There is no industry in the county, and therefore it enjoys the advantage of not being burdened by air or water pollution. In 1992, the county decided to proceed with the economic development of the county based on the creation of "the *Lentinula* economy." Within 5 years (1997), the value of the mushroom production reached U.S. $81 million, which represented 32% of the total value of agricultural production in the county.

Since the small rushlike/plastic shed and big bag method of Biyang has been developed and adopted, the average income of the farmer has increased 5.5-fold between 1991 and 1997. In the mountain ranges of the county, the cultivation of *Lentinula* along with other mushrooms has permitted the government to eliminate poverty in only a few years. Now the Biyang method has been introduced to 120 counties of 15 provinces in China. In 1997, 300 million mushroom bags were planted for a total production value of U.S. $375 million. As a result of this new technique, many farmers can now produce high-quality (flower/cracked) *Lentinula* using sawdust mixed with other lignocellulosic biomass materials and thereby free themselves from the poverty they endured for years.

Biyang County is the second county to hold an International Symposium sponsored by the International Society for Mushroom Science (ISMS) on *Lentinula* in China in 2002. More than 350 people, including 70 foreigners from five continents, participated in this fruitful event.

VIII. FRUITING IN LIQUID MEDIA

A Experiments with *Lentinula*

Although the time required for fruiting body formation in *Lentinula* is usually too long to make it practical to obtain fruiting bodies on agar medium, even for experimental studies, there are some

TABLE 13.8
Three Chemically Defined Formulas for Cultivation of *L. edodes* in Liquid Media

	Leatham (L)	Fruiting (F)	Fruiting + (F+)
Dextrose	25.0 g	20.0 g	20.0 g
L-Glutamic acid	2.5 g	0	0
D-Glucuronic acid	4.0 g	0	0
Asparagine	0	1.5 g	1.5 g
KH_2PO_4	2.0 g	0.46 g	0.46 g
K_2HPO_4	0	1.0 g	1.0 g
$MgSO_4 \cdot 7H_2O$	2.0 g	0.5 g	0.5 g
Thiamine-HCl	(in C below)	0.12 mg	0.12 mg
(A) Mineral solution	10 ml		10 ml
(B) Trace element solution	1 ml		1 ml
(C) Vitamin solution	1 ml		1 ml
(D) Salicylic acid solution	0.1 ml		0.1 ml

Note: Solution A contained 3.67 g $CaCl_2 \cdot 2H_2O$, 4.39 g $MgSO_4 \cdot 5H_2O$, and 2.20 g $ZnSO_4 \cdot 7H_2O$ per liter. Solution B contained 14.1 g Fe $(NH_4)_2(SO_4) \cdot 6H_2O$, 784 mg $CuSO_4 \cdot 6H_2O$, 81 mg $CoCl_2 \cdot 6H_2O$, 51 mg $Na_2MoO_4 \cdot 2H_2O$, 81 mg $NiCl_2 \cdot 6H_2O$, 38 mg $SnCl_2 \cdot 2H_2O$, and 2 ml concentrated HCl per liter. Solution C contained 1000 mg I-inositol, 1000 mg thiamine-HCl, 100 mg pyridoxine-HCl, 100 mg nicotinic acid, 100 mg sodium pantothenate, 100 mg *p*-aminobenzoic acid, 100 mg riboflavine, 30 mg biotin, 10 mg folic acid, and 10 mg cyanocobalamin per liter. Solution D contained 10 g salicylic acid per liter of 95% ethanol.

reports of fruiting on agar medium,[12,26,27] but fruiting in liquid medium is of more frequent occurrence.

Leatham[13] reported that he obtained fruiting of a dikaryotic stock of *L. edodes* (ATCC 48085) on a chemically defined medium within 45 days after inoculation and that sometimes fruiting occurred as early as 27 days. Miles and Chang[15] examined the fruiting ability of different dikaryotic stocks of *L. edodes* on this chemically defined liquid medium and also on modifications of a defined medium that has been used in studies of *Schizophyllum commune.*[18] The studies had two different interests. One interest was to determine whether stocks differ significantly in their nutritional requirements for fruiting. This is part of a general study of the genetic control of fruiting, which would be facilitated by consistent and rapid fruiting. Another interesting problem involving fruiting in a chemically defined liquid medium arises from the possibility that in the future research may lead to the development of a type of hydroponic system for the development of this mushroom. Success would mean that *Lentinula* could be grown in factory-type buildings anywhere, in all seasons. Some preliminary experiments of the growth and fruiting of various dikaryotic stocks of *L. edodes* in liquid media reported by Miles and Chang[15] are now described briefly.

The ingredients of these chemically defined media are given in Table 13.8. The pH values of the media were adjusted to 4.0 prior to being dispensed in 25-ml aliquots into 250-ml Erlenmeyer flasks, which were cotton-stoppered and then sterilized by autoclaving at 121°C for 20 minutes. On cooling, each flask was inoculated with five mycelial-agar disks cut from the growing fringe of an agar plate culture with a sterile cork borer of approximately 2 mm diameter. Incubation was in darkness until half the containers for a treatment had mycelial growth covering the surface. At this time the culture flasks or jars were brought under ambient laboratory conditions of illumination with a temperature of 23 ± 2°C.

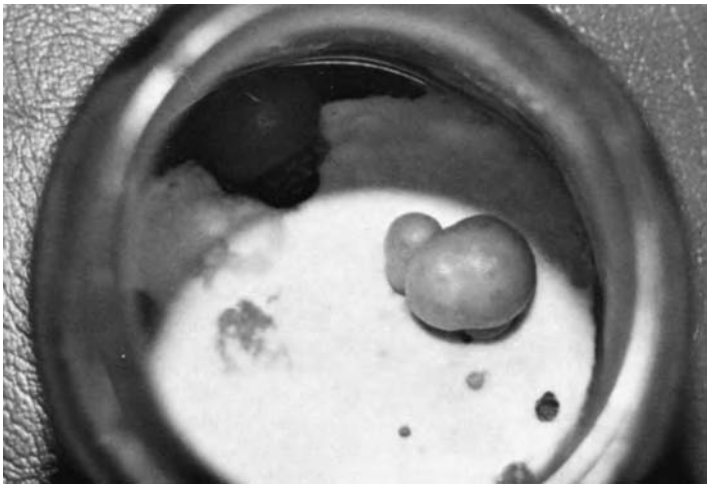


FIGURE 13.13 *Lentinula* mushrooms cultivated in liquid medium in culture vessel.

In some of the experiments, substances were added that would support the mycelium and fruiting bodies, because the weight of the fruiting bodies frequently caused them to topple into the liquid medium in the absence of a supporting material. Both inorganic substances (glass-wool, vermiculite, and perlite) and organic substances (ground-up corncobs, peanut hulls, cotton wool, and disposable wooden chopsticks) were tested as supporting materials. When supporting materials were used, the containers were either deep culture dishes (8.5 cm in diameter × 4.5 cm high) or wide-mouthed screw cap bottles (7.0 cm in diameter × 9.0 cm high) to facilitate the removal of fruiting bodies for subsequent spore collection.

The times required for formation of primordia (Figure 13.13) and for mature fruiting bodies were used as measurements of fruiting. Mycelial growth was estimated visually by the amount of surface covered and by the density of the mycelium.

An initial study of growth and fruiting of eight stocks of *L. edodes* on three defined liquid media — Leatham (L), fruiting (F), and fruiting containing the four stock solutions present in the Leatham medium (F+) — produced the following results:

1. Growth of all dikaryotic stocks was similar on both L and F+ media and substantially better on these media than on F.
2. Mature fruiting bodies appeared only on L medium although primordia formation was similar on both L and F+.
3. The *Schizophyllum* fruiting medium (F) was inadequate for fruiting of *Lentinula*.
4. Growth of one stock was as good or superior to that of all other dikaryotic stocks on each medium, but this clamp-bearing mycelium failed to produce even fruiting body primordia by 71 days.
5. Another stock was also slow to produce primordia in this experiment, whereas only two stocks produced mature fruiting bodies by 71 days.

In this preliminary screening experiment, the similarity of growth and fruiting of L and F+ media and the poor growth and lack of fruiting on F medium indicated that it might be possible to detect specific requirements for good growth and/or fruiting by elimination of specific solutions from the formula of the F+ medium. The results of this experiment in which the media employed were L, F+, F+ − A (lacking minerals), F+ − B (lacking trace elements), F+ − C (lacking vitamins), F+ − D (lacking salicylic acid), and F are as follows:

1. Growth of all stocks was fair to good on L and F+ media, and in five stocks growth was better on L than F+.
2. Two stocks had less exacting nutritional requirements for both growth and fruiting in that they showed fair to good growth on F medium and on media from which specific solutions were withheld from the F+ medium (i.e., F+ – A, F+ – B, F+ – C, and F+ – D).
3. One stock produced fair to good growth on all media except F, but primordia and fruiting bodies were formed only on L, a behavior in regard to primordia formation that was in marked contrast to several other stocks.
4. Six stocks fruited on L medium and five on F+, and in two cases fruiting was earlier on L than on F+.
5. Primordia formed on all but one of the stocks on L medium and all but three stocks on F+. Time of formation was generally the same or earlier on L than on F+. The medium lacking the mineral supplementation was poorest in primordia formation for most stocks, but there were two stocks for which this was not true. Therefore, differences among stocks in regard to primordia formation exist, just as for growth and fruiting.

To prevent the weight of developing fruiting bodies from tearing away from the membranous mycelium and becoming partially submerged in the liquid medium, supporting substances were placed in the media. Organic supporting substances, which have been used in media for plastic bag cultivation of *Lentinula,* were tried. These substances were peanut hulls, crushed corncobs, and wooden sticks (disposable chopsticks), which were placed in L medium. The following results were obtained:

1. Satisfactory mycelial growth was obtained with all stocks and with all treatments.
2. All stocks formed primordia and all but one stock formed fruiting bodies.
3. Fruiting bodies were obtained with some stocks for each of the supporting substances, although the results obtained indicated better fruiting with the wooden sticks and crushed corncobs than with the peanut hulls.

To be able to study the genetics of fruiting under chemically defined conditions, inorganic supporting substances (perlite, vermiculite, and glass wool) were included in L medium. The following results were obtained:

1. Good growth was obtained with all stocks and all treatments.
2. With two of the stocks no primordia or fruiting bodies were formed, but in the other six stocks fruiting bodies were formed.
3. With each of the inorganic supporting substances there were some stocks that fruited, but glass wool and vermiculite gave the best results.

The observation was made that prior to fruiting body formation the mycelial coat usually took on a brownish pigmentation. An experiment involving three dikaryotic stocks was conducted to check this observation and to determine if the formation of the pigment was associated with light. The evidence presented in Table 13.9 indicates that light is required for the formation of the brownish pigmentation, but primordia formation can occur in darkness. Stocks TMI-830 and L-38 both formed fruiting bodies by 56 days when they had been in the light for 22 days, but not if they had been in the light for only 2 days.

B. Generalizations

The results reported here are only preliminary findings of some exploratory studies concerning fruiting of *Lentinula edodes* in liquid media. There are, nevertheless, some generalizations that can

TABLE 13.9
Effect of Light on Pigmentation and Fruiting

		TMI-830[a]	L-35	L-38
Group A	44 days (10 days in light)	Pigmented; primordia	Pigmented; primordia	Pigmented; primordia
Group B	44 days (0 days in light)	Unpigmented; primordia	Unpigmented; primordia	Unpigmented; primordia
Group A	56 days (22 days in light)	Coat with brown patches; primordia and fruit bodies	Coat with brown patches; some primordia	Coat with brown patches; primordia and fruit bodies
Group B	56 days (2 days in light)	White coat with occasional brown spots; primordia	White coat with some primordia beginning to brown	White coat with some primordia beginning to brown

[a] Each culture dish (9 cm inner diameter × 5 cm tall) was inoculated with five cylindrical plugs (~2 mm diameter) of mycelia from the margin of a colony growing on an agar plate. Each culture dish contained 25 ml of liquid Leatham medium, with glass wool used as a supporting substance. Ten dishes were inoculated with each stock. After inoculation, all dishes were incubated in the dark. Group A dishes (five inoculated with each stock) were brought into the light at 34 days, but Group B dishes (five inoculated with each stock) were left in the dark until 54 days. Scoring as to pigmentation, primordia formation, and fruiting was done at 44 days and 56 days.

be made from these studies, which should prove helpful in future experimentation on the genetics of fruiting of this commercially important edible species.

1. Greater variation was found among the dikaryotic stocks in the formation of primordia and fruiting bodies than in vegetative growth. The differences in the time of primordia formation and of fruiting among the stocks, in the absence of substantial differences in growth, is indicative of genetic control of the fruiting process.
2. The differing results obtained for growth and fruiting on the three chemically defined media indicate a role for the solutions present in L and F+ media; but, since the growth on F medium was much less than that on either L medium or F+ medium, it cannot be stated that the nutritional requirement is specifically for fruiting. The requirement could be simply for growth, which is a prerequisite for fruiting.
3. Various dikaryotic stocks displayed specific growth and fruiting differences in nutritional requirements, related to the solutions used to supplement F medium. Some stocks showed depressed growth in the absence of supplemental solution A, which contained $CaCO_3$, $MnSO_4$, and $ZnSO_4$. This may indicate a role of one of the metal ions as a cofactor in an enzymatic reaction important in growth. With two stocks, fruiting body primordia failed to form in the absence of this same solution (A). Because growth was only slightly reduced in the case of one of them, the possibility that one of these ions plays a role in fruiting body primordia formation of this stock also exists. The most obvious result of this experiment is the difference in behavior of the various stocks in regard to fruiting. Even on L medium, which supported fruiting better than any of the other media, there was a range in fruiting expression extending from absence of primordia formation, primordia formation only, to stocks that produced fruiting bodies at 40 days.

A role for a light requirement in the formation of a brownish pigment on the mycelial coat was demonstrated (Table 13.9). The results obtained with regard to the formation of primordia and fruiting bodies in this experiment suggest that light is not required for primordia formation but does play a role in subsequent fruiting body maturation.

TABLE 13.10
Fresh Weight to Dry Weight Conversion of *Lentinula* Based on Conditions from Pinning to Harvest

Condition	Time from Formation of Pinhead to Harvest	Ratio of Fresh to Dry Weight
After rain	7–10 days	12–14:1
Normal growth	12–15 days	6–8:1
Huaku (donku — thick cap)	10–25 days	4.2–4.5:1

Definitions: (Japanese) Donku = (Chinese) Dong-gu/Xiang-gu — Thick caps with cracks in the outer surface of the pileus; this is the best quality. (Japanese) Koshin = (Chinese) Xiangshin/Xyangshin — Thin, smooth cap.

Note: Usually there is good dong-gu if the temperature ranges from 2.7 to 13.8°C during fruiting, with a difference of approximately 11°C. The mushroom grows slowly, and the pileus increases in thickness with cracks and grooves forming in the pileus. The air-dried mushroom has 13% moisture.

Source: Data from Tokimoto, K., *Rep. Tottori Mycol. Inst.* (Japan), 11, 23–28, 1974.

Fruiting occurred with all three inorganic supporting substances (glass wool, vermiculite, and perlite). The increase in surface area provided by the supporting substances seemed to facilitate vegetative growth. Use of such substances is important in keeping the gills of the fruiting body from becoming submerged in the liquid medium. Such submersion makes it impossible to obtain basidiospores for genetic studies.

Although various stocks in these experiments displayed some fruiting differences, in general, three stocks appeared to be genetically good fruiters; three stocks were variable or intermediate fruiters; and two were generally poor fruiters. It has been shown in *Schizophyllum commune* by Raper and Krongelb[19] that in *S. commune* fruiting is under polygenic control and that all dikaryotic stocks do not fruit equally well (see Chapter 8). While we do not at this time have evidence concerning the number or mode of operation of genes controlling fruiting in *L. edodes*, it is evident that all dikaryotic stocks of this species do not behave similarly in regard to fruiting, and we believe that fruiting is genetically programmed as well as under environmental regulation.[14]

IX. DRYING AND STORAGE

Lentinula can be eaten fresh or preserved by drying, canning, or pickling in vinegar; however, the dried form is the most popular. Mushrooms preserved by drying have a good flavor, and the drying both prevents deterioration and is convenient for long-term storage and transportation. The moisture content of fresh mushrooms varies in the range of 70 to 95%, depending on the harvest time and environmental conditions, whereas it is about 10 to 13% in dried mushrooms. The percentage of dried mushrooms converted from fresh mushrooms depends on the weather at harvest time and the grade of the mushroom (Table 13.10). In winter with clear weather, the highest conversion rate is 32 to 37% for the best grade of dong-gu, sometimes caused by the cracking of the skin of the cap of the mushroom due to the extreme fluctuation of cold temperatures; and the conversion rate is 18 to 24% for ordinary dong-gu; and 12 to 14% for Hyangshin. In the spring, the percentage for Hyangshin is 9 to 10% with clear days and 6 to 8% with rainy days.

Methods for drying *Lentinula* can be divided into sun drying and thermal power drying. In general practice, the picked mushrooms are cut off at the basal part of the stalks (Figure 13.14)

FIGURE 13.14 Freshly picked *Lentinula* mushrooms being cut off at the basal part of the stalks and sorted for drying.

FIGURE 13.15 *Lentinula* mushrooms on bamboo racks in drying room.

and are arranged in single layers on shelves, which are either exposed to the sun or placed within a drying oven (Figure 13.15). The time needed for sun drying varies depending on the season when the mushrooms are harvested — 2 to 4 days with continuous sunshiny days. The process of thermal power drying should begin at a relatively low temperature, 35°C for mushrooms grown with sunny days, and 30°C for mushrooms grown during a rainy season. After 5 hours of heating for mushrooms grown under ordinary conditions and 7 hours of heating for those grown during a rainy season, the room temperature can be raised gradually and then kept at 40 to 60°C for 12 to 18 hours. Drying, in addition to preserving the product, can enhance the flavor and appearance of the mushroom.

After drying, the moisture content of the mushrooms ranges from about 10 to 13%. As dried fruiting bodies of *Lentinula* are highly hygroscopic and apt to absorb moisture from the air, they should be properly stored. If the moisture content reaches about 20%, insects and molds will easily infect the mushrooms, and the gloss of the cap surface may disappear. The surface of the body may also have a white powder, and the gills may be changed to a brown color rather than the original yellowish white. Therefore, the dried mushrooms should be put into a polyethylene bag,

sealed, and stored in a dry, chilly, and dark place, if possible. For prolonged storage, they should be packed in cartons or wooden boxes and stored at 2 to 5°C in a low-temperature storehouse.

REFERENCES

1. Campbell, A.C. and Slee, R.W., Commercial cultivation of shiitake in Taiwan and Japan, *Mushroom J.*, 170, 45–53, 1987.
2. Chang, S.T., Mushroom research and development — equality and mutual benefit, in *Mushroom Biology and Mushroom Products*, Royse, D.J., Ed., Pennsylvania State University, University Park, 1–10, 1996.
3. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinula edodes* (Berk.) Sing. in China, *Int. J. Med. Mushrooms*, 1, 291–300, 1999.
4. Chang, S.T. and Miles, P.G., Historical record of the early cultivation of *Lentinus* in China, *Mushroom J. Trop.,* 7, 31–37, 1987.
5. Declaire, J.R., Evolution de la consommation des champignons comestibles dans le monde au cours de la periode 1977–1981, *Mushroom Sci.*, 10(Part 2), 863–893, 1979.
6. Declaire, J.R., Place et role des champignons cultives comme source de proteins humaines en 1'ans 2000, *Mushroom Sci.*, 11(Part 1), 1–18, 1981.
7. Diehle, D.A. and Royse, D.J., Shiitake cultivation on sawdust: evaluation of selected genotypes for biological efficiency and mushroom size, *Mycologia*, 78(6), 929–933, 1986.
8. Gruen, H., Mushroom growing in Japan, *Boston Mycol. Club Bull.*, 1(Suppl. 1), 1964.
9. Huang, N.L., The reasons for formation of undesirable and abnormal *Lentinus* mushrooms in bag culture, *Edible Fungi China*, 3, 27–28, 1987.
10. Ito, T., Cultivation of *Lentinus edodes*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 461–473, 1978.
11. Kendrick, B., *The Fifth Kingdom*, Mycologue Publications, Waterloo, Canada, 1985.
12. Komatsu, M. and Kimura, K., Studies on abnormal fruit bodies of hymenomycetous fungi. V. Fruit bodies with white pilei of *Lentinus edodes* (Berk.) Sing., *Rep. Tottori Mycol. Inst.* (Japan), 6, 9–17, 1968.
13. Leatham, G.F., A chemically defined medium for the fruiting of *Lentinus edodes, Mycologia*, 75, 905–908, 1983.
14. Miles, P.G. and Chang, S.T., The collection and conservation of genes of *Lentinus,* in *Proc. Int. Symp. Scientific and Technical Aspects of Cultivation of Edible Fungi*, Elsevier Science, Amsterdam, 227–234, 1987.
15. Miles, P.G. and Chang, S.T., Fruiting of *Lentinus edodes* (shiitake) in liquid media, *MIRCEN J.*, 3, 103–112, 1987.
16. Mori, K., *Mushrooms as Health Foods*, Japan Publications, Tokyo, 1974.
17. Nakamura, K., *An Historical Study in Shiitake (Mushroom) Culture*, Tosen Shuppon, Tokyo, 1983.
18. Raper, J.R. and Miles, P.G., The genetics of *Schizophyllum commune, Genetics*, 438, 530–546, 1958.
19. Raper, J.R. and Krongelb, G.S., Genetic and environmental aspects of fruiting in *Schizophyllum commune* Fr., *Mycologia*, 50, 707–740, 1958.
20. Royse, D.J., Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom, *Mycologia*, 77(5), 756–762, 1985.
21. Royse, D.J., Schisler, L.C., and Diehle, D.A., Shiitake mushrooms: consumption, production and cultivation, *Interdiscip. Sci. Rev.*, 10, 329–335, 1985.
22. Sato, Narihiro [or Seiyu], Onkosai Guzui Hen [or Kyozinroki, Notes on Surprising Mushrooms] or Record of Jingshiang (Jingshiang means "shocking the mushroom"), 1796.
23. Singer, R., *Mushrooms and Truffles*, Leonard Hill, London, 1961.
24. Suzuki, S. and Oshima, S., Influence of shiitake (*Lentinus edodes*) on human serum cholesterol, *Mushroom Sci.*, 9(Part 1), 463–467, 1976.
25. Ting, H.G., The key points for management and time of removal of plastic bags in field cultivation of *Lentinus edodes, Edible Fungi*, 6, 31–32, 1986.
26. Tokimoto, K., Formation of callus-like aberrant fruit bodies on agar cultures of *Lentinus edodes* (Berk) Sing., *Rep. Tottori Mycol. Inst.* (Japan), 11, 23–28, 1974.

27. Tokimoto, K. and Kawai, A., Nutritional aspects of fruit body development in replacement culture of *Lentinus edodes* (Berk) Sing. *Rep. Tottori Mycol. Inst.* (Japan), 12, 25–30, 1975.
28. Wang Cheng, *Book of Agriculture*, China, 1313.
29. Yamanaka, K., Production of cultivated mushrooms, *Food Rev. Int*., 13, 327–333, 1997.
30. Yao, S.X., World production and trade of *Lentinus edodes* [in Chinese], in *The Development Strategy of Lentinus edodes Cultivation in North China,* Tin, J.H., Ed., Association of Edible and Medicinal Mushrooms in Northeast Three Provinces in China, 19–32, 1998.
31. Yao, S.X., Developing trends in production and international trade of Chinese *Lentinus edodes* [in Chinese], *Edible Fungi of China,* 19(Suppl.), 41–44, 2000.
32. Zhang, S.-C., *Book of Agriculture* by Wang Cheng and (consideration of) the location of the Mushroom Village, *Edible Fungi*, 2, 46–48, 1981.

14 *Volvariella* — A High-Temperature Cultivated Mushroom

I. INTRODUCTION

Although more than 100 species, subspecies, and varieties of *Volvariella* have been described throughout the world,[57,59] all the cultivated forms of *Volvariella* in Southeast Asia are probably members of the species *Volvariella volvacea* (Bull. Ex Fr.) Sing.[15] *Volvariella volvacea*, commonly known as the straw mushroom, paddy straw mushroom, or the Chinese mushroom,[12] belongs to the family Pluteaceae Kotl. & Pouz of the Basidiomycetes.[58] It is an edible mushroom of the tropics and subtropics, and it began to be cultivated in China as early as 1822.[11] The "Nanhua mushroom," which was in fact *Volvariella*, was named for the Nanhua Temple of Chaohsi in northern Guangdong Province, China. The Buddhist monks of that temple apparently cultivated the mushroom for their own table, and by 1875 it was sent as a tribute to the royal family. As it took many years for a food to be considered a proper tribute to the royal family, cultivation of *Volvariella* must have begun before the 18th century, almost 300 years ago.[14] Much earlier references to the cultivation of mushrooms in China have been found, but they deal with species that prefer cooler climates, and the authors in earlier times might well have been ignorant of species cultivated in the subtropical and tropical regions of China. Around 1932 to 1935, *V. volvacea* was introduced into the Philippines, Malaysia, and other Southeast Asian countries by overseas Chinese.[1,3] Since then, its cultivation has been mainly concentrated in various countries of the region.

Volvariella volvacea is referred to as a "warm mushroom" because it can grow at relatively high temperature, i.e., vegetative growth at about 32 to 34°C. It is a fast-growing mushroom; the time required from spawning to harvesting is only about 8 to 10 days. No other vegetable or cultivated mushroom can be served as a table dish within such a short time from its planting; but, under favorable conditions, *V. volvacea* can do this. However, the ability of its mycelium to become colonized with its substrate is rather weak; therefore, its mycelial network in the substrate is easily broken and disconnected if the inoculated substrate is disturbed. The yield of mushrooms can be reduced drastically by mismanagement or improper care.

Volvariella volvacea can use cellulose materials more effectively than other cultivated mushrooms; e.g., the optimum C:N ratio for *V. volvacea* is about 40 to 60, for *Agaricus bisporus* it is 17 to 18, and for *Lentinula edodes* it is 20 to 25 during the mycelial running stage. It can grow quickly and easily in uncomposted substrates such as paddy straw and cotton wastes or other high cellulosic organic waste materials. Under favorable environmental conditions and growth medium, the primordium of the mushroom can be formed 4 to 5 days after spawning. In light of the above-mentioned special biological characteristics of the mushroom, it has been considered one of the easiest mushrooms to cultivate, but it should be noted that the biological efficiency of this mushroom is lower than other commonly cultivated mushrooms. In this sense, it is a rather difficult mushroom to grow. Details of the biological nature of this mushroom can be found in previous reports.[12,22]

In recent years the cultivation of the mushroom has gradually extended to the northern provinces of temperate climate in China where summer months are hot, and the mushroom can be cultivated under the shade of trees or with a covering of plastic sheets or straw. Four to six crops of the mushrooms can be harvested, and each crop lasts about 20 days for two flushes. High-temperature mushrooms, including *Volvariella*, possibly can become a source of inexpensive mushrooms, not only in the traditional region of the tropics and subtropics, but also in temperate regions in warm summer months. Another paddy straw mushroom (*V. diplasia*) can also be cultivated during summer as a gap-filling crop,[63] because during a hot summer it is not possible to grow the increasingly popular mushrooms, the white button mushroom (*A. bisporus*) and the phoenix-tail mushroom (*Pleurotus sajor-caju*), which are usually cultivated in low and moderate climates. A preliminary screening for low-temperature spawns of the straw mushroom indicated that it might be possible to adapt or develop a straw mushroom cultivar suitable for commercial cultivation in the southernmost area of temperate regions.[55]

II. BIOLOGICAL CHARACTERISTICS

Since the early 1960s, scientists at the Chinese University of Hong Kong have undertaken research studies on the morphology, cytology, genetics, nutrition, and cultivation of *Volvariella volvacea*.[10,12,18–26,44] Fungal cytology is comparatively difficult because of the minute size of nuclei, the study of which requires meticulous technique and tedious observations. A detailed description of the morphology and morphogenesis of *V. volvacea* should prove useful in many ways for both basic and practical studies.

A. Morphological Characteristics

For convenience in description and discussion, the basidiocarp of the straw mushroom is roughly divided into six different stages in development, namely, pinhead, tiny button, button, egg, elongation, and mature stages (Figure 14.1). Each stage has its own typical morphological and

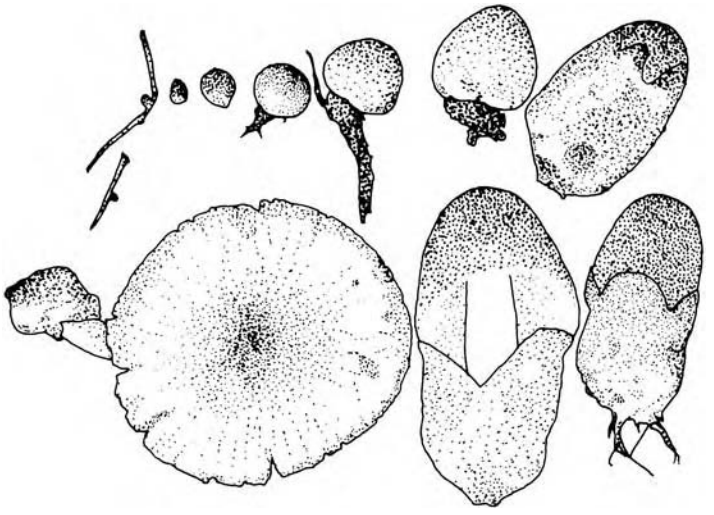


FIGURE 14.1 Different stages in the development of straw mushroom. The largest one at the left is the "mature" fruiting body. The second from the left, showing the volva, which has been disconnected from the pileus by the rapid elongation of the stipe, is the "elongation" stage. The next two are "egg" stages showing that the universal veils have been ruptured. The rest are "button," "tiny button," and "pinhead" stages, consecutively.

anatomical characteristics, but the elongation and mature stages are the most interesting with regard to the formation of basidia and basidiospores.

1. Mature Stage

At the mature stage, the whole structure is divided into three regions: (1) the pileus or cap, (2) the stipe or stalk, and (3) the volva or cup. The volva is a thin sheet of interwoven hyphae around the bulbous base of the stalk. In fact, every part of the mushroom is made up of interwoven hyphae. The volva is fleshy, white, and cup shaped, but it has an irregular margin. At its base is the rhizomorph for the absorption of nutrition from the substrate. The hyphae making up the rhizomorph are different from those that make up the core of the basidiocarp. The former are thicker and loosely interwoven and have many brown bodies and swollen cells, which are considered to serve as storage of food; and the latter are thin and compactly interwoven without brown bodies and swollen cells. The stipe is attached to the center of the lower surface of the pileus and connects it to the volva. The length of the stipe varies according to the size of the pileus. It is usually about 3 to 8 cm long and 0.5 to 1.5 cm in diameter. It is white and fleshy and without an annulus. The fully expanded pileus is circular (Figure 14.2), with an entire margin and a smooth surface, which is dark gray at the center, but light gray near the margin. The diameter is about 6 to 12 cm; the size varies according to nutrition and other environmental factors. It has been found that the cap grows fastest at the edge and gradually slower toward the center. The lower surface of the pileus bears many lamellae (Figure 14.3). The number varies from 280 to 380. They are straight and have an entire margin. There are several kinds of lamellae: the full size, the three quarter size, the half size, and the one quarter size. But even in the full size, the lamellae do not touch the stipe but are separated from it by about 1 mm. The number of different kinds of gills related to the various stages in development

FIGURE 14.2 The upper part of the pileus of a mature fruiting body. The diameter is about 6 to 12 cm, the size varying according to nutrition and other environmental factors.

FIGURE 14.3 The lower surface of a mature fruiting body showing three regions: pileus with lamellae, stipe, and volva.

of the straw mushroom has been studied, but the data are obscure and quite different from the findings of Levine[42] in *Coprinus*. Further cytological study on the origin and differentiation of gills in *Volvariella* is now in progress. Under the microscope, each lamella is seen to be composed of three layers of interwoven hyphae (Figure 14.4). The innermost layer where the hyphae are loosely woven and running obliquely is called the inverse trama.[58] The differences between the regular and the inverse trama have been described by Bessey:[4] "The regular trama consists of elements clearly parallel. In the inverse trama the young lamella has the regular structure with a distinct

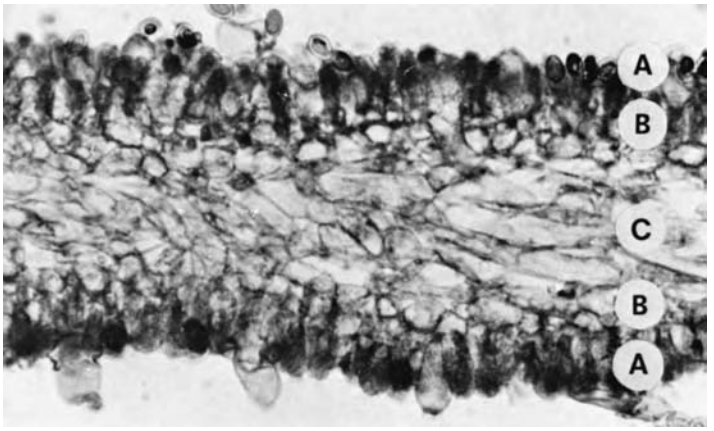


FIGURE 14.4 Cross section through a piece of gill from a fruiting body at mature stage. (A) Hymenium (basidia with basidiospores or sterigmata and the swollen cystidia); (B) subhymenium; (C) inverse trama.

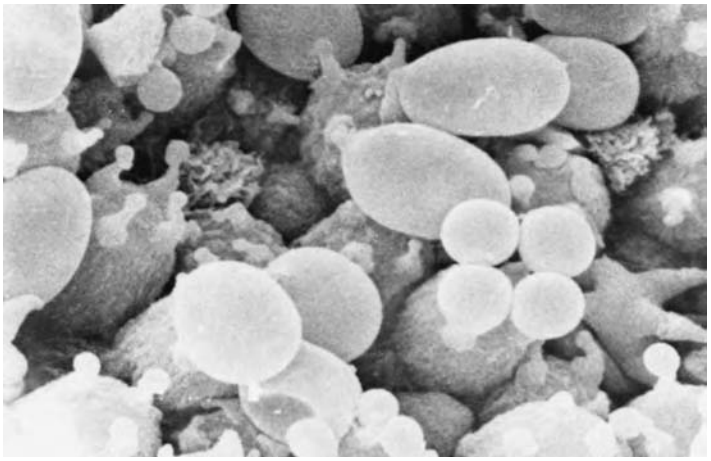


FIGURE 14.5 Scanning electron micrograph of the hymenial layer of a mature fruiting body of *V. volvacea*. Note the various stages of development of basidiospores.

subhymenium. As maturity approaches, hyphae grow from the subhymenium obliquely inward, filling the space formerly occupied by the vanished median portion of the trama." It must be recognized that these two types grade into one another and that at times it is difficult to decide which type is present, especially if the gills under study are not exactly the same age. The middle layer is called the subhymenium, and here the hyphae are densely woven. The outermost layer (i.e., the two surfaces of a lamella) is called the hymenium, and here the terminal cells of the hyphae remain narrow to form the paraphyses or swell to form the club-shaped basidia and the cystidia. The basidia bear spores called basidiospores. Usually four basidiospores are attached to each basidium by means of four sterigmata (Figure 14.5). The photomicrograph was taken from the gill surface of a normal fruiting body at an early mature stage, which means that the pileus has just completely expanded and has light pink-colored lamellae. The tetrad of basidiospores was clearly demonstrated. The basidiospores of this species are mostly asymmetric with a slight tendency to be egg shaped, but spherical or ellipsoidal spores are not uncommon. It is to be noted that the outline of the spore will vary depending on the plane in which it is viewed. The average length of the egg-shaped spores is 7 to 9 μm, the width of the widest end is 5 to 6 μm, and the narrow end is 3 to 4 μm. The spores are smooth but have a thick covering. At the narrow end there is a short, triangular spine protruding. When viewed microscopically with transmitted light, the color of the cell wall and of the cell contents varies from transparent and light yellow to pink and dark brown. The change of spore color is a result of its maturation. When basidiospores are collected on a piece of white paper under a bell jar, the spore print obtained is brownish in color. Thus, it is the color of the mature basidiospores that makes the lamellae change from white to brown on maturity. The total number of basidiospores in each mature mushroom is tremendous and must be discussed in terms of billions. The cystidia are of two kinds based on their location; those located along the edge of a lamella are called cheilocystidia, and those along the face are pleurocystidia. The pleurocystidia are large, stout, and extend across the space between two lamellae, thus holding the lamellae apart.

2. Elongation Stage

Previous to this mature stage is the elongation stage. It is similar in shape to the mature stage, except that the pileus is not opened and the size is smaller. The stipe is extended to almost full length at this stage; hence, the name elongation stage is given. A small portion of volva and pileus at the egg stage was cut off, and the stipe marked at equal intervals up its length with uniform dots of waterproof drawing ink. Then the development was measured periodically. It is noted that

elongation takes place mainly in the upper portion of the stipe. Along most of the stipe, the ink spots remain round; but two spots placed within a certain sharply defined zone next to the pileus stretch out into a vertical line. By anatomical studies, it was found that the meristematic region is the region of the stipe just below the pileus and this is the portion that takes up much safranine, thus demonstrating the presence of an abundance of nucleic acid. It is explained thusly by Baker:[2] "When dividing cells are colored with a cationic (basic) dye, each metaphase chromosome takes up far more of it than an equal volume of cytoplasm does. This results partly from the fact that nucleoprotein is much more acidic (basophilic) than the cytoplasm, but partly also from the fact that anionic (acid) dyes also color chromosomes more deeply than the cytoplasm." This may be the reason that the material picked up from this portion to obtain a tissue culture for pure spawn seems to be better than others.[8] It was also found that the filaments of the elongation portion below the meristematic region are lined up parallel to one another, but the filaments of the rest of the stipe remain a tangled mass of thread. The hyphae in the parallel region of the stipe are 5 to 8 μm in width; the distance between two adjacent septa may range from 35 to 83 μm. The lamellae hang below the pileus in the form of thin strips of tissue radiating from the margin toward the stalk. Under the microscope, various stages of basidial development can be seen. The terminal cell of a hypha in the hymenium gradually enlarges to form a club-shaped cell. One nucleus is seen. As the cell enlarges, the nucleus undergoes meiotic divisions at the base of the basidium, and four haploid nuclei are present. In the meantime, four sterigmata push out at the distal end of the basidium and their tips swell, forming the basidiospore initials. The four nuclei, together with cytoplasm, migrate upward and squeeze through the sterigmatal passage into the enlarging portions. A crosswall is eventually formed at the base of the enlargement, and the cell cut off is a basidiospore. What remains behind is an empty basidium.

3. Button and Egg Stages

The button stage and the egg stage (Figure 14.6) are sold in the market at a premium price. Both stages are ovoid in shape. In the button stage, the whole structure is wrapped by a coat, which is called the universal veil. Inside the universal veil is the closed pileus. The stipe can only be seen in a longitudinal section of the whole structure. In the egg stage the pileus is pushed out of the veil, which remains to form the volva. Here again the stipe is hidden. The pileus in these two stages is similar to that in the above two stages except that it is smaller in size. Under the microscope, the lamellae of the egg stage do not bear basidiospores, although there may be basidia in the stage of sterigmata formation. For the button stage, only the cystidia and the paraphyses are seen.

FIGURE 14.6 Straw mushrooms grown on cotton waste compost in the button and egg stages.

4. Pinhead Stage

There are two more stages formed from interwoven hyphae. They are the pinhead stage and the tiny button stage. The universal veil is much thicker. On its removal, the tiny pileus can be seen with a dark gray center and white margin. The basal portion of the entire structure is quite large compared to the pileus. For the younger tiny buttons, only the top of the universal veil is brown, the rest is white. The tiny buttons are round in shape. If a vertical cut is made through a very young tiny button, the lamellae are seen as a narrow band on the lower surface of the comparatively thick pileus. The pinhead stage, by its name, is of the size of a pinhead. The universal veil is spotlessly white. With a vertical section, the pileus and the stipe cannot be seen. The whole structure is a tiny knot of hyphal cells.

5. Germination and the Germling

Germination of basidiospores starts with the protrusion of a tubular germ tube through the germ pore, which is an unthickened spot in the spore wall at the hilum. It continues to grow to become a hypha, which usually extends to a certain length before branching occurs (Figure 14.7). The number of nuclei in a nonseptate germ tube is variable, ranging from 1 to 15. As many as 21 nuclei were counted in a germ tube 244 μm long and recorded 48 hours after germination at 40°C. The length of a germ tube varies from 28 to 267 μm and the width from 2.5 to 4 μm. The nuclei are not evenly distributed in the cytoplasm of each hyphal cell, but there is no concentration of nuclei in the proximal portion of the germ tube. In most germinated spores, the cellular contents are found to have migrated to the hypha, leaving an empty spore wall behind.[10] As the germ tube grows, simple septa are formed and hyphal cells of about 43 to 182 μm result, with a nuclear number ranging from 2 to 12 per cell. In the germinating basidiospores there are membranous structures that are free in the cytoplasm or associated with plasma membranes against the wall.[23,25] The membrane of these structures consists of two electron-dense lines separated by a light interspace, the so-called unit membrane structure, which is about 90 Å thick. These structures are also observed within vacuoles. Their origin, nature, and functions have not yet been clearly defined. Since the line facing the cytoplasm is more densely stained than the other facing the lumen, it may be that these membranous structures are formed by the invagination of the cytoplasmic membrane.[25]

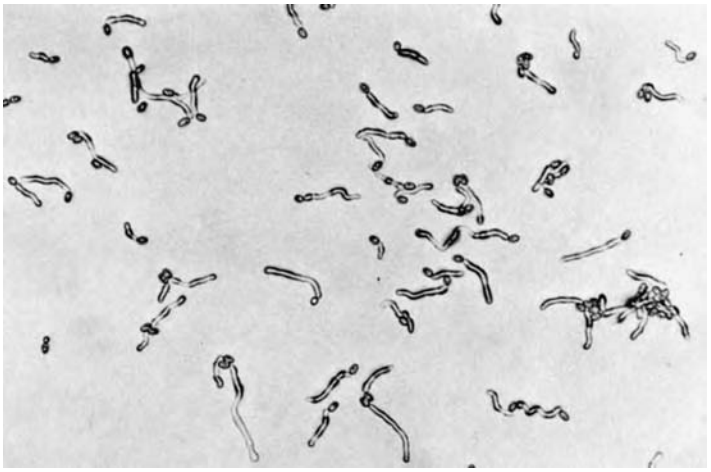


FIGURE 14.7 Basidiospores germinated at 32°C after 28-hour incubation. Note that only one unbranched germ tube emerges from the spore.

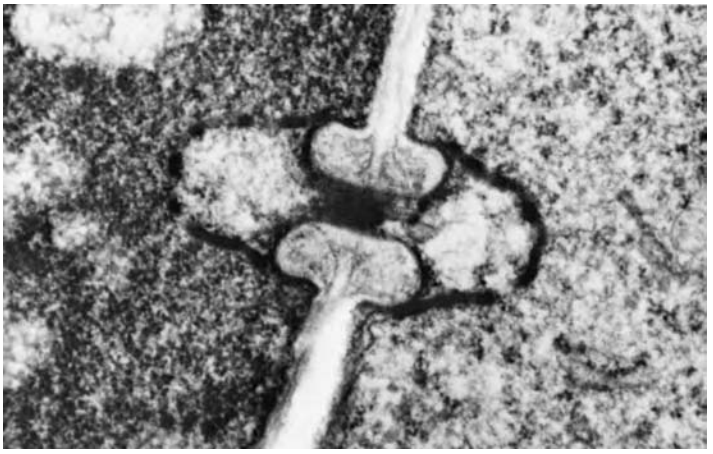


FIGURE 14.8 Near median section of a dolipore septum of a *V. volvacea* hypha.

6. Vegetative Hyphae

Further growth and branching of the germlings give rise to mycelium. In addition to those started from the basidiospores, mycelial cultures can also be obtained from the internal tissue (pseudoparenchyma) of the fruiting body.

In the Basidiomycetes with defined sexuality, the uninucleate haploid mycelium formed directly from the basidiospore is called the primary mycelium. Dikaryotic mycelia result from plasmogamy of compatible primary mycelia and are termed secondary mycelia, which generally bear clamp connections.[60] However, in the case of *V. volvacea*, the mycelium from the very beginning is multinucleate. Clamp connections are absent in all mycelial cultures, and it is still unknown whether the mycelium is haploid. Therefore, distinction of primary vs. secondary mycelium is not justified.

As the hypha grows, septa are formed at certain distances, and branching may occur. Near the center of a septum there is a circular swelling that surrounds the septal pore.[25] A thickened, porous electron-dense septal pore cap covers the septal swelling and septal pore. The cap is formed from the endoplasmic reticulum, which lies close to and parallel to the cross walls on both sides. Since 1961, this complicated septal structure has been termed the dolipore septum (Figure 14.8) by Moore and McAlear.[48] It is obvious that there are two types of hyphae in one mycelial culture. The straight, relatively broad, and uniformly wide hyphae grow parallel to each other forming hyphal bundles, and they branch only occasionally. In the second type, the branches are of irregular width, and they branch very frequently. These hyphae appear as clusters rather than parallel bundles.

The number of nuclei per cell is reported to be high, ranging from 3 to 105 in a sample of 123 cells with a mean value of 22.1 ± 1.5 nuclei per cell.[21] The location of nuclei is not restricted to the central region of the hypha but is randomly distributed in the cells. The size of the nuclei has been reported to vary from 1.68 to 4.22 μm in diameter. It should be kept in mind, however, that such measurements are of little practical importance because they depend heavily on the cytological techniques of staining, etc., but the measurements can be compared with the median size of living cells revealed by phase contrast photomicrographs, but not to this degree of resolution. The fine structure of a growing hyphal tip has been studied with electron microscopy.[62] The apical zone (the first 5 μm) of the tip is rich in apical vesicles and devoid of nuclei, mitochondria, and other organelles. Nuclei are found first in the subapical zone, which begins at about 40 μm from the tip. The structure of those apical vesicles is similar to the membranous structures found in the germlings mentioned before. Crystalline structures of a high electron density have been found throughout the apical zone. They vary greatly in size from 0.2×0.4 μm to 1.0×1.4 μm and are of rhombic form. No information about their function and nature is yet available.

7. Chlamydospores

In some mycelial cultures, thick-walled chlamydospores are found. Macroscopically they can be detected as brownish-red patches among the hyaline hyphae. Detailed examination reveals that they are borne either singly or in clusters; their formation can be either intercalary along the hyphae or terminal at hyphal apices. They are found only on the much-branched side branches and seldom along the parallel broad branches. The chlamydospores are variable in shape and volume, generally spherical, sometimes globose, with a smooth surface, and the average diameter is about 40 to 60 µm. It has been suggested that swollen cells along the side branches are precursors of the chlamydospores.[43] They are multinucleate and are easily detached from the hyphae at maturity. Formation of chlamydospores takes place when the environmental conditions become adverse; however, since they are found in some young, 3-day-old agar cultures of straw mushrooms, their nature and function need to be explored. With suitable environmental conditions, germination of chlamydospores occurs readily. One to several germ tubes grow out at various points of the thick spore wall,[10] and their further growth leads to formation of a mycelial culture.

B. Requirements for Mycelial Growth

Traditionally, *V. volvacea* has been grown on rice straw;[9] that tradition earned it the common name of paddy straw mushroom. Most substrates composed of other agricultural wastes have resulted in poorer yields. The exception appears to be cotton waste.[13] We know that cotton is almost pure cellulose; however, the cotton wastes from spindle mills often contain cotton seed hulls, cotton leaves, and even oil dust from sorting and clearing operations — which means that the wastes also contain minerals, low-molecular-weight carbohydrates, nitrogen, and even some oil. It appears that *V. volvacea* prefers pure cellulose to some of the other natural waste materials. In other words, *V. volvacea* prefers herbaceous materials, in which along with cellulose there is hemicellulose, which is not always present in logs and sawdust.

In laboratory tests, Chandra and Purkayastha[7] found that most carbohydrates supported substantial growth; however, sorbose, lactose, and sucrose supported little or no growth whereas glucose and polymers of glucose supported the greatest amount of growth. Chang-Ho and Yee[27] noted that *V. volvacea* showed a marked preference for asparagine nitrogen and that, with optimum nitrogen (nitrate) supply, cellulolysis was greater at a C:N ratio of 24:36. However, Tzeng[64] found that a C:N ratio of 60:1 was optimum for his basal medium, but when 0.5% yeast extract was added, the maximum growth was at a ratio of 80:1, which was also the maximum ratio tested. It seems reasonable to conclude that the optimum C:N ratio is determined as much by the other components of the substrates as by the primary sources of carbon and nitrogen.

The mycelium of *V. volvacea* grew best at pH 7 and in the temperature range of 30 to 35°C.

C. Requirements for Fruiting Body Formation

The transition from the vegetative hyphae to the formation of primordia and the subsequent development of fruiting bodies relies on many factors, genetic as well as environmental. Once the mushroom compost has been spawned, the genetic constitution cannot be altered, and morphology and cytology can only describe what changes occur. Environmental factors, however, can provide the tools to control and improve the yield, time of fruiting, and other characteristics of the crop up to its genetic potential. Before the crop is spawned, environmental factors are also important in the planning and preparation for the crop. The optimal temperature for fruiting in *V. volvacea* is 28 to 30°C and is generally lower than the optimal temperature for mycelial growth (34 to 36°C). Light is required to act as a "trigger" for fructification. Good ventilation can provide more O_2 and remove the excess CO_2. These factors also can help the transition from vegetative stage to the stage of fruiting body formation. Based on this knowledge, cultivation of the straw mushroom can now be more or less monitored,[15] but the genetic aspect of its life cycle is as yet undefined. In spite of the

known mechanisms of sexuality in Basidiomycetes, it is probable that the fungus is homothallic, based on the average of 76% of the monosporous progeny being fertile.[26] Graham[34] reported that a single basidiospore selection of *V. volvacea* could stabilize and rejuvenate cultures, which, after continuous subculture on artificial media, had lost their productivity. This may be accomplished by eliminating "inefficient" nuclei or by the selection of "efficient" combinations of nuclei. Although selection of single-basidiospore isolation in certain stocks of *V. volvacea* could be useful in improving the yield and stabilizing spawn performance, this is not always the case in other strains. Acceptance of the homothallic hypothesis for *V. volvacea* makes it difficult to explain the variation of mycelial colonial morphology, growth rate, and self-fertility of the single-spore isolates from the same fruiting body.[44]

In a heterothallic species with normal meiotic segregation, only those spores that receive two of the meiotic products can possibly be fertile. In their cytological studies, Chang and Ling[21] counted the nuclei in 2123 basidiospores. Of these, 142 were found to be binucleate. Assuming that these spores were binucleate, as a consequence of receiving two nuclei by migration and not as a result of postmeiotic mitosis, and that an incompatibility system was present, only 6 to 7% of all spores could possibly be fertile. Chang and Yau[26] have provided an explanation for these data assuming that an incompatibility system is present and that fertility can be related to gene dosage. However, Raper[52] pointed out that such a system would be unprecedented and favored the views that *V. volvacea* is homothallic and that the infertility of some isolates is due to secondary genetic characters. In *Armillaria mellea*, Ullrich and Anderson[65] demonstrated that diploid is the normal state of the fertile mycelium. Elliott and Challen[33] proposed a new secondary homothallic model with a diploid/tetraploid nuclear cycle for *V. volvacea* and *V. bombycina*. Cytological observations of the quadrivalent pairings at pachytene in the tetraploid nuclei of *Saccharomyces cerevisiae*[6] have never been recorded in *V. volvacea* and *V. bombycina*. Chiu and Chang[29] reported that karyogamy and meiosis took place in basidia of *V. bombycina*. Most basidiospores were uninucleate. Yet a postmeiotic mitosis might take place. A monosporous germling formed a hyaline, multinucleate, clampless mycelium. Siblings show variation in colony morphology, fertility, and growth rate; nevertheless, segregation of a mating type gene, if any exists, was not detected. Multinucleate chlamydospores were the asexual means to propagate the species. Under favorable conditions, most isolates fruited without mating and completed the life cycle autonomously. At this time it is believed that *V. bombycina* is a haploid primary homothallic species. The life cycle of *V. bombycina* is similar to that of *V. volvacea*. By using gamma radiation as the tool of investigation, Quaye[51] reported that the basidiospores of *V. volvacea* were divided into two classes, one class radiosensitive and a second class radioresistant; then he suggested that the basidiospores are haploid. Furthermore, based on the data from single and joint segregation of biochemical loci of this fungus, Royse et al.[53] confirmed that basidiospores of *V. volvacea* are haploid. These data would support the cultural and cytological data from Chang and his co-workers[10,21,26] from which they proposed a primary homothallic life cycle for *V. volvacea*.

III. CULTIVATION METHODS

A. Production of Spawn

Spawn can be simply defined as a medium impregnated with mushroom mycelium that serves as the "seed" for mushroom cultivation. It must be prepared under strict aseptic conditions.

1. Starting Cultures

Volvariella mushroom cultures can be obtained in any one of the following four ways as a start of a culture: (1) obtain a pure culture from a friend who has isolated the mushroom desired or purchase one from a company that specializes in the collection and sale of mushroom cultures, (2) gather mushroom spores as a spore print and then germinate the spores to make a single-spore culture,

(3) make a multispore culture, and (4) pick a fresh mushroom and make a tissue culture. All tissues in the life cycle of the mushroom can be grown vegetatively in culture. The steps of making a spore print for the multispore culture and the technique for making a tissue culture are as follows:

a. Spore Print Technique

- Obtain a mature (opened) mushroom, cut off the cap near the upper end of the stalk, and place it on a piece of clean paper. Leave it there for 10 minutes. Discard this first print, because bacteria and other microorganisms may be present.
- Place the mushroom on a second sheet of clean paper and cover it with a clean beaker to eliminate air currents. Spores will be shed on the paper in 20 to 30 minutes. This print is ready for inoculation.

b. Multispore Culture Technique

- Before inoculation, clean the working area with 3% Lysol, and disinfect hands with 70% alcohol.
- Sterilize the inoculating loop by passing it through the flame of a spirit lamp two or three times, until it is red hot. Dip the loop into sterile distilled water; then gently touch the spore print with the loop.
- Using aseptic technique, transfer the material onto an agar slant for preparation (see below) by just touching the surface of the agar with the loop. Immediately after drawing the loop out of the test tube, run it over the alcohol lamp to prevent further dissemination of spores.
- Incubate the culture at 30 to 32°C for 4 to 5 days.

c. Tissue Culture Method (Use a Mature, Unopened Mushroom)

- Wash the mushroom thoroughly in water and dry it gently with tissue paper.
- Clean the working area with 3% Lysol (or similar disinfectant). Disinfect hands with 70% alcohol. Also, wipe the mushroom button gently with 70% alcohol.
- Sterilize the knife blade by heating it until red hot over a flame. Let it cool. Cut the bottom of the button slightly. Pull the mushroom into two halves and avoid touching the inner surface.
- Transfer a piece of pileus tissue from the center of the mushroom onto an agar plate. Repeat step three more times.
- Incubate these plates at 30 to 35°C for 4 to 5 days.
- By now there should be mycelium growing out from the tissue onto the agar medium. If there is contamination, transfer a tiny bit of mycelium onto another agar plate and incubate again. If the culture is uncontaminated, cut out agar blocks with mycelium on them and transfer them onto agar slants. These slants are incubated at 30 to 32°C for 48 hours.
- Use cultures directly in spawn substrates.

2. Culture Media

There are various media on which mushroom cultures can grow. Following are a few examples.

a. PDA (Potato Dextrose Agar) Medium

- Wash, peel, and dice 200 g potatoes. Place in a pan or flask and add 1000 ml distilled water. Boil until potatoes are soft enough to be eaten but not overcooked.

- Strain the decoction through cheesecloth (or thin layer of cotton) in a funnel and collect the liquid in a graduated cylinder. Restore the volume of the decoction to 1000 ml by adding fresh distilled water and put it back in the pan or flask. Set aside the potatoes.
- Add 20 g dextrose and 15 g agar. Heat to boiling while stirring occasionally until the agar is completely dissolved.
- Transfer the medium into 10-ml test tubes and/or 250-ml Erlenmeyer flasks and plug with cotton wool.
- Put the tubes or flasks in a wire basket and sterilize in an autoclave at 121°C for 15 minutes.
- Slant the test tubes while still hot but take care not to let the medium touch the cotton plug.

b. V8 Medium (1 liter water, 50 ml V8® juice, 20 g agar, and 0.2 g $CaCO_3$)

- The preparation of this medium is similar to that mentioned above. All media should be sterilized in the autoclave for 20 minutes at 121°C.
- It should be noted that most mushrooms prefer a neutral to slightly acidic medium, i.e., a pH of about 5.5 to 6.5. However, *V. volvacea* prefers a medium with a higher pH of 6.8 to 7.8; therefore, it is important to make sure that the pH of the medium is correct for a particular mushroom.

3. Spawn Media

A number of materials, alone and in different combinations, can be used as spawn substrates — e.g., rice straw cuttings, sorghum and rye grains, cotton wastes, used tea leaves, etc. Spawn should be incubated at 32°C for about 2 to 3 weeks. By that time the mycelium should fully fill the container, and usually patches of brown chlamydospores will have appeared on the inner surface of the container. Two kinds of spawn substrates are described here for practical purposes.

a. Grain Spawn (e.g., Rye/Sorghum/Wheat)

The approximate amounts of grain and water to use are 100 g of grain with 150 ml of distilled water and 2 g of calcium carbonate. It may be necessary to adjust the proportions up to 10% to account for natural differences in varieties or the original moisture content of the grain.

b. Straw Spawn (e.g., Paddy Straw)

The rice straw is first soaked in water for 2 to 4 hours, and then cleaned and cut into pieces 2.5 to 5 cm long. It is mixed with 1% calcium carbonate and 1 to 2% rice bran and placed into clean wide-mouthed quart bottles.

c. Used Tea Leaves Spawn

Used tea leaves can be collected from the local Chinese restaurants. They should be first washed thoroughly with water to remove any debris, drained, and 2% calcium carbonate added to adjust the pH value to the range of 6.8 to 7.8. Then the substrate should be mixed thoroughly. The substrate should either be put into a glass bottle having a plastic screw cap (a central hole should be bored in the substrate), or it should be packaged in heat-resistant plastic bags (e.g., polypropylene). In the latter case, a plastic ring should be pulled over the upper part of the bag and the bag opening plugged with cotton. Each opening should be covered loosely with aluminum foil.

d. Cotton Waste Spawn

The cardfly grade of cotton waste is usually chosen for the spawn-making substrate. Its treatment in spawn making should be similar to that for the used tea leaves, and 2% calcium carbonate is also added. The $CaCO_3$ neutralizes the acids that are produced by mycelial metabolism.

e. Manure-Husk Spawn

A mixture of fresh horse manure and lotus seed husks has been found to be suitable.[40] The compost is made by mixing fresh horse manure thoroughly with an equal quantity of lotus seed husks that have been steeped in water until they have absorbed enough moisture to prevent their drying out during composting. The manure should be well moistened but not too wet. After mixing, the compost is piled into a pyramid about 1 m high on a level floor (well-packed earth, or preferably cement) with a roof overhead for protection from the sun and rain. During the next 4 to 5 days, the temperature of the compost rises steadily, sometimes going as high as 65°C when the first turning is made. If too dry, the compost should be thoroughly mixed and slightly watered and then piled as before. The temperature will then rise again but will eventually come down. After four or five turnings at 4- to 5-day intervals, the temperature will drop to about 40 to 45°C, when the compost is ready for bottling. To see if the fermented compost has the right moisture content, it is pressed in the palm of the hand; if no water drips between the fingers and if the compost retains its shape when the pressure is released, the moisture content is just right.

The compost is then placed in bottles or in airtight aluminum cans. Insert the pointed end of a stick about halfway through the compost to make a hole. After sterilization, the mycelium is inoculated. Within 2 weeks, the spawn should run well and be ready for use.

The entire process of production of spawn is summarized in Figure 14.9.

B. Mushroom Production

A variety of waste materials have been used for cultivation of the *Volvariella* mushroom. They include: paddy straw,[9] water hyacinth,[28] oil palm bunch,[49] oil palm pericarp waste,[35,67] banana leaves and sawdust,[32] cotton waste,[13,37,66] sugarcane bagasse,[37–39] composted mixtures of tropical wood wastes and pineapple skin waste,[45] wood waste,[50,56] and some other substrates.[31] All mushrooms use lignocellulose as a substrate. Lignocellulose is often divided into two macromolecular groups, lignin and cellulose. In addition there is hemicellulose, which is present in herbaceous materials, but not always present in logs or sawdust. It is more variable in structure, but also more easily metabolized. Experience has shown that not all lignocellulose is equally useful to every cultivated species. Furthermore, examination of the lignocellulolytic enzyme profiles of the three important commercially cultivated species, *Lentinula edodes, V. volvacea,* and *Pleurotus sajor-caju*, show this diversity to be reflected in qualitative variations in the major enzymatic determinants (i.e., cellulases and ligninases) required for substrate bioconversion.[5] For example, *L. edodes*, which is cultivated on highly lignified substrates such as wood or sawdust, produces two extracellular enzymes that have been associated with lignin depolymerization in other fungi (manganese peroxidase and laccase). Conversely, *V. volvacea*, which prefers high cellulose, low lignin-containing substrates produces a family of cellulolytic enzymes including at least five endoglucanases, five cellobiohydrolases, and two glycosidases, but none of the recognized lignin-degrading enzymes. *Pleurotus sajor-caju* contains both groups of these enzymes, and it is the most adaptable of the three species in that it can be grown on a wide variety of agricultural waste materials of differing composition in terms of polysaccharide/lignin ratio. Since *V. volvacea* appears to lack a ligninol- or lignin-transforming system, this reduces the capacity of this mushroom to grow and fruit in a lignified substrate. The cultivation of *Volvariella*, although less sophisticated than that of *Agaricus* and *Lentinula*, is extensive and rewarding in tropical and subtropical climates.[61]

Before 1970, paddy rice straw was practically the only material used for preparing the medium for cultivation of *Volvariella* under natural conditions. In 1971, cotton wastes (discarded after sorting in textile factories) were first introduced as a heating material as shown in Figure 14.10 for growing the straw mushroom in controlled conditions.[12, 66] By 1973, cotton wastes had completely replaced the traditional paddy straw for cultivation of the mushroom in indoor conditions in Hong Kong. This is a turning point in the history of straw mushroom cultivation, because cotton waste compost gives a higher and more stable yield (30 to 40%), as well as earlier fructification and harvesting (pinheads,

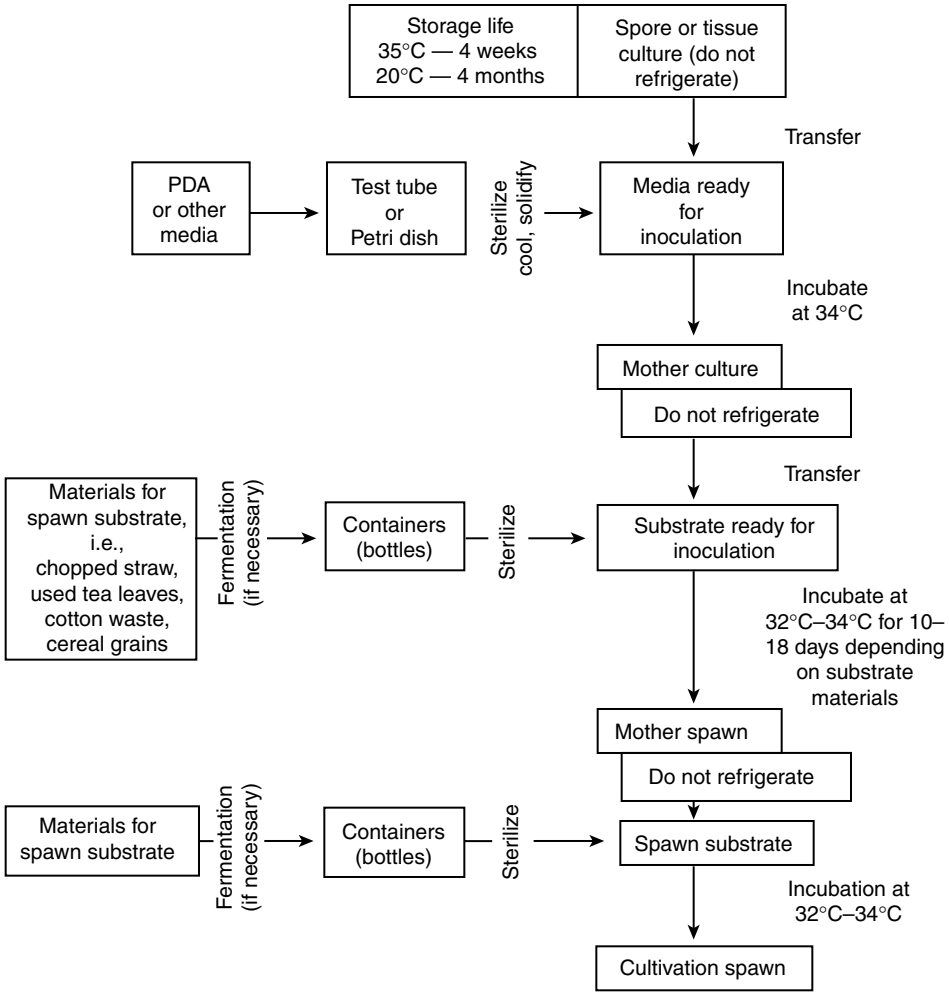


FIGURE 14.9 Spawn production flowchart for *V. volvacea*.

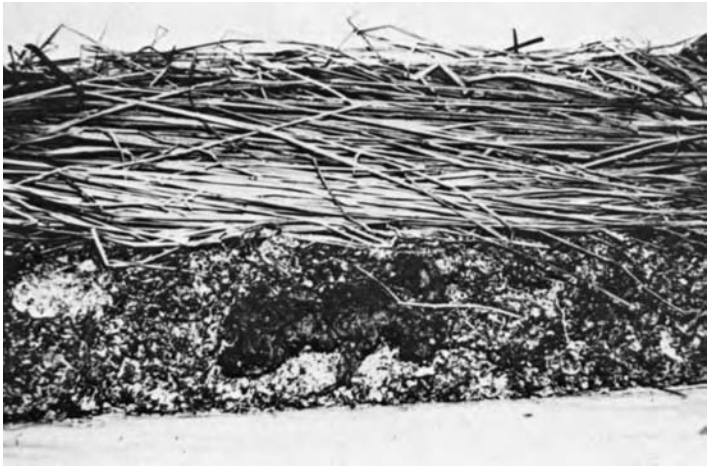


FIGURE 14.10 Cotton waste arranged under straw as a kind of heating material for upper layer of straw.

4 days; first harvest, 9 days after spawning), than that obtained using straw as substratum under the same conditions. The good characteristics of cotton waste compost have led to the cultivation of the mushroom becoming semi-industrialized in Hong Kong, Taiwan, Indonesia, China, and Thailand.[47,66]

Three common methods for the growing of straw mushroom are described below.

1. Without Pasteurization (Indoor Cultivation)

a Making of the Box

Two kinds of boxes can be used. One is with a wire screen at the bottom, and the other is with both ends open (frame type). Sizes of the boxes are (1) 80 × 80 × 10 cm, with screen, and (2) 60 × 40 × 30 cm, frame type.

b. Preparation of the Boxes

Cut the bedding material (rice straw or dried banana leaves) to a uniform length of 20 cm. Fill the boxes tightly with bedding material arranged parallel with the length of the box in anticipation of the loosening of the pack when soaked in water. Trim the boxes of any protruding and dangling bedding material to avoid obstruction during watering. Soak the boxes containing bedding materials for at least 2 hours in 2% $CaCO_3$ water, or until the straw becomes dark brown or the banana leaves exhibit a certain degree of transparency. Remove the boxes from the soaking area and drain excess water immediately prior to planting.

c. Planting of the Spawn

Young (10 to 14 days old) spawn should be used. Remove thumb-size pieces from the bottle and distribute them on the surface of the box. Bury the spawn 5 cm deep in the bedding material, but first plug the opening with newsprint previously soaked in water for 5 to 10 minutes. Four pieces of spawn should be equally distributed along the width and five pieces along the length at a distance of 5 cm from the sides of the box. Massage the surface to close the open spaces resulting from the insertion of spawn and plugging with paper. Repeat the same procedure on the other side of the box.

d Incubation of Boxes

The boxes may be either placed in specially built incubation rooms of high temperature (35 to 38°C) and high relative humidity (at least 75%) or simply covered with plastic sheets. Remove the boxes from the incubation room as soon as a good spawn run has been obtained. This is usually attained in 3 days, but takes 5 days if they are wrapped with plastic sheets.

e. Care of the Boxes

If the boxes are incubated in incubation rooms, be sure that the conditions in the growing house do not fluctuate too much so as to minimize serious stress on mushroom mycelial growth. Start to lower the temperature after the first 24 hours of incubation by opening ventilators or introducing fresh air to the growing room. Maintain the temperature at 28 to 30°C with a relative humidity of 75 to 85%. If the boxes are wrapped in plastic, simply remove the sheets and gradually reduce the temperature and relative humidity after 24 hours. Aerial spraying with a superfine mist will help to maintain the desired relative humidity in the growing room. Controlling the ventilators used for aeration will keep the temperature at the proper level. If the bedding material appears to be getting dry, water it with a fine mist to avoid destroying the delicate mycelial threads of the mushroom.

2. Without Pasteurization (Outdoor Cultivation)

The best places to make the mushroom growing beds are in shade created by trees or creepers. The beds are usually built in an east–west direction, and the base is raised to ensure that the straw is not flooded in the rainy season. On the other hand, the straw must not be allowed to dry out and the trenches are dug around the bed to distribute water when this is required to maintain damp conditions. The raised base of the bed is usually made from soil, but if this is too sandy to make

FIGURE 14.11 Preparation of the straw bed showing the raised base (front), young mushrooms, and bamboo hoops on which a plastic sheet is used for covering.

a firm platform, then bamboo poles, bricks, or wooden planks may be used to ensure that the base will withstand erosion.

The actual mushroom compost is made from either rice straw or other materials. Rice straw is tied up in bundles, about 10 cm in diameter, using ropes made from banana leaf sheath fibers. These bundles are trimmed to 40 cm in length and soaked overnight in fresh, preferably running, water or in 2% $CaCO_3$ water.

A stout bamboo pole is then driven into the center of each end of the bed, and the bundles of material are laid down in neat layers, with each layer spawned before the next is added. The growing bed may only have four layers in the dry season but as many as seven are permissible during the rainy times of the year. The bed is finally topped with a 20-cm-deep layer of rice straw, and the whole construction is covered with a polythene sheet supported on bamboo framework (Figure 14.11). The polythene sheet cover plays a dual role in that it increases both the temperature and humidity. However, after 4 days the sheet is removed and on the sixth day the bed is carefully sprinkled with water. Spraying may be unnecessary in the rainy season, and in any event it should not be carried out after the mushroom pinheads appear on the surface of the bed.[17,36]

3. With Pasteurization

Preparation of the growing substrate is shown in the flowchart (Figure 14.12). Details of each step are given below.

a. Substrate

The substrate for cultivation is cotton waste, which is rubbish from textile factories. The material is basically a complex of cellulose and hemicellulose.[41] It is particularly desirable in straw mushroom cultivation. Unlike *Agaricus bisporus*, the straw mushroom culture is unable to utilize lignin but depends on hemicellulose and cellulose as its major food sources. This feature makes cotton waste a better substrate than rice straw or banana leaf for this mushroom. In addition, the fine texture of cotton waste is good in moisture retention, which minimizes requirements for irrigation and thus avoids damaging the delicate fruiting primordia. Table 14.1 compares the composition of various popular materials for straw mushroom cultivation.

b. Preparation of Compost

The cotton waste is first provided with water and lime, 1% dry weight, to adjust to a 65% moisture content and pH 7 to 8. To aid uniform distribution, a square wooden rack (92 × 92 × 28 cm) is

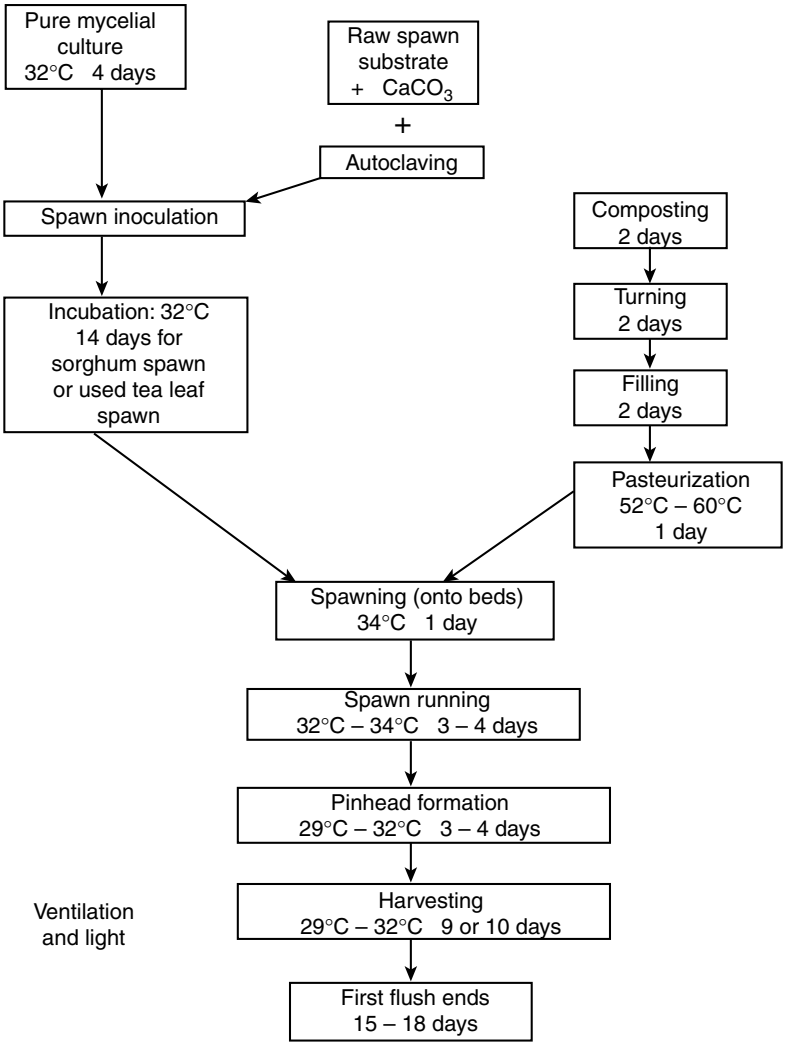


FIGURE 14.12 Flowchart for *Volvariella* mushroom production.

TABLE 14.1
Major Forms of Carbohydrate in Three Popular Substrates for Straw Mushroom Cultivation

	Cotton Waste[41]	Rice Straw[46]	Banana Leaf[17]
Total N	1.22	0.61	1.71
Total C	49.94	54.26	50.52
C:N	40.90	84.00	29.50
Hemicellulose	8.73	17.11	19.95
Cellulose	56.76	29.68	10.85
Lignin	10.47	12.17	18.21

used to hold a layer of cotton waste about 30 cm deep, which is sprinkled with one portion of lime. Then water is hosed in as the worker treads on the cotton to help absorption. The rack is raised when the first layer is trodden and another layer applied. The work is repeated until the total amount of cotton waste for one cultivation shed has been prepared. On average, 1.5 tons of cotton waste (enough for a 100-m bed) are available in sheds.

The pile of wet cotton waste is left to ferment in the open, but covered when there is rain or it is cold. Turning over once or twice is usually practiced to produce more uniform compost. The fermentation process takes about 3 days to 1 week. The central temperature of the pile of compost can reach more than 60°C in 2 days and lower to about 40°C before bedding, i.e., putting onto tier shelves in the cultivation sheds.

c. Bedding and Pasteurization

The thickness of cotton waste compost on shelves varies according to the season, i.e., about 5 to 8 cm in summer but over 10 cm in winter. Thick compost helps preservation of moisture and heat, which is the key to success in the cold dry months. The compost is spread evenly on the shelves and the surface made flat by pressing lightly.

After 8 to 12 hours the doors and vents are then tightly closed and stream introduced from the generator into the shed via a rubber hose about 6 cm in diameter. The steam generator and the polystyrene cladding are capable of producing and preserving an air temperature of 62 to 65°C against winter colds as low as 10°C. The temperatures of the pasteurization are 62°C for 2 hours and 50 to 52°C for 8 to 16 hours depending on whether the compost has been well fermented before bedding.[15,36] There can be a high concentration of ammonia or hydrogen sulfide if the cotton waste compost is not well fermented. Aeration to expel the gases and re-steaming would then the necessary. This is a popular practice when there is an upsurge in market prices and the grower is anxious to shorten the working days by shortening the fermentation process.

After steaming, the shed is left closed to let the air temperature lower gradually to 34°C or the compost temperature to 38°C before it is opened for spawning. This will usually take 16 hours or overnight.

d. Spawning

The air temperature in the mushroom house is cooled to 35°C, with a bed temperature 36 to 38°C, which is suitable for spawning. The amount of spawn used is 1.4% (dry weight) or 0.4% (wet weight) of the compost. The pure culture spawn should be taken out of the container (bottle) and placed on a tray for easy handling. The spawn is pressed and broken into small pieces (about the size of a small peanut). The pieces are inserted into the compost, which has been scooped out to a depth of 2 to 2.5 cm, at intervals of 12 to 15 cm. The inserted spawn is then covered with the displaced compost. Finally, the beds are covered with thin plastic sheets. The temperature of the room is maintained at 32 to 34°C during the period of spawn running. Full growth may be achieved within 3 to 4 days, depending on the compost quality and the temperature.

e. Fructification

During the 3-day period of spawn running, no water or light, but a little ventilation, is needed. At the end of 3 days, white light (by means of fluorescent lamps) is introduced into the room, and more ventilation is given. Under good composting and pasteurization conditions, unidentified species of Actinomycetes and *Humicola fuscoatra* Traaen will develop in and on the beds, together with the mycelia of *Volvariella* during the spawn running period.[16] After removal of all plastic sheets and after sprinkling the beds with water on the fourth day, growth of Actinomycetes and *Humicola* are retarded, but *Volvariella* continues to grow. On the fifth day after spawning, primordia of fruiting bodies usually appear on the surface of the beds. After another 4 to 5 days, the first flush of mushrooms is ready for harvesting.

The initiation and development of fruiting bodies require a rather marked change in environment. Aeration is required, and light is also a stimulating factor. Fluorescent lights are provided

inside the cultivation shed at this stage whenever sunlight diffusing through the foam boards appears to be insufficient. A lower relative humidity of about 80% and a temperature around 30°C would aid better quality. It takes about 5 days from the appearance of pinheads, i.e., mushroom primordia, to attain marketable size. The quick growth rate demands an ample supply of water and oxygen, which are unfortunately antagonistic to each other in practice. Aeration generally reduces humidity, which in turn dries out the compost; this is more obvious under the dry winter climate. Watering the compost often brings a disadvantage, in that the bed temperature is lowered and can hardly be raised back to the high of 30°C in cold months. Although such a problem does not exist in summer, too much water will suffocate the tiny primordia and reduce yield. Crop management to achieve the best possible combination of light, temperature, ventilation, relative humidity, and compost moisture is in fact an art of judgment, experience, and effort.

IV. HARVESTING AND PROCESSING

A. Harvesting

The straw mushrooms are usually not left to grow to their maximum size but they are picked for selling at the stages before the volva breaks or just after rupture. As described in Section II.A, the former is the button stage and the latter, the egg stage. Because the mushrooms grow in rather high temperature and humidity conditions, they are fast growing. To harvest mushrooms of good quality, the straw mushrooms are harvested twice a day, in the morning and in the afternoon; and, sometimes, an additional crop is picked at noon.

Under normal conditions, it usually takes about 3 days from the appearance of minute fruiting bodies to the development of mushrooms at marketing stages. The first crop of mushrooms is usually harvested 8 to 10 days after spawning. The first flush normally constitutes three successive days of harvesting, and 70 to 90% of the expected yield is obtained, depending on the growing substrates and environmental conditions. During a rest period of 3 to 5 days after the first flush, thorough watering of the compost may be resumed. Likewise, during this intervening period, proper conditions in the growing room must be maintained. The second flush may also require 2 to 3 days for harvesting, but it gives a lower yield. The second flush adds only 10 to 30% to the total crop, making the expected biological efficiency 10 to 45%, with a production of 2.4 to 7.3 kg/m in a cycle of 15 to 20 days.

When the fruiting bodies reach harvest size, they should be carefully separated from the bed/substrate base by lifting, while shaking slightly left and right, and then twisting them off. This way of harvesting could prevent damage to the growing mycelium and the developing primordial, which are adjacent to the fruiting bodies being harvested. It should be noted that the mushroom should not be cut off by knives or scissors from the base of the stalk, because the stalks left behind on the bed/substrate might cause rot, be attacked by pests, and contaminated by molds, which could, in turn, destroy the mushroom bed.

B. Processing

Like all fruits and vegetables, straw mushrooms are perishable and, after being harvested, they continue to undergo metabolic activities and often change in ways that make them of lower commercial value or even unacceptable for human consumption.

The mushrooms can be processed by canning, pickling, and drying, but the quality of the finished product often is not comparable to the quality of fresh mushrooms. The fresh mushrooms undergo autolysis at 4°C, and their shelf life stored at 10 to 15°C is about 3 days.[54] It has been suggested that for the short-term storage, preservation at 15 to 20°C or controlled atmosphere storage may provide a solution.[30] Straw mushrooms are imported by Hong Kong in large numbers. In regular practice, the straw mushrooms from China are packed in wooden cases and transported

FIGURE 14.13 The authors examining the mushroom bed on a rural straw mushroom farm in Ping-Shan County, Hebei Province, China, July 13, 1986.

by train or boat. The case is divided into three compartments — mushrooms are placed in the central compartment and ice is in the other two. On the other hand, mushrooms from Taiwan and Thailand are packed in bamboo baskets and transported by airfreight. An aeration channel is located at the center of the basket, and dry ice wrapped in paper is placed above the mushrooms. Certainly, canning should be considered to be the best method for preservation of straw mushrooms; the main advantages are a longer period of storage and less risk of damage during transport. Research is necessary on the canning of the straw mushroom similar to that which has been done with *Agaricus* on the effect of blanching, postharvest storage, soaking, or other chemical treatments before canning, in order to increase the drained weight and to improve the quality of the canned product.

V. SPECIAL CULTIVATION PRACTICE

A. Rural Spawn Station in Ping-Shan County, Hebei Province, China

The authors (Figure 14.13) visited, on July 13, 1986, a recently constructed rural spawn-making station that was in operation at that time. The station physically separated the operations of substrate mixing, bagging the substrate, sterilization, inoculation, and incubation, all within a small compound.

1. Substrate

The spawn substrate consisted of 20% wheat straw and 80% cottonseed hulls. Spawn substrate (100 kg) was soaked in a solution containing 1.5 kg of $CaCO_3$. The soaking lasted for 3 hours, at which time the pH of the substrate became 8 to 9. During the soaking, the cotton seed hulls became black.

2. Bagging

Several workers were engaged in bagging the spawn substrate. Each bag contained approximately 250 g of spawn substrate. The moisture content was about 60 to 65%. A plastic ring was used to form a circular opening at the open end of the plastic bag, and this opening was then closed with a cotton plug.

3. Sterilization

Two cement towers, approximately 2.7 m tall, heated by a common coal-burning furnace, constituted the sterilizing chambers. The bags were placed in six layers of 80 to 90 bags per layer. The bags for each layer were placed in a round metal basket with a wire bottom. Each basket had six short supporters upon which the basket above rested. This provided an air space of a few centimeters between each layer of bags. A metal cover was placed on the top of each tower to contain the steam, and a thick quilt was placed on the top of this cover to further retard loss of steam.

Once the temperature in the sterilization towers reached 95°C, the heating was continued for 7 hours. Each tower could hold approximately 500 bags of spawn substrate; therefore, 1000 bags could be sterilized at one time. The cost of constructing the towers was only about 1000 yuan (U.S. $275), as the bricks, cement, and some other raw materials were already available in the area.

4. Inoculation

A small inner room with an ultraviolet lamp for general sterilization of the area served as the inoculation room. In the room was a locally constructed inoculation box with work openings on two sides. The inoculation box was of the usual design in which the operator's hands are inserted through sleeves attached to the box. Routinely, 70 bags are placed inside the box for surface sterilization, which is accomplished by vapors from a mixture of 5 g potassium permanganate and 10 ml of Formalin placed in a saucer. One half hour after this mixture is placed in the inoculation box, inoculation operations can begin. Two persons, one on each side of the box, can inoculate 70 bags in 1 hour.

5. Incubation

A culture room for incubation of the spawn was maintained at 30 to 35°C. The culture room held 6000 to 7000 bags. After incubation for 10 to 14 days, the bags are ready for use as spawn.

6. Comments on Management

The spawn station has 4 full-time workers and 20 summer workers. The Hebei Academy of Sciences supported the station by loaning money for construction costs and providing technical advice. Most importantly, to help initiate this project the academy provided a scientific advisor who worked at the site for 2 months. In addition, the spawn station was fortunate in having the benefit of the experience of two able and enthusiastic volunteers — one of whom was a retired teacher and the other who was also a retired civilian officer. The management of the spawn factory did not require the growers to pay for the spawn upon delivery, but permitted the growers to pay when they had harvested and sold their crop. The price to the farmers for the spawn is 0.25 ¥ per bag (about U.S. $0.07), and the cost of production is 0.21 ¥ (about U.S. $0.06).

7. Implications of This Experimental Project

This project in Ping-Shan County, Hebei Province, China, can serve as a model to be followed by developing countries interested in forming rural mushroom industries.

Although it is the overall planning and careful attention to details that are undoubtedly essential for the success of a project of this nature, there are certain features of the development of the Ping-Shan County Rural Spawn Station that we feel should be emphasized:

1. The initial stages of planning and development of the physical plant received encouragement and support, both moral and financial, from government.

2. Persons experienced in mushroom cultivation were present at the site during the building of the plant and in the stages of its initial operation to help train the personnel, to help resolve unforeseen problems, and to become aware of local conditions so that, after the departure of the advisor from the site, appropriate advice and assistance could be given when necessary.
3. The confidence of the spawn station in their product was exemplified by deferment of payment for the spawn by the farmers until after harvesting and selling the crop. This served to give the farmers confidence and to permit those with insufficient finances to become growers. The initial low price set on the spawn was also indicative of a cooperative, noncompetitive attitude on the part of the spawn station. For successful mushroom cultivation the importance of having reliable spawn at a reasonable price cannot be overemphasized.
4. Research at the Hebei Academy of Sciences will play a continuing role in assisting the growers and the spawn station. A close working relationship between researchers and growers is very valuable to both groups.

B. Technique of Cultivation of Straw Mushrooms in Green Poplar Village, Ping-Shan County, Hebei Province, China

When the authors visited Green Poplar Village, Ping-Shan County, Hebei Province, China, on July 13, 1986, the farmers in this area were cultivating straw mushrooms with the initial advice and guidance of scientists of the Hebei Academy of Sciences. In this area there is a supply of wheat straw that can be used for this purpose, and the summer temperature is suitable for the cultivation of *V. volvacea*, the straw mushroom. The technique for cultivation of the straw mushroom in Green Poplar Village is outlined in detail, as we feel that it may be useful in rural communities elsewhere.

1. Preparation of Compost

The compost is prepared in the following manner:

1. Wheat straw, which has been cut into pieces about 10 to 15 cm in length, is soaked in 1% $CaCO_3$ overnight.
2. The soaked straw is placed on the ground for 6 to 8 hours to drain off excess water.
3. The straw is piled for composting and covered with a plastic sheet. The compost is turned when the temperature in the center of the compost pile reaches 50°C. After 1 or 2 days, depending on the weather, the compost is ready for bedding.
4. The compost is placed in a frame approximately 70 × 35 × 22 cm. This contains approximately 5 kg of air-dried wheat straw and about 12 to 13 kg of wet weight straw.
5. The substrate block consists of four layers arranged in the following way in a mold: a layer of compost is placed on the bottom. Around the edge of this layer on the four sides is placed the spawn and some wheat bran. The second layer of compost is placed on top of the first, then the spawn and wheat bran around the edges. The third layer is added. The fourth layer is added with the spawn and wheat bran around the edges.
6. The compost costs the farmers nothing since they use their own materials.

2. Arrangement of Bed Blocks (Figure 14.13)

1. The soil base is raised several centimeters using the soil from the ditches, which surround the base.
2. The bed blocks are arranged in two rows and separated from one another by 20 to 25 cm.
3. Poplar branches are bent in a bow shape to form the frame over the bed blocks.

4. A plastic sheet is spread over this frame, and this, in turn, is covered by straw mats.
5. Temperature is roughly maintained at 33 to 35°C by removing or adding the straw mats. The plastic sheet is necessary to maintain the proper moisture conditions and concentration of CO_2 to stimulate vegetative growth.

3. Harvesting of Mushrooms

1. Pinheads of the mushrooms appear 4 days after spawning.
2. Usually 5 days after the appearance of pinheads, the first mushrooms in the egg stage can be harvested. Mushrooms develop very rapidly, and they should be picked three times daily (morning, noon, and evening). This first flush lasts 3 days, and gives about 75% of the total yield.
3. After the first flush, the bed blocks are watered with 0.5% $CaCO_3$ and covered.
4. A few days later, mushrooms of the second flush appear. The mushrooms of this second flush amount to approximately 25% of the total yield.
5. One crop usually consists of two flushes. A crop takes about 18 to 20 days.
6. The biological efficiency (fresh weight of mushrooms/air dry weight of substrate × 100) is about 20%.
7. Under the climatic conditions of northern China, e.g., Shijiazhuang area (Hebei), four to five crops can be obtained each year.

4. Spent Compost

1. After growing the straw mushrooms, the spent compost can be dried and saved for autumn to grow *Pleurotus sajor-caju*. On such spent compost the biological efficiency for production of *P. sajor-caju* is 80%.
2. The spent compost, following the production of *P. sajor-caju*, can be used directly as a very good soil conditioner.

5. Conclusion

Wheat straw, the basic substrate material for this mushroom production method, is sometimes burned. It may be used as bedding material for animals or as a soil conditioner, after a long period of composting. When the straw is used for the production of mushrooms, however, it is quickly converted to a highly acceptable food for human beings that is rich in protein. We recommend this use of straw over burning whenever and wherever possible.

VI. SOME SPECIAL METHODS AND THEIR RATIONALE

A. Phenomenon of Early Fruiting

Cultivation of the straw mushroom (*Volvariella volvacea*) generally requires from 4 to 6 days from spawning to pinhead formation, but with certain conditions pinheads will appear within 3 days after spawning. This is called here the phenomenon of early fruiting.

The early fruiting bodies usually appear in the area immediately surrounding the site of spawning or even on the spawn substrate itself. At this time, the mycelium is scattered only over the surface of the compost and has not yet formed a robust hyphal network throughout the compost. The fruiting bodies from such early fruiting are usually small, and the yield is low. Some mushroom farms in Indonesia and in Hong Kong have been troubled by this phenomenon of early fruiting. In seeking an explanation for this, and thus corrective measures, we must examine the developmental stages.

Development of mushrooms can be divided conveniently into vegetative and reproductive stages as mentioned in previous chapters. The development of mycelium (the vegetative stage) consists not only of a quantitative increase but also of some qualitative changes, which are not necessarily revealed by morphological features. The qualitative changes are, nevertheless, extremely important in that through them the mycelium becomes robust, mature, and accumulates the abundant nutrients, which provide the basic materials from which the fruiting bodies develop. This is not a unique developmental pattern but is also found in the cultivation of other edible mushrooms.

The development of *V. volvacea* requires a high temperature (28 to 35°C), high humidity (80 to 85%), nutrients, and a slightly alkaline pH; but the vegetative and reproductive stages require slightly different environmental conditions, particularly temperature. The optimal temperature for vegetative growth is 32 to 35°C, and for fruiting body development 28 to 32°C. An increase or decrease of temperature can directly influence a change in the developmental stage. For example, if the air temperature in the mushroom house and the bed temperature are lower than the optimum for growth and development of mycelium, this can cause not only retardation in the growth of the mycelium, but also an enhancement of the formation of fruiting bodies. Thus, (1) if the mycelium has not yet completed the quantitative and qualitative changes inherent in a mature mycelium that is established for fruiting, and (2) if the environmental conditions are those that favor fruiting, then the fruiting bodies will form sooner and be smaller than would be the case with a mycelium that was robust and had reached maturation.

The reason for the phenomenon of early fruiting is, therefore, a lower than optimal temperature for vegetative growth or dropping the temperature to stimulate fruiting too early. It is extremely important in the practice of cultivating straw mushrooms to exercise strict control of the temperature in the mushroom house and the bed. To avoid the phenomenon of early fruiting the following points should be noted:

1. During composting, the moisture content of the compost should be controlled and adjusted to the range of 60 to 65%, because with this moisture content the necessary temperature of the compost can be controlled more easily.
2. After pasteurization, when the temperature has come down to 35°C, spawning should be performed immediately and the bed should be covered with plastic sheets. The high temperature and high humidity conditions created by this treatment will assure the rapid development of the mycelium. The plastic covering should be used for 4 days.
3. Ventilation should not be given during the first 3 days following spawning because ventilation would lower the temperature and moisture in the mushroom house, and this would stimulate early fruiting.
4. At 4 to 6 days after spawning the plastic sheet should be removed and the bed surface gently sprinkled with water, and at the same time ventilation should be given to decrease the temperature and moisture for the stimulation of fruiting.

B. Insect Enemy of Straw Mushrooms — Nematodes

Occasionally there is a problem involving the death of a great number of pinheads of the straw mushroom during cultivation. One cause of this is damage by nematodes. Nematodes will occur in high numbers if the compost has not been properly pasteurized. When the compost has high water content, proper fermentation does not occur, with the consequence that the temperature does not rise sufficiently high and the compost is immature. Even when steam is used, the compost will not be satisfactorily pasteurized. The high moisture content makes it difficult to raise the temperature in the center of the bed sufficiently high (+50°C) to kill the nematodes. Thus, the nematodes harbored in the center of the compost in the bed survive the pasteurization treatment, and, after spawning, the bed temperature gradually decreases. This permits the nematodes in the center of the compost to multiply rapidly, as the optimal temperature for multiplication of nematodes is

almost the same as that for formation of fruiting bodies of *Volvariella* (28 to 32°C). Nematodes are likely to eat mushroom mycelium, and, when the mycelium beneath a pinhead is eaten by nematodes, the pinhead dies quickly because of loss of the supply of nutrients and water.

Nematodes are well protected against adverse environmental conditions of moisture and temperature. Consequently, the mushroom house, the tools used in mushroom production, the materials of the compost, and the place of composting are all capable of harboring nematodes for their subsequent spread to the compost in the beds. The active stage of the nematodes cannot tolerate a temperature of 50°C. With this understanding of the manner in which nematodes can be spread and their lethal temperature, the following procedure has been developed for controlling damage by nematodes:

1. In preparation of the compost, the moisture content should be strictly controlled at 60 to 65%. At this moisture content, the formation of clumps of compost, made by the footprints of the workers preparing and mixing the compost, is minimized.
2. Steam should not be applied immediately after the beds are filled. A period of at least 10 to 12 hours should elapse before steaming begins. The reason for this is to keep the room temperature lower than the bed temperature. The nematodes in the center of the compost will then migrate from the center of the compost where the temperature is relatively high to the outer layer or surface of the compost where the temperature is lower. In the outer regions of the compost, the nematodes will be killed more easily when steam is applied.

If the above procedure is not followed and steam is injected into the room immediately after filling the beds, the room temperature will be raised quickly, resulting in a higher temperature of the surface and outer layer of compost than is present in the center. With this situation the nematodes will quickly migrate from the outer layer to the center of the compost. If in the center there are some moist clumps of compost harboring nematodes, the steam during pasteurization will not kill the nematodes because it will not penetrate the clumps as freely as it does well-mixed, unclumped compost. The nematodes surviving in the clumps can spread through the compost and propagate when the temperature drops, and, after the compost has been spawned, they can seriously damage the crop.

C. *Coprinus* — Fungal Competitor of *Volvariella*

In the biological world, competition between species for survival is a common phenomenon. In the practice of cultivation of straw mushrooms, contamination by another mushroom species is frequently encountered in mushroom farms. As an example, we cite the following case involving a mushroom farmer in Indonesia in 1978.

The mushroom farmer decided not to remove the compost used for the first flush, but to add some urea to it, believing that this treatment would increase the yield of a second flush. What his treatment did was to produce a crop of *Coprinus* without a single straw mushroom! The straw mushroom has several fungal competitors, but *Coprinus* is the most frequently encountered. *Coprinus* completes its life cycle in a shorter time than does the straw mushroom, taking only 1 week, whereas *V. volvacea* takes 9 to 10 days. This makes *Coprinus* a strong competitor of *V. volvacea* when the latter is being cultivated.

When the fruiting body of *Coprinus* forms, the pileus opens quickly, and then overnight the mushroom undergoes autodigestion, leaving the spores present in a black, inky fluid. After the fruiting bodies of *Coprinus* in the mushroom house have completely decayed, a strong odor emanates from the residue. This is often followed by the growth of a green mold that is usually *Trichoderma*.

Growth of *Coprinus* damages the bed of the straw mushroom. Its role as a competitor stems from the fact that it has almost the same requirements as *V. volvacea* for environmental conditions, and

with its shorter life cycle it can grow and fruit on the substrate sooner than the straw mushroom. For example, both mushrooms have the same optimal temperature, and both can efficiently break down cellulose, hemicellulose, soluble starch, and glucose and other sugars for their carbon. The same nitrogen sources can be utilized, but the nitrogen sources used most effectively by the two fungi are different, and *Coprinus* requires a much higher nitrogen concentration — approximately four times higher — than does *Volvariella*. Thus, in compost high in nitrogen, *Coprinus* is apt to appear.

The pH requirements of *Coprinus* and *Volvariella* are different; however, the range of pH for *V. volvacea* includes values in which *Coprinus* can also grow normally. The optimal pH value for growth is 7.0 for *V. volvacea* and about 5.0 for *Coprinus*.

It is with consideration of the characteristics that have just been given of the two species that the mushroom grower must determine the most suitable cultivation conditions to promote vigorous growth of the straw mushroom mycelium, conditions that at the same time will be less favorable for, or even inhibit, the growth of mycelium of competitor fungi. The following suggestions are made in this connection for cultivation of the straw mushroom:

1. In the preparation of compost, the C:N ratio should be adjusted to the requirement for mycelial growth of *V. volvacea*. This C:N ratio is 40:1 to 50:1. When nitrogen (e.g., urea or ammonium chloride) is added to the compost material, be it cotton waste or paddy straw, it should be added at the beginning of the composting procedure. The reason for this is that during fermentation the ammonia will be utilized by other microorganisms in the compost, thereby keeping the nitrogen in the compost and not allowing it to be lost to the atmosphere as gaseous ammonia.
2. The water content of the compost should be maintained in the range of 60 to 65% to have high-temperature fermentation, which is essential for the production of a good-quality compost. If the compost is too wet, it will cause decay, lower the pH, and thus promote the growth of *Coprinus*.

REFERENCES

1. Baker, J.A., Mushroom growing in Wellesley and Penang Provinces, *Malay. Agric. J.*, 22, 25–28, 1934.
2. Baker, J.R., *Cytological Technique*, Methuen, London, 1960.
3. Benemerito, A.N., Mushroom culture in Canton, *Philipp. Agric.*, 24, 624–634, 1936.
4. Bessey, E.A., *Morphology and Taxonomy of Fungi*, Hufner, New York, 1964.
5. Buswell, J.A., Cai, Y.J., Chang, S.T., Peberdy, J.F., Fu, S.Y., and Yu, H.S., Lignocellulotic enzyme profiles of edible mushroom fungi, *World J. Microb. Biotechnol.,* 12, 537–542, 1996.
6. Byers, B. and Goetsch, L., Electron microscopic observations on the meiotic karyotype of diploid and tetraploid *Saccharomyces cerevisiae, Proc. Natl. Acad. Sci. U.S.A.*, 72, 5056–5060, 1975.
7. Chandra, A. and Purkayastha, R.P., Physiological studies on Indian edible mushrooms, *Trans. Br. Mycol. Soc.*, 69, 63–70, 1977.
8. Chang, S.T., The influence of cultural methods on the production and nutritive content of *Volvariella volvacea, Chung Chi. J.*, 4, 76–84, 1964.
9. Chang, S.T., Cultivation of the straw mushroom in S. E. China, *World Crops*, 17, 47–49, 1965.
10. Chang, S.T., A cytological study of spore germination of *Volvariella volvacea, Bot. Mag.*, 82, 102–109, 1969.
11. Chang, S.T., The studies of *Volvariella volvacea* in the past and in the future, *J. Hortic. Soc. China* [in Chinese], 18, 55–58, 1972.
12. Chang, S.T., *The Chinese Mushroom (Volvariella volvacea) — Morphology, Cytology, Genetics, Nutrition and Cultivation*, Chinese University Press, Hong Kong, 1972.
13. Chang, S.T., Production of the straw mushroom (*Volvariella volvacea*) from cotton wastes, *Mushroom J.*, 21, 348–354, 1974.
14. Chang, S.T., The origin and early development of straw mushroom cultivation, *Econ. Bot.*, 31, 374–376, 1977.

15. Chang, S.T., *Volvariella volvacea*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 573–603, 1978.
16. Chang, S.T., Cultivation of *Volvariella volvacea* from cotton waste composts, *Mushroom Sci*., 10(2), 609–618, 1979.
17. Chang, S.T., Cultivation of *Volvariella* mushrooms in Southeast Asia, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 221–252, 1982.
18. Chang, S.T. and Chang, K.Y., Quantitative and qualitative changes in proteins during morphogenesis of the basidiocarp of *Volvariella volvacea, Mycologia*, 65, 355–364, 1973.
19. Chang, S.T. and Chu, S.S., Nuclear behavior in the basidium of *Volvariella volvacea, Cytologia*, 34, 293–299, 1969.
20. Chang, S.T. and Chu, S.S., Factors affecting spore germination of *Volvariella volvacea, Physiol. Plant.*, 22, 734–741, 1969.
21. Chang, S.T. and Ling, K.Y., Nuclear behavior in the Basidiomycetes, *Volvariella volvacea, Am. J. Bot*., 57, 165–171, 1970.
22. Chang, S.T. and Quimio, T.H., Eds., *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chinese University Press, Hong Kong, 1982.
23. Chang, S.T. and Tanaka, K., The ultrastructure of complex membranes in the germinating spore of *Volvariella volvacea, 7th Int. Cong. Elec. Micro.* (Grenoble, France), 3, 437–438, 1970.
24. Chang, S.T. and Tanaka, K., Culturing and embedding of filamentous fungi for electron micrography in the plane of the filaments, *Stain Technol.* (U.S.A.), 45, 109, 1970.
25. Chang, S.T. and Tanaka, K., An electron microscopy study of complex membranous structures in basidiomycete *Volvariella volvacea, Cytologia*, 36, 639–651, 1971.
26. Chang, S.T. and Yau, C.K., *Volvariella volvacea* and its life history, *Am. J. Bot*., 58, 552–561, 1971.
27. Chang-Ho, Y. and Yee, N.T., Comparative study of the physiology of *Volvariella volvacea* and *Coprinus cinereus, Trans. Br. Mycol. Soc*., 68, 167–172, 1977.
28. Cheng, S. and Mok, S.H., Preliminary experiment of water hyacinth used as a medium for the cultivation of padi straw mushroom, *J. Hortic. Soc. China* (Taiwan) [in Chinese with English summary], 17, 194–197, 1971.
29. Chiu, S.W. and Chang, S.T., *Volvariella bombycina* and its life history, *Mushroom J. Trop*., 7, 1–12, 1987.
30. Cho, K.Y., Yung, K.H., and Chang, S.T., Preservation of cultivated mushrooms, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 63–86, 1982.
31. Chua, S.E., Cultivation of straw mushroom (*Volvariella volvacea*) using different substrates in multi-tiered racks, *Mushroom Sci*., 9(1), 701–706, 1976.
32. Chua, S.E. and Ho, S.Y., Fruiting on sterile agar and cultivation of straw mushroom (*Volvariella* species) on padi straw, banana leaves and sawdust, *World Crops* (London), 25, 90–91, 1973.
33. Elliott, T.J. and Challen, M.P., The breeding system of the silver-silk straw mushroom, *Volvariella bombycina, Mushroom Newsl. Trop.*, 6(1), 3–8, 1985.
34. Graham, K.M., Studies on the padi mushroom (*Volvariella volvacea*). V. Performance of single-basidiospore isolates, *Malay. Agric. Res*., 5, 31–36, 1975.
35. Graham, K.M. and Yong, Y.C., Studies on the padi mushroom (*Volvariella volvacea*). II. Effect of bed depth on yield in oil palm pericarp waste, *Malay. Agric. Res*., 3, 1–6, 1974.
36. Ho, K.Y., Indoor cultivation of straw mushroom in Hong Kong, *Mushroom Newsl. Trop*., 6(2), 4–9, 1985.
37. Hu, K.J., Song, S.F., and Liu, P., Experiments on Chinese mushroom cultivation, I. The comparison of cultivating materials, *J. Taiwan Agric. Res*., 22, 145–148, 1973.
38. Hu, K.J., Song, S.F., and Liu, P., The comparison of composts made of different raw materials for *Volvariella volvacea, Mushroom Sci*., 9(1), 687–690, 1976.
39. Hu, K.J., Song, S.F., Liu, P., and Peng, J.T., Studies on sugarcane rubbish for Chinese mushroom culture and its growth factor, *Mushroom Sci*., 9(1), 691–700, 1976.
40. Jalavicharana, K., Culture of mushroom in Thailand, *Dept. Agric. Bull*., 1, 1950.
41. Kwan, H.S. and Chang, S.T., Biological studies of cotton waste compost during the cultivation of *Volvariella volvacea, Mushroom Sci*., 11, 585–594, 1981.

42. Levine, M., The origin and development of lamellae in *Agaricus campestris* and in certain species of *Coprinus, Am. J. Bot*., 9, 509–533, 1922.
43. Li, S.F., Morphology of *Volvariella volvacea*, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 119–137, 1982.
44. Li, S.F. and Chang, S.T., Variation in the homothallic basidiomycete, *Volvariella volvacea, Mushroom Sci*., 10(1), 171–184, 1979.
45. Lim, G., Tee, L.L., and Avadhani, P.N., Cultivating padi-straw mushrooms on industrial wastes, *Philipp. Agric*., 65, 209–214, 1982.
46. Lin, C.T. and Hu, G.S., Studies on mushroom nutrition. I. The chemical composition of rice straw compost, *Bull. Tai. Agric. Res. Inst*., 25, 1967.
47. Martinez-Carrera, D., Chang, S.T., and Mok, S.N., Cultivation of the edible mushroom *Volvariella volvacea* on three different composts in Hong Kong, *Rev. Mex. Mic*., 1, 227–238, 1985.
48. Moore, R.T. and McAlear, J.H., Fine structure of mycota. V. Lomasomes — previously uncharacterized hyphal structures, *Mycologia*, 53, 194–200, 1961.
49. Naidu, N.R., Cultivation of padi straw mushroom *Volvariella volvacea* (Fr.) Sing. using soil palm bunch waste as a medium, *Planter*, 47, 190–193, 1971.
50. Ofosu-Asiedu, A., Schmidt, O., and Liese, W., Growth studies with isolates of *Volvariella volvacea* for cultivation on wood waste, *Mater. U. Organismen*, 19, 241–251, 1984.
51. Quaye, J.R., Evidence for haploidy of the basidiospores of *Volvariella volvacea* from gamma radiation studies, *Mushroom J. Trop*., 7, 39–44, 1987.
52. Raper, C.A., Sexuality and breeding, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 83–117, 1978.
53. Royse, D.J., Jodon, M.H., Antoun, G.G., and May, B.P., Confirmation of intraspecific crossing and single and joint segregation of biochemical loci of *Volvariella volvacea, Exp. Mycol.*, 11, 11–18, 1987.
54. San Antonio, J.P. and Fordyce, C., Jr., Cultivation of the paddy straw mushroom, *Volvariella volvacea* (Bull. Ex Fr.) Sing., *HortScience*, 7, 461–464, 1972.
55. San Antonio, J.P., Hepperly, P.R., and Shaffer, R.L., Straw mushroom in the Western Hemisphere, *Mushroom Newsl. Trop*., 4(4), 3–5, 1984.
56. Schmidt, O., Experiments with mushroom cultivation on wood waste, *Plant Res. Dev.*, 24, 85–92, 1986.
57. Shaffer, R.L., *Volvariella* in North America, *Mycologia*, 49, 545–579, 1957.
58. Singer, R., *Mushroom and Truffles: Botany, Cultivation and Utilization*, Leonard Hill, London, 1961.
59. Singer, R., *The Agaricales in Modern Taxonomy*, J. Cramer, Vaduz, West Germany, 1975.
60. Snell, W.H. and Dick, E.A., *A Glossary of Mycology*, rev. ed., Harvard University Press, Cambridge, MA, 1971.
61. Sukara, E., Hendrarto, I.B., and Dickinson, C.H., The cultivation of the paddy straw mushroom, *Bull. Br. Mycol. Soc*., 19(2), 129–132, 1985.
62. Tanaka, K. and Chang, S.T., Cytoplasmic vesicles in the growing hyphae of the Basidiomycete, *Volvariella volvacea, J. Gen. Appl. Microbiol*., 18, 165–178, 1972.
63. Tewari, R.P., Paddy (rice) straw mushroom (*Volvariella diplasia*) cultivation in a moderate climate, *Mushroom J*., 150, 213–215, 1985.
64. Tzeng, D.S., Studies on nutritional requirements and the improvement of techniques in cultivation of straw mushroom *Volvariella volvariella* (Bull. Ex Fr.) Sing., M.Sci. thesis, National Chung Hsing University, Taichung, Taiwan, 1974.
65. Ullrich, R.C. and Anderson, J.R., Sex and diploidy in *Armillaria mellea, Exp. Mycol*., 2, 119–129, 1978.
66. Yau, C.K. and Chang, S.T., Cotton waste for indoor cultivation of straw mushroom, *World Crops*, 24, 302–303, 1972.
67. Yong, Y.C. and Graham, K.M., Studies on the padi mushroom (*Volvariella volvacea*). I. Use of oil palm pericarp waste as an alternative substrate, *Malay. Agric. Res*., 2, 15–22, 1973.

15 *Flammulina* and *Pholiota* — Low-Temperature Cultivated Mushrooms

I. INTRODUCTION

In the previous chapter we examined the straw mushroom *Volvariella volvacea*, which fruits at relatively high temperatures and thus is commonly cultivated in subtropical and tropical regions. In this chapter we consider two mushrooms, *Flammulina velutipes* (Curt.: Fr.) Sing. and *Pholiota nameko* (T. Ito) S. Ito et Imai, which have low-temperature requirements for fruiting and thus are commonly cultivated in regions of temperate climate.[13] Both mushrooms have their greatest popularity in Asian countries, especially China, Japan, and South Korea, and those are their principal places of production.

Flammulina was fifth in total worldwide production of edible mushrooms in 1997; in the same year, *Pholiota* ranked ninth.[5] Production of these mushrooms continues to increase, particularly *Flammulina* in China where its production increased rapidly from 15,000 MT in 1997 to 389,204 MT in 2002, a 26-fold increase. Production of *Pholiota* in China also increased rapidly although it is unlikely that the anticipated spurt in production for *Flammulina* will occur for *Pholiota.* Its production in 1997 was only 3100 MT and in 2002 increased to 51,516 MT, a 16.6-fold increase. These mushrooms also increased steadily in Japan, with a production for *Flammulina* of 109,324 MT in 1997 and 113,713 MT in 1999, and an increase from 24,522 MT in 1997 to 25,771 MT in 1999 for *Pholiota.*[18] Although *Flammulina* and *Pholiota* can only be fruited in low-temperature conditions, these mushrooms can be massively produced year-round under controlled low-temperature conditions or by rotating crops geographically to places where the temperature will be suitable. If these mushrooms become more popular in Europe and America, it is possible that there will be a demand for increased production.

Flammulina has greater popularity than *Pholiota*, and because of its taste, texture, and pleasing appearance it is readily accepted in Western countries. It is likely that in the developed countries, where the cost involved in providing temperature control of the mushroom houses is a less restrictive factor than it is in developing countries, there will be increased production of *Flammulina*. These mushrooms are mainly cultivated for gourmet and tonic purposes.

II. BIOLOGICAL CHARACTERISTICS OF *FLAMMULINA*

Flammulina velutipes (Curt.: Fr.) Sing. is a member of the family Tricholomataceae. It bears the common names of winter mushroom or velvet stem and is known in Japan as enokitake (Figure 15.1 and Figure 15.2).

FIGURE 15.1 Fruiting bodies of *Flammulina velutipes* grown on sawdust substrate in bottles. The cone-shaped or almost cylindrically shaped plastic sheets are placed around the mouths of the bottles to support the elongation of the fruiting bodies.

A. Morphology

The pileus of *Flammulina velutipes* is 2 to 10 cm on mushrooms growing in nature, but under cultivation techniques it is small and more commonly 2 to 3 cm. Initially the pileus, or cap, is hemispherical in shape, but with growth it opens to a plane. The surface of the cap is orange-red, yellow tinged at the edges and darker in the center. The gills are white or pale yellow and slightly adnexed. The stipe is 5 to 10 × 0.4 to 0.8 cm in nature and 2 to 9 × 0.2 to 0.8 cm in cultivation and is slightly tapered toward the base. The basidiospores are white, elliptical, smooth, and 7 to 10 × 3 to 5 μm in size. The range of sizes and colors varies in the fruiting bodies depending on the conditions in nature, and there are differences between fruiting bodies produced in nature and by the conditions of artificial cultivation.

The hyphae of the monosporous mycelium of *F. velutipes* have septa and are about 2.1 to 3.2 μm in diameter. The hyphae of the dikaryotic mycelium have clamp connections with branches commonly forming just below the clamps and less commonly above the clamp.[10] Both monokaryotic and dikaryotic hyphae of *F. velutipes* produce uninucleate oidia.[4]

B. Natural History

The life cycle is that of a typical basidiomycete with a few modifications. The formation of haploid or monokaryotic fruiting bodies is frequently reported,[16] and dedikaryotization is accomplished by the formation of uninucleate oidia on dikaryotic hyphae.[4,15] *Flammulina velutipes* is tetrapolar; i.e., it displays bifactorial heterothallism and has frequently been used for experimental studies of the details of the genetic control of sexuality.

Flammulina velutipes is worldwide in distribution, growing on a variety of broad-leaved trees. It is frequently found on the trunks and stumps of elms from late autumn to early spring, hence the name winter mushroom. The capacity of fruiting bodies of *F. velutipes* to withstand drying and freezing has been studied.[11] It was found that if a fruiting body is floated on water after it has ceased to discharge spores and become dry, it will rehydrate and then discharge spores again. This procedure was repeated, and again spores were discharged. Almost daily observations were made of fruiting bodies of *F. velutipes* growing on elms killed by Dutch elm disease during an extremely cold winter in England when night frosts sometimes reached −12°C.[11] It was possible on any day to collect fruiting bodies that would discharge spores, but since individual fruiting bodies remained

FIGURE 15.2 (Top) Cultivation of *Flammulina velutipes* on sawdust media in plastic bags. Note the elongated stipes resulting from the extension of the plastic bags above the substrate surface. (Bottom) "Bouquets" of *F. velutipes* held (from left to right) by Prof. D.J. Lu, Prof. X.M. Yang, and Prof. S.T. Chang. Also in the photograph are Prof. S.I. Lu and Mr. N.L. Huang.

active for only 1 to 2 weeks, it was concluded that the regularity of spore discharge was due to the maturation of new fruiting bodies and not the survival of ones that had matured much earlier.

C. Requirements for Mycelial Growth

Because *F. velutipes* has been used in many experimental studies of a basic or fundamental nature, it is not surprising that there is a good deal of information about its nutritional needs. The requirements are similar to those of many wood-rotting Basidiomycetes: a nitrogen source of either amino acid or the ammonium ion; a carbon source, which is satisfied by a variety of monosaccharides, disaccharides, or polysaccharides; mineral salts (magnesium sulfate, potassium phosphate, and ferrous sulfate); trace elements; and thiamine (vitamin B_1).

Mycelial growth occurs as follows: (1) over a range of pH from 4.5 to 8.6, (2) in a range of temperature from below 0°C to 33 to 34°C with the optimal temperature around 25°C, and (3) optimally with a substrate moisture concentration of 60 to 65%. The growth at 0°C is slow, but significant.[11] It is interesting to note that the mycelium does not die at such low temperatures but is killed after a short period at 34°C.

D. Requirements for Fruiting Body Formation

Fruiting body formation of *F. velutipes* will occur on chemically defined liquid media. The nutritional requirements are essentially the same as those for mycelial growth. The temperature that is required for formation of primordia is in the range of 10 to 20°C with the optimum at 15°C. At temperatures outside the optimal range, a longer time is required for fruiting.

Light is frequently a factor that triggers the formation of primordia of fruiting bodies, but this is not the case in *F. velutipes* in that primordia will form in cultures that have been kept in the dark. Many morphogenetic studies of the fruiting process have been made with *F. velutipes* and, although primordia and stipes form in the dark, the formation and expansion of pilei require light.

With an increasing concentration of CO_2, as can occur with mushrooms grown in a confined area, resulting in the accumulation of metabolic CO_2, it has been found that pileus expansion is depressed, and with a 5% CO_2 concentration the pileus does not develop beyond a tiny rudiment at the tip of the stipe.[14]

Among the other morphogenetic studies of fruiting body formation of *F. velutipes* are those demonstrating a rotation of the stipe during growth and a hormonal effect of a substance produced in the gills upon the elongation of the stipe.[6–8]

These basic studies are of great value in understanding growth and the fruiting process for the development of cultivation methods.

III. BIOLOGICAL CHARACTERISTICS OF *PHOLIOTA*

Pholiota nameko (T. Ito) S. Ito et Imai is a member of the family Strophariaceae. It bears the Japanese name *nameko* and is sometimes referred to as the "viscid mushroom" because of a glutinous substance on the cap that makes it sticky to the touch. The mushrooms have a good flavor and this, together with their viscous nature, makes them popular in many Japanese dishes, including miso soup (Figure 15.3).

A. Morphology

The pileus of *P. nameko* is from 2 to 15 cm in diameter, depending largely on the nutritional and environmental conditions in which the mushroom has developed. The cap is smooth and shiny in appearance because of a viscous substance that covers it. It is brown at the center, becoming lighter toward the margin. The gills are of different lengths and differ in their degree of attachment to the

FIGURE 15.3 Fruiting bodies of *Pholiota nameko.*

stipe. Gills are initially white or yellow and become brown when mature. The hymenial layer of the gill of *P. nameko* has two types of cells, the basidia and the cystidia, which are present in a palisade-like arrangement at right angles to the long axis of the gill. The hyphal cells of the trama of the gill are long and arranged parallel to the long axis of the gill.[2]

The mature basidiospores of *P. nameko* are more frequently binucleate than uninucleate as a result of a postmeiotic mitosis that occurs in either the basidium or in the basidiospores. The basidia are four spored. The basidiospores are 4 to 6 × 2.5 to 3 μm. No germ pore structure was revealed by the electron microscope[2] although in germination the germ tube is always located at the apical part of the spore. Viewed microscopically, the spores are reddish brown and the color of a spore print is dull brown.

The stipe is attached centrally to the lower side of the cap. It varies in length and is usually 2.9 to 7.0 cm but may sometimes be almost three times that length. The diameter of the stipe is 0.3 to 1.8 cm and is almost completely solid. An annulus is present.

The germ tube also commonly branches before septation after the hypha formed from the germ tube has become fairly long. These hyphae are homokaryotic, having originated from a haploid basidiospore (a meiospore), but the hyphal cells contain different numbers of nuclei. The number of nuclei in the terminal cell of a hypha ranges from 1 to 50. The intercalary cells usually have few nuclei (one to four).

Pholiota nameko is a bipolar heterothallic species, which has multiple alleles at the single mating type locus.[3] Thus, a confrontation between two homokaryotic mycelia with different alleles at the *A* mating type locus will lead, following hyphal fusion and nuclear migration, to the formation of dikaryotic hyphae. The dikaryotic hyphae have clamp connections.

Both homokaryotic and dikaryotic hyphae produce asexual spores. Arthroconidia (oidia are examples of this type) are formed by fragmentation of a conidiophore. Both homokaryotic and dikaryotic hyphae form arthroconidia. They may contain one or two nuclei, which are alike in the case of homokaryotic hyphae, but in the conidia from dikaryotic hyphae the two nuclei may be a dikaryon (compatible nuclei) or they may be homokaryotic.

Another type of conidium produced by both homokaryotic and dikaryotic hyphae is the aleuroconidium. Such conidia are usually thick walled and arise singly as the blown-out end of the tip of a conidiophore or hyphal branch. They are hyaline and cylindrical in shape. The nuclear composition of aleuroconidia produced on homokaryotic hyphae is monokaryotic, but that of conidia produced on dikaryotic hyphae may be dikaryotic if both nuclei move into the primordium of the conidium, or monokaryotic if only one member of the dikaryotic pair enters into the developing conidium. Germination of conidia is, in general, similar to that of basidiospores, but dikaryotic conidia give rise to dikaryotic hyphae when they germinate.

B. Natural History

Sexuality in *P. nameko* is heterothallic with unifactorial control, i.e., it is bipolar. In addition to the sexual cycle in which there is the formation of a dikaryon when compatible mycelia (mycelia with different alleles at the *A* mating type locus) are brought together, there are five different asexual cycles.[1]

1. The conidia formed on homokaryotic mycelium may form haploid or homokaryotic fruiting bodies, which produce basidiospores that develop into homokaryotic mycelia.
2. Conidia formed on homokaryotic mycelia give rise to homokaryotic mycelia.
3. Dedikaryotization occurring when homokaryotic conidia are produced from dikaryotic mycelium results in homokaryotic mycelium.
4. Dikaryotic conidia formed from dikaryotic mycelium germinate to form dikaryotic mycelium with clamp connection.
5. Dikaryotic mycelium may undergo dedikaryotization without conidia formation and form the component homokaryons of the original dikaryon.

Like *Flammulina velutipes, P. nameko* grows in nature on the dead trunks or stumps of deciduous trees. This species is found only in Japan and in Taiwan at high elevations, but other species of *Pholiota* are more widely distributed. Studies of *P. nameko* distribution in Japan indicate a close correlation with the distribution of the hardwood *Fagus crenata* in Japan, especially in districts along the Japan Sea. The rainy season in this area coincides with the fruiting season for *P. nameko* in nature, and it is known that this mushroom requires more moisture for fruiting than most other mushrooms.

Pholiota nameko differs from most other Hymenomycetes in the frequency of homokaryotic fruiting.[2] In one experiment, almost all 70 monosporous mycelia from two dikaryotic stocks produced fruiting bodies on a sawdust–rice–bran medium. These homokaryotic fruiting bodies were generally smaller than dikaryotic fruiting bodies, and sometimes they were not completely developed. Spores obtained from these haploid fruiting bodies germinated well, and all from the same fruiting body were of a single mating type. It is important to note that homokaryotic fruiting bodies are not encountered in nature. Over a 15-year period, 200 isolates from different localities were invariably dikaryotic.[2]

C. Requirements for Mycelial Growth

Pholiota nameko does not have unusual nutritional requirements for mycelial growth. It is reported that the optimal glucose concentration is 3%, that nitrate is not satisfactory as a nitrogen source, but the ammonium ion and organic nitrogen are suitable (placing *P. nameko* in Class 3 according to the Robbins scheme of nitrogen utilization). Yeast extract doubled the growth of mycelium, indicating a probable requirement for a vitamin. Many of the nutritional studies have been concerned with growth on natural substrates, such as the sawdust of various woods, with the goal of identifying substrates for use in commercial cultivation. In this connection, an interesting aspect is that the mycelia of *P. nameko* can grow well on the sawdust of conifers although it is isolated in nature from broad-leaved trees.[1]

Because of the common occurrence of dedikaryotization, it is difficult to measure growth of strictly dikaryotic mycelium. That is, during the course of an experiment, the growth of the culture will frequently include both homokaryotic and dikaryotic mycelia, which has the effect of giving variable results and differences in the cardinal points reported by various investigators. For mycelial growth, a minimum temperature less than 8°C, an optimum in the range 24 to 26°C, and a maximum of 32°C were obtained for dikaryotic stocks examined carefully during the experiment to ascertain freedom from dedikaryotization.[1]

The optimal pH for mycelial growth varies greatly with the type of medium and conditions of growth, but in general the various experiments indicate an initial pH on the acid side of neutrality is best, and an initial pH in the range of 4.5 to 6.0 comes close to the optimal values reported for many media and conditions.

D. Requirements for Fruiting Body Formation

Because fruiting bodies of *P. nameko* can be obtained when the dikaryotic mycelium is cultured on some artificial media, there is information available about the nutritional requirements of this mushroom for fruiting. Maltose produced larger fruiting bodies than glucose but required a longer time to do so. Sucrose gave good mycelial growth, but no fruiting occurred when it was used as the sole carbon source.[1] This would seem to indicate the desirability of having more-detailed studies on the interrelationship of the metabolism of these substrates with growth factor requirements, trace elements, and conditions of culture for a more complete understanding of the fruiting process in *P. nameko*, which might have commercial significance. The nitrogen source had a significant effect on fruiting with highest yield on a medium containing L-aspartic acid, and slower and malformed fruiting bodies on media containing ammonium tartrate as the nitrogen source.[1]

It was previously mentioned that in a study of a large number of stocks of *P. nameko*, fruiting separated into two temperature groups — high temperature (8 to 20°C) and low temperature (5 to 15°C). Fruiting at temperatures above 15°C results in mushrooms with small caps and slender stipes, whereas fruiting at low temperatures (less than 8°C) gives larger fruiting bodies, but the cap is late in opening. Although there are these temperature relationships with fruiting, a fluctuation in temperature is not necessary to trigger the initiation of primordia.

There is a close relationship of moisture with the fruiting of *P. nameko*. A moisture-loving fungus, *P. nameko* requires more moisture for fruiting than most other cultivated mushrooms. The areas in which it is found in nature in Japan and the season in which it fruits are related to this moisture requirement.

Light is necessary for normal fruiting. In the dark, the stipes are long and the caps are thin and not well developed. The development of the cap is also hindered in conditions of poor aeration with an elevated CO_2 level.

IV. CULTIVATION METHODS

A. *Flammulina*

Cultivation of *Flammulina velutipes* has an interesting history that has been described by Tonomura.[17] Initially, cultivation was by wood logs, but now cultivation on sawdust is the method employed in Japan, Taiwan, and China. The method of cultivation using sawdust was begun around 1928, and Hasagawa, a junior high school teacher, established a method of cultivation of *F. velutipes* on sawdust medium in bottles as a demonstration for his students of principles of fungal biology. Demonstration of this culture method to mushroom growers soon led to its use in cultivation and to his obtaining a patent on the process. In the mid-1960s the cultivation process was mechanized, air conditioning was used to regulate the temperature, and varieties were selected for this type of cultivation on sawdust substrate in polypropylene bottles. With air conditioning, cultivation can take place throughout the year. In the temperate regions the necessary low temperature for fruiting is reached in winter, but heating is required in the cultivation rooms in this season for mycelial running.

Hardwood sawdust is superior to the sawdust of most conifers for mycelial growth and the initiation of fruiting body primordia, but mixtures of the two are very satisfactory. It is a practice in Japan to use sawdust from *Cryptomeria, Chamaecyparis*, and *Pinus*, after it has been seasoned for a year or so to soften it, because this sawdust will absorb and hold much water, which is essential for mushroom development. In addition to the sawdust (80%), rice bran (20%) is incorporated in the medium. The rice bran provides vitamins such as thiamine, which is known to be required by *F. velutipes*. The ingredients are thoroughly mixed, water is added to achieve a moisture content of 58 to 60%, and the medium is mixed again. Polypropylene bottles of 800 ml size are mechanically filled with the substrate mixture, which will contain approximately 540 g of substrate per bottle. The bottles are then capped and sterilized in an autoclave. Sterilization is accomplished by autoclaving at low pressure for 4 hours at a temperature of 95°C or at high pressure for 1 hour at 120°C.

After the sterilized bottles have cooled to 20°C, they are inoculated with sawdust spawn. The sawdust spawn substrate consists of ten parts of sawdust mixed with one part of rice bran and a suitable amount of water. One bottle of spawn (1 liter) contains sufficient spawn to inoculate 50 to 60 polypropylene bottles of substrate for cultivation of *F. velutipes* mushrooms.

The inoculated bottles are placed in an incubation room at 18 to 20°C for mycelial running. This is slightly below the optimal temperature of 25°C for mycelial growth, but it is used for economic considerations.

After 20 to 25 days, when the mycelium covers 90% of the bottle, the cap is removed, the spawn inoculum is lifted off, and the surface of the substrate is raked smooth for uniform fruiting.

The bottles are placed in the dark at 10 to 12°C at a humidity of 80 to 85% to stimulate formation of fruiting body primordia. Primordia form 10 to 14 days after the bottles are placed at 10 to 20°C. The formation of good fruiting bodies requires careful moisture control.

Rapid growth of the fruiting bodies occurs at 10 to 12°C, but the stipes are long and slender. Therefore, the temperature is lowered to 3 to 5°C. At this temperature and with air currents the fruiting bodies formed are stiff, white, and drier. This treatment is continued for 5 to 7 days — from the first appearance of the cap until the stipe is approximately 2 cm in length.

At this time (stipe approximately 2 cm long), the bottles are placed at a temperature of 5 to 8°C, and the humidity is raised to 75 to 80% — conditions suitable for fruiting body growth. When the stipes have elongated to within about 2 to 3 cm from the mouth of the bottle, a thick waxed paper or plastic sheet is placed around the mouth to form a cone-shaped or almost cylindrically shaped support for the fruiting bodies as they emerge. In Fujian Province, China, a slightly different technique is used to accomplish the same purpose. The substrate is contained in cylindrically shaped plastic bags. When the bags have been opened and the stipes start to elongate, the top of the bag, which has been folded down, is pulled out to form a support for the developing mushrooms.

The purpose of the paper or plastic extension from the culture bottle is to keep the mushroom from bending over, resulting in a more uniform growth of the fruiting bodies. This supporting structure also reduces the supply of oxygen around the fungus and increases the concentration of carbon dioxide. The consequence of this type of gaseous composition is an inhibition of the development of the pilei and a promotion of the elongation of the stipes. It has also been reported[9] that the degree of permeability of the cylinder to gases affects the uniformity, elongation, quality, and yield of the mushroom, depending on the season and the amount of moisture. More permeable paper is used during the rainy season and less permeable during the dry season, but, generally speaking, a wax paper cylinder is most commonly used.

The time of attaching the paper extension is very important. If the cylinders are placed too early, the oxygen within the cylinder is insufficient and the stipes become very weak, lack uniformity, and the yield of mushrooms is low. Also, when the moisture content of the mushroom is too high, the mushrooms cannot be stored for very long, and this lowers the price. It is recommended that the pilei and stipes be white and dry before covering with the cylinder, which limits evaporation of water from the fruiting bodies. To establish this condition, the mushrooms should not be watered just prior to covering, and the relative humidity of the room should be reduced to 70 to 80%, either by increasing the bed temperature with electric soil heaters or by use of fans to blow away the extra moisture in the room. The fan should not be left on too long, however, because this will stop the growth of the mushrooms. Turning on the fans 2 to 3 days before harvesting is recommended.

The supporting paper or plastic is removed when the fruiting bodies are around 13 to 14 cm long. The fruiting bodies are now pulled from the bottle and packed. A second flush can be obtained, but it is lower in yield and quality. The total yield for the crop by this technique is in the range of 160 to 220 g per 800-ml bottle. This mushroom is marketed both fresh and canned.

B. *Pholiota*

The history of cultivation of *Pholiota nameko* is similar to that of many other wood-rotting edible mushrooms. In 1921 in Japan, cut wood logs were used. Logs, upon which *Pholiota* had grown, were soaked in water, and this water was then spread on the cut wood logs. Sawdust spawn was first used in 1931, and after 1960 sawdust plus wheat bran was used for the commercial cultivation of *P. nameko*. Most fungi cannot use freshly cut wood as effectively as wood that has been cut for a sufficient length of time for the cells to be dead, but *Pholiota* can utilize wood containing live cells. For this reason, some have considered *P. nameko* to be a semiparasitic fungus.[12]

Pholiota nameko is commercially produced on sawdust. In general respects, its cultivation is similar to the methods used for *Flammulina velutipes*. In accord with the description for fruiting requirements of *P. nameko* given in Section III.C, cultivation management requires special attention

to (1) moisture concentration in the beds, (2) the species of wood from which the sawdust is made and the inclusion of rice bran, and (3) the temperature. Temperature management for *P. nameko* involves fewer changes than that for *F. velutipes*, as temperature fluctuation is not required for primordia formation by *P. nameko*. Light must be available to avoid the formation of abnormal fruiting bodies, but there are no stringent requirements regarding intensity or wavelength that require special attention. Aeration is also important, as it is with all cultivated mushrooms, to avoid accumulation of CO_2 and the attendant inhibition of mushroom development and to provide the necessary oxygen.

REFERENCES

1. Arita, I., *Pholiota nameko*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 475–496, 1978.
2. Arita, I., Cytological studies on *Pholiota, Rep. Tottori Mycol. Inst*. (Japan), 17, 1–118, 1979.
3. Arita, I. and Takemaru, T., Some problems in the mating system of *Pholiota nameko* (T. Ito) S. Ito et Imai [Japanese with English summary], *Rep. Tottori Mycol. Inst*. (Japan), 2, 1–10, 1962.
4. Brodie, H.J., The occurrence and function of oidia in the Hymenomycetes, *Am. J. Bot*., 23, 310–327, 1936.
5. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China, *Int. J. Med. Mushroom*, 1, 291–300, 1999.
6. Gruen, H.E., Growth and rotation of *Flammulina velutipes* fruit bodies and the dependence of stipe elongation on the cap, *Mycologia*, 61, 149–166, 1969.
7. Gruen, H.E., Promotion of stipe elongation in *Flammulina velutipes* by a diffusate from excised lamellae supplied with nutrients, *Can. J. Bot*., 54, 1306–1315, 1976.
8. Gruen, H.E. and Wu, S., Promotion of stipe elongation in isolated *Flammulina velutipes* fruit bodies by carbohydrates, natural extracts, and amino-acids, *Can. J. Bot*., 50, 803–818, 1972.
9. Huang, N.W., *Cultivation of Edible Mushrooms,* translation, Fujian Training Center of Edible Fungi, Fujian, China, 1984.
10. Ingold, C.T., Mycelium, oidia and sporophore initials in *Flammulina velutipes, Trans. Br. Mycol. Soc*., 75(1), 107–116, 1980.
11. Ingold, C.T., *Flammulina velutipes* in relation to drying and freezing, *Trans. Br. Mycol. Soc*., 76(1), 150–152, 1981.
12. Jiang, H.C., New technique for high yield production of *Tremmella* by bag method, *Edible Fungi China*, 6, 39–40, 1985.
13. Lou, L.H., Chu, H.C., and Chu, P.W., *Biology and Cultivation Technique of Edible Fungi,* China Forestry Press, Beijing, 1983.
14. Plunkett, B.E., The influence of factors of the aeration complex and light upon fruit body form in pure culture of an agaric and a polypore, *Ann. Bot*., 20, 563–586, 1956.
15. Takemaru, T., Genetics of *Collybia velutipes*. II. Dedikaryotization and its genetical implication, *Jpn. J. Genet.*, 29, 1–7, 1954.
16. Takemaru, T., Genetical studies on fungi. X. The mating system in Hymenomycetes and its genetical mechanism, *Biol. J. Okayama Univ*., 7, 133–211, 1961.
17. Tonomura, H., *Flammulina velutipes,* in *Edible Mushrooms and Their Cultivation*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 409–421, 1978.
18. Yamanaka, K., Production of cultivated mushrooms, *Food Rev. Int.*, 13, 327–333, 1997.

16 *Pleurotus* — A Mushroom of Broad Adaptability

I. INTRODUCTION

The *Pleurotus* mushroom is generally called the oyster mushroom because the pileus or cap is shell-like, spatulate, and the stipe eccentric or lateral. The species of *Pleurotus ostreatos* (Jacq: Fr.) Kummer has been used as food or for medicinal purposes for a long time, and at present plays an important role as a commercial edible mushroom. More than 1000 species of the oyster mushroom have been described throughout the world, in more than 25 related genera. However, only approximately 50 valid species are recognized in the genus *Pleurotus*.[13] *Pleurotus ostreatus* (Jacq.: Fr.) Kummer is one of the best known among the oyster mushrooms. Other commonly cultivated species include: *P. sajor-caju* (Fries) Sing. (gray oyster mushroom or phoenix-tail mushroom), *P. cystidiosus* O.K. Miller (abalone mushroom), *P. ostreatus* var. *florida nom. prov.* Eger (white oyster mushroom), *P. citrinopileatus* Sing. (golden oyster-mushroom), *P. flabellatus* (Berk. and Br.) Sacc (pink oyster mushroom), and *P. sapidus* (Schulzer) Kalchbremer (black oyster mushroom). They belong to the family Tricholomataceae, order Agaricales, and class Basidiomycetes. They grow naturally mainly in the temperate zones or in the cooler seasons in subtropical areas on rotting trees such as oak, elm, maple, bass, poplar, holly, and laburnum. They have a fragrant odor and delicious flavor. However, *Pleurotus* has been reported as parasitic on several trees.[9,27] Species of *Pleurotus,* such as *P. cornucopiae* (Paul.: Pers.) Roll., *P. cystidiosus, P. ostreatus*, *P. tuberregium* (Fr.) Sing., are known to attack and consume living nematodes, through special structures named microdroplets, as reported by Barron and Thorn[2] and Hibbett and Thorn.[15]

As *Pleurotus* species are comparatively easy to grow and of broad adaptability, they are cultivated worldwide and their production has increased rapidly in recent years.[17] It was the third most popular cultivated edible mushrooms in 1997[4] after *Agaricus bisporus* and *Lentinula edodes*. Although the 2002 world production figures of *Pleurotus* have not been available, *Pleurotus* production in China was estimated to be 2,594,000 MT in 2002 leading all cultivated edible mushrooms. The second and third mushrooms produced in greatest amount in 2002 in China were *Lentinula,* 2,072,000 MT, and *Agaricus*, 743,000 MT. In addition to the conventionally cultivated species*, P. ostreatus* and *P. sajor-caju* and others mentioned above, there are several new species of *Pleurotus* that have been very well established and developed in recent years in China, such as *P. eryngii* (DC.: Fr.) Quel., *P. ferulae* Lenzi, *P. eryngii* (DC.: Fr.) Quel. var. *nebrodensis* Inzenga, *P. citinopileatus* Sing., *P. djamor* (Fr.) Boedjin *sensu* Lato, and *P. tuberregium* (Fr.) Sing.[16] Methods of cultivation have now been worked out and the techniques for growing them are simple and inexpensive. A wide range of plant wastes, such as sawdust, paddy straw, bagasse, cornstalks, waste cotton, stalks and leaves of bananas, can all be used for *Pleurotus* mushroom production without a requirement for costly processing methods and enrichment materials. It has frequently been suggested that edible mushrooms, because of their high protein content, should be produced in greater amounts to alleviate protein deficiencies in places where the prospect of starvation is a life-threatening problem. Recently, it was suggested that the most important of the edible mushrooms that could be used in this way are species of *Pleurotus* and *Stropharia*. Some species of *Pleurotus*

FIGURE 16.1 The two mushrooms have well-developed gills and show the eccentric orientation of the stalks.

produce very high yields. In a matter of a few weeks these fungi can convert 100 g of dry waste plant materials into 50 to 70 g of fresh *Pleurotus* mushrooms.[22]

The genus *Pleurotus* is now widely consumed as food worldwide. One reason is that oyster mushrooms are by far the easiest and least expensive to grow of all industrially cultivated edible mushrooms. Another reason is that there is a wide choice of species available for cultivation under different climatic conditions. There can be year-round production of the mushrooms using different species or varieties in different seasons. Therefore, *Pleurotus* will no doubt continue to make a global impact on the mushroom industry as well as on human welfare.

However, *Pleurotus* has some adverse features for which improvements should be obtained: (1) some fruiting bodies are rather brittle, making it difficult for them to be packaged and transported to market without damage; (2) they discharge spores in tremendous numbers during the process of cultivation, and this frequently causes allergies and respiratory problems to workers in the mushroom houses, as mentioned in Chapter 3; and (3) in some countries there is a problem convincing the population to accept the mushroom.

To overcome these drawbacks, research is needed on the improvement of the quality and breeding of sporeless mutant varieties as well as nutritional education programs to further expand this group of mushrooms in the normal diet of people all over the world.

II. BIOLOGICAL CHARACTERISTICS

A. Morphology of Sporophores

The oyster mushrooms are soft and are variously colored, including dark blue, white, cream-to-brown, yellow, and pink. The pileus is usually shell and tongue shaped or, in the case of older stages, depressed. The color intensity may alter according to changes in environmental factors, e.g., light and temperature. In general, the color will be darker in conditions of more light and cold weather, and the color will be lighter in weak light and hot weather. The size can vary from 5 to 30 cm in diameter. The mushroom is generally smaller on wood and larger on straw cotton waste substrates. The gills are whitish or gray. The stipe is usually eccentric or lateral (Figure 16.1), but the stipe of sporeless mushrooms is close to the center of the cap[6] (Figure 16.2).

B. Sexuality

On each basidium there are commonly four basidiospores. Occasionally, five or more have been observed. It has been reported that *P. ostreatus,*[8] *P. sajor-caju,*[29] and species related to *P. ostreatus*[26]

FIGURE 16.2 Fruiting bodies of a sporeless strain of *Pleurotus ostreatus* var. *florida* (right) showing their stalks close to the centers of the caps, compared with a spore-producing strain (left), which bears eccentric stalks.

are clearly heterothallic and tetrapolar. Differences between rates of growth and vigor of the various haploid strains were evident, and the possibility of developing high-fruiting and good-quality strains for the efficient production of sporophores is promising through a proper breeding program.

C. Requirements for Mycelial Growth

The carbon sources suitable for mycelial growth are starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose, and lignin. Ethanol is also a source of carbon for mycelial growth; however, citrate, oxalate, and other organic acids are not beneficial to the growth of the mycelium.[21]

The nitrogenous sources utilized by *Pleurotus* are peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulfate, asparagine, serine, alanine, and glycine. The utilization of urea is rather poor.

The optimal temperatures for growth of the mycelium are around 25 to 28°C, and the range of pH is about 5.5 to 6.5. The tolerance of mycelia for CO_2 is rather strong. The mycelia of *Pleurotus* spp. can still grow flourishingly at a CO_2 concentration of 15 to 20%.[31] Only when the concentration of CO_2 is raised to 30% does the growth of mycelia rapidly decrease.

D. Requirements for Fruiting Body Formation

Although the mycelium of *Pleurotus* can tolerate a high concentration of CO_2, the fruiting body of the oyster mushroom cannot endure high CO_2. When the CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06%), the stipe elongates, and the growth of the caps will be prevented.[21,32]

The requirements for light by the oyster mushroom are different for the various stages of growth. The growth of mycelium does not need any light, and cultivation of the oyster mushroom in a dark place is better than in a bright place. The formation of primordia and the growth of fruiting bodies does require light, however. The former requires light of 200 lux intensity for over 12 hours. The growth of the fruiting body requires light of 50 to 500 lux intensity.

The color of the caps is closely related to the intensity of light, and if it is low, then the color will be pale. When the golden oyster mushroom receives light of an intensity over 300 lux, the caps will be golden in color. If the intensity is below 100 lux, the color will be yellowish pale, and, when it is lower than 10 lux, the color will be nearly white.

The optimal temperatures for the development of fruiting bodies can range from 10 to 28°C, depending on the species, e.g., *P. ostreatus* var. *florida* and other *P. ostreatus* strains, 14 to 18°C; *P. sajor-caju*, 20 to 24°C; and *P. cystidiosus*, 26 to 28°C.

III. NUTRITIONAL VALUES AND MEDICINAL PROPERTIES

A. Nutritional Values

The protein content expressed as a percentage of dry weight is from 10 to 30% and even up to 40%, varying considerably among and within the species. *Pleurotus* contains all the essential amino acids, which comprise 40% of the total amino acid content. The lipid content is about 3 to 5% on a dry weight basis and is generally higher in the stalk than in the cap. Fresh mushrooms generally contain 3 to 28% carbohydrate and 3 to 32% fiber on a dry weight basis. *Pleurotus ostreatus* has an extremely high (57%) carbohydrate content and 14% crude fiber content, with a large proportion of 47% consisting of dietary fibers.[3]

B. Medicinal Properties

According to *Icons of Medicinal Fungi from China,*[30] the medicinal part of the oyster mushroom is in its fruiting body. According to diverse bibliographic sources, Guzman[13] summarized that *Pleurotu*s is used in traditional medicine to prevent or assist in more than 30 diseases or disorders.

Antitumor activity was found in the polysaccharide fractions of the fruiting bodies of almost all *Pleurotus* species.[12] These polysaccharides belong to (1→3)-β-D-glucans.[19] Different glucans from *Pleurotus* have been found to enhance the activity of natural killer cells and lymphokine-activated killer cells. In addition to modulating the immune system, *Pleurotus* spp. have hypoglycemic activity, antithrombotic effects, inhibit tumor growth, reduce inflammation, and lower blood pressure and plasma lipid concentration.[11] In addition, they have antioxidant activity.[1] The investigation of antioxidant activity of different extract fractions — acidic, phenolic, alkaline, neutral — showed that the highest activity level was present in the phenolic fraction. It was also demonstrated that the extracts of fruiting bodies had higher antioxidant activity than mycelium and cultured liquid extracts. Differences in antioxidant activity between samples of extracts may be related to different fatty acid composition of their lipids. Thus, the content of unsaturated fatty acids in fruiting body extracts was higher than in mycelium and cultured liquid extracts.

IV. CULTIVATION METHODS

A. Production of Spawn

The chief problem of the spawn manufacturers involves the isolation and maintenance of stable strains that will fulfill the expectation of the growers. A poor strain ultimately will be unsatisfactory no matter how ideal are the conditions in the spawn plant or in the growing house itself. There are certain principles guiding how to develop new strains and to make appropriate selections,[7,28] but they will not be discussed here. Many different types of materials, which may or may not be the same as the substrate used in cultivation, alone or in various combinations, can be used as substrate for spawn making. A few examples are rice straw cuttings, cotton waste, cotton seed hulls, rice hulls, sorghum grains, rye grains, etc. There has been a tendency to overemphasize the importance of the type of substrate the spawn is growing in rather than the particular strain itself. Actually, the substrate is merely the carrier or vehicle of the strain in a convenient medium that can be used to inoculate the beds. Nevertheless, the spawn substrate does influence the growth habit of the mycelium to some extent. Some spawns may grow (run) more quickly and the beds fill out more rapidly than with other spawns. In choosing a suitable spawn substrate, the cost and availability of

the raw material, as well as the growth of mycelium on it, should be considered. The details on the preparation of grain spawn and straw spawn are described here.

1. Grain Spawn

The grains (e.g., rye, sorghum, wheat) are cooked in water until they swell but do not burst. The excess water is then drained off. Lime (calcium carbonate 2% w/w) is mixed in, and the glass bottles or polypropylene plastic bags are filled loosely (3/4 full only) and plugged with cotton wool. The bottles and plastic bags with their contents are sterilized in an autoclave for 30 minutes at 121°C and then allowed to cool. The bottles are inoculated with the prepared pure culture and incubated at the proper temperature. When the mycelium has run over the whole surface as well as permeated through the substrate, the spawn is ready for use.

2. Straw Spawn

The straw (e.g., paddy rice straw, wheat straw) is cut into pieces approximately 5 cm long, which are soaked in water for 5 to 10 minutes. Lime (2% w/w) is mixed in, and the procedure described above is followed.[5]

The inoculation and incubation of spawn should be done under strictly aseptic conditions. The normal atmosphere is loaded with fungal spores and bacteria, which float around freely in the air currents. If even one such spore happens to enter a bottle of fresh medium, it may grow vigorously and render the spawn unusable. To avoid this, strict precautions are necessary.

B. Production of Mushrooms

There are various types of methods for the cultivation of oyster mushrooms. Some are cultivated by using cut wood logs, some are cultivated on culture media contained in jars or plastic bags, and still others are cultivated by placing the culture substrates on the surface of the ridge, bed frame, or by filling different sized cases. The substrates used for cultivation of this group of mushrooms can be wood logs and trunks, several straws, and other kinds of plant waste materials, such as coffee pulp[14] (Figure 16.3), and pulp mill sludges[25] (Figure 16.4). All of these have been used for growing *Pleurotus*. Comprehensive literature on the cultivation of *Pleurotus* spp. has been

FIGURE 16.3 *Pleurotus ostreatus* grown on coffee-pulp. The bags on the lower shelf are completely colonized, and on the upper shelf have mushrooms ready for harvest. (Courtesy of G. Guzman and D. Martinez.)

FIGURE 16.4 Oyster mushrooms (*Pleurotus sajor-caju*). Cellulosic pulp mill sludges are suitable substrates for *Pleurotus* cultivation. (Courtesy of J.C. Mueller.)

summarized by Zadrazil, [32] Leong,[23] Kurtzman and Zadrazil,[21] and Zadrazil and Kurtzman.[33] Here the cultivation of *P. sajor-caju* on cotton waste is described briefly as an example.

Pleurotus sajor-caju is comparable to the high-temperature strains of *P. ostreatus* in its temperature requirement for fructification.[20,24,31] It was originally isolated in India.[18] This mushroom has been identified to be the best strain for cultivation in the tropical and subtropical areas. The desirable attributes are rapid mycelial growth, high ability for saprophytic colonization, and simple, efficient, relatively inexpensive cultivation techniques. Therefore, it has a promising future in the tropical and subtropical countries. [8,32] The cultivation method that has been tested and found to be successful is now described.

Cotton waste is used as the substrate. Large pieces are torn into smaller parts. Lime (2% w/w) is added and mixed with sufficient water to a moisture content of about 70%. The mixture is piled up, covered with plastic sheets, and allowed to stand overnight. The substrate is put into small baskets or on shelves for pasteurization, which is done in the same way as described in Chapter 5. After cooling to approximately 25°C, about 2% (w/w) spawn is mixed thoroughly with the substrate and packed into columns 60 cm long, which have hard plastic (PVC) tubing 100 cm (4 cm in diameter) as a central support (Figure 16.5). Plastic sheets are used as an outside wrapping.

These columns are incubated at about 24 to 28°C, preferably in the dark. When the mycelium of *Pleurotus* has ramified throughout the entire column of substrate (after 3 to 4 weeks), the plastic wrapping is removed and a white light is switched on. The column is watered occasionally to keep the surface from drying. Approximately 3 days later white primordia should appear over the entire surface. After another 2 days, the *Pleurotus* mushrooms are ready for harvesting. During the cropping period, watering is very important because many flushes occur.

An average biological efficiency (fresh weight of mushrooms divided by air-dried substrates × 100) can range from 80 to 120% for *P. ostreatus*, 90 to 150% for *P. sajor-caju*, and 60 to 80% for *P. cystidiosis*.

V. HARVESTING AND PROCESSING

The harvesting standards for the oyster mushroom are different for different products. They should be harvested before the mushrooms show slightly curled edges. For some uses, small caps 20 to 25 mm are requested. The fresh mushrooms are usually packed in plastic cases for sale. The caps of those oyster mushrooms to be canned or preserved in brine can be 40 mm in diameter or even larger.

The oldest method of preserving *Pleurotus* mushrooms is by air-drying, and they should be cleaned before they are dried. Circulating air at 45 to 60°C will produce an acceptable dried

FIGURE 16.5 *Pleurotus sajor-caju* on cotton waste compost in cylindrical sack.

mushroom (Figure 16.6) that will rehydrate when it is soaked in water. The rehydrated mushrooms will not have the texture of the fresh ones, but they will have a firmer texture than canned mushrooms.[21]

In recent years, in addition to canning mushrooms for export or storage, *Pleurotus* mushrooms have been preserved in brine. This method is as follows:

1. **Boiling:** After washing, the fresh mushrooms are put in the boiling water for precooking. The small mushrooms are kept in the boiling water for 3 to 5 minutes and the larger ones for 7 to 8 minutes.
2. **Salting:** After precooking, the water is decanted, and the mushrooms are steeped in a solution of sea salt (not rock salt) at a concentration of 22 to 25%.[16] The salt water must cover the mushrooms, and the mixture must be stirred every 2 days. Every 2 to 3 days the salt concentration must be tested, and if the concentration is below 18 to 20%, more sea salt must be added. Generally, the mushrooms are kept in the salt solution for 10 to 14 days, and sometimes for 5 to 6 weeks, so that the osmotic pressure reaches equilibrium. Next, the mushrooms are placed in a vessel for drainage to remove the salt water.
3. **Preservation:** The mushrooms are placed in barrels or other containers (Figure 16.7), which contain a brine solution consisting of 18 to 20% sea salt to which 80 g of citric acid is added for each 100 kg of salt solution, in order to reduce the pH of the solution to 3.5. Relatively high concentrations of acetic acid, or other food acids, tend to maintain the texture better than canning in a simple brine. Before consumption, the preserved mushrooms should be desalted by washing gently in warm water.

A study of the quality of preserved oyster mushrooms has determined that steam blanching causes a loss of flavor,[10] while others reported little or no loss of flavor from hot water blanching.[21] It also has been reported that water removes less flavor than steam, but a loss of flavor can occur if the mushrooms are cooled too slowly. It should be noted that there is no objective method for evaluating taste, which is the most important of all criteria.

FIGURE 16.6 Dried *Pleurotus* mushrooms (*P. sajor-caju*). The mushrooms on the lowest tray are ready for packaging, and the fresh ones have just been placed for drying as shown in the upper tray.

VI. SPECIAL CULTIVATION PRACTICE

The authors traveled to the Hwai-yen County Chemical and Food Plant in Datong District of Shanxi Province, China, on July 23, 1986. On this field trip, we were taken to a mushroom farm that uses a type of house in which trenches are dug and then roofed with plastic. *Pleurotus sajor-caju* was grown in these houses. The houses were of two types.

1. **Type I:** The Type I house was oriented in an east–west direction. The trench was 2 m deep and 2.5 m wide. The roof was made of branches bent to form a bow shape, covered with plastic and then with a thick, waterproof cloth. The height to the top of the plastic roof from base level was 3 m. The trench was 30 m in length. This was a two-tiered house with four shelves per tier. The shelves were made of branches (willow). We observed the seventh flush of *P. sajor-caju*. There was very little contamination even after seven flushes. The temperature in the house was 18 to 22°C. The east–west orientation of the house provided good aeration, since the prevailing winds are in an east–west direction. A biological efficiency of 150% was anticipated.
2. **Type II:** The Type II house was oriented in a north–south direction. The trench was 2 m deep and 1.5 m wide. From base level to roof, the height was 2.5 m (Figure 16.8). The length of the trench was 18 m, and a single-tier system was employed (Figure 16.9). We observed the third flush. They had just finished harvesting, and there was little contamination. The temperature ranged from 18 to 22°C. The procedure was to cover the bed with plastic at the end of the flush for 10 days, remove the plastic, and then the next flush occurred. This is repeated every 11 to 12 days. The biological efficiency is 80 to 100%, on average.

FIGURE 16.7 Preserved *Pleurotus* mushrooms are placed into plastic barrels for transport. One barrel usually contains 50 kg preserved mushrooms.

FIGURE 16.8 Entrance of roofed-over trench in which *Pleurotus* mushrooms are cultivated.

FIGURE 16.9 Mushroom shelves in the trench.

REFERENCES

1. Babitskatya, V.G., Bisko, N.A., Scherba, V.V., Mitropolskaya, N.Y., and Puchkova, T.A., Some biologically active substances from medicinal mushroom *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. (Agaricomycetideae), *Int. J. Med. Mushrooms,* 1, 345–349, 1999.
2. Barron, G.L. and Thorn, R.G., Destruction of nematodes by species of Pleurotus, *Can J. Bot*., 64, 774–778, 1987.
3. Burns, P.J., Yeo, P., Keshavarz, T., Roller, S., and Evans, C.S., Physiological studies of exopolysaccharide production from the Basidiomycetes *Pleurotus* sp. *florida*; effect of C source and N source on polysaccharide production for potential as a hypocholesterolemic, antitumor and a fat mimetic, *Enzyme Microb. Technol.*, 16, 566–572, 1994.
4. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China, *Int. J. Med. Mushrooms,* 1, 291–300, 1999.
5. Chang, S.T. and Li, S.F., Mushroom culture, in *Advances in Agricultural Microbiology*, Subba Rao, N.S., Ed., Oxford/IBH, New Delhi, 677–691, 1982.
6. Chang, S.T., Yu, M.L., and Lau, W.C., A sporeless strain in *Pleurotus florida, Mushroom Newsl. Trop*., 5(3), 19–21, 1985.
7. Eger, G., Biology and breeding of *Pleurotus*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 497–519, 1978.
8. Eugenio, C.P. and Anderson, N.A., The genetics and cultivation of *Pleurotus ostreatus, Mycologia*, 60, 627–634, 1968.
9. Farr, D.F., Bills, G.F., Chamuris, G.P., and Rossman, A.Y., *Fungi on Plants and Plant Products in the United States,* APS Press, St. Paul, MN, 1252, 1989.
10. Gormley, T.R. and O'Riordain, F., Quality evaluation of fresh and processed oyster mushrooms, *Pleurotus ostreatus, Lebensm. Wiss. Technol*., 9, 75–78, 1976.
11. Gunde-Cimerman, N., Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales, Basidiomycetes), *Int. J. Med. Mushrooms,* 1, 69–80. 1999.
12. Gunde-Cimerman, N. and Plemenitas, A., Hypocholesterolemic activity of the genus *Pleurotus* (Jacq.: Fr.) P. Kumm. (Agaricales, Basidiomycetes), *Int. J. Med. Mushrooms,* 3, 395–397, 2001.
13. Guzman, G., Genus *Pleurotus* (Jacq.: Fr.) P. Kumm. (Agaricomycetideae): diversity, taxonomic problems, and cultural and traditional medicinal uses, *Int. J. Med. Mushrooms,* 2, 95–123, 2000.
14. Guzman, G. and Martinez, D., *Pleurotus* growing on coffee-pulp in a semi-industrial plant — a new promising mushroom cultivation technology in the subtropics of Mexico, *Mushroom Newsl. Trop*., 6(3), 7–10, 1986.

15. Hibbett, D.S. and Thorn, R.G., Nematode-trapping in *Pleurotus tuberregium*, *Mycologia,* 86, 696–699, 1994.
16. Huang, N.L., The collection, processing and exporting of wild mushrooms, *Edible Fungi*, 4, 46–47, 1987.
17. Huang, N.L., *Cultivation of Eighteen Precious and Delicious Edible Fungi,* Chinese Agricultural Press, Beijing, China, 164, 1997.
18. Jandaik, C.L., Artificial cultivation of *Pleurotus sajor-caju* (Fr.) Sing., *Mushroom J*., 22, 405, 1974.
19. Karacsonyi, S. and Kuniak, L., Polysaccharides of *Pleurotus ostreatus*: isolation and structure of pleuran, an alkali insoluble β-D-glucan. *Carbohydr. Polym*. 24, 107–111, 1994.
20. Kurtzman, R.H., Jr., Mushrooms convert waste to food, *New Technol*., 42(Nov.–Dec.), 45, 1976.
21. Kurtzman, R.H., Jr. and Zadrazil, F., Physiological and taxonomic considerations for cultivation of *Pleurotus* mushrooms, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 299–348, 1982.
22. Lelly, J., Edible mushrooms as a weapon against starvation, *Mushroom J*., 173, 170, 1987.
23. Leong, P.C., Cultivation of *Pleurotus* mushrooms on cotton waste substrates in Singapore, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 349–361, 1982.
24. Li, S.F., Studies on the tolerance to elevated temperatures in *Pleurotus ostreatus* (Jac1. Ex Fr) Kummer — a contribution to taxonomy and the genetics of the fruiting process, *Bibl. Mycol*., 76, 1–86, 1980.
25. Mueller, J.C. and Gawley, J.R., Cultivation of phoenix mushrooms on pulp mill sludges, *Mushroom Newsl. Trop*., 4(1), 3–8, 1983.
26. Ohira, I., *Pleurotus ostreatus* and its related species, *Tottori Mycol. Inst.* (Japan), 15, 29–37, 1977.
27. Petersen, R.H. and Ridley, G.S., A New Zealand *Pleurotus* with multiple-species sexual compatibility, *Mycologia,* 88, 198–207, 1996.
28. Raper, C.A., Sexuality and breeding, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 83–117, 1978.
29. Roxon, J.E. and Jong, S.C., Sexuality of an edible mushroom, *Pleurotus sajor-caju, Mycologia*, 69, 203–205, 1977.
30. Yin, J., Mao, X., Ma, Q., Zong, Y., and Wen, H., *Icons of Medicinal Fungi from China,* Science Press, Beijing, 575, 1989.
31. Zadrazil, F., The ecology and industrial production of *Pleurotus ostreatus, Pleurotus florida, Pleurotus cornucopiae* and *Pleurotus eryngii, Mushroom Sci.*, 9, 621–652, 1974.
32. Zadrazil, F., Cultivation of *Pleurotus*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 521–557, 1978.
33. Zadrazil, F. and Kurtzman, R.H., Jr., The biology of *Pleurotus* cultivation in the tropics, in *Tropical Mushrooms — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 277–298, 1982.

17 *Tremella* — Increased Production by a Mixed Culture Technique

I. INTRODUCTION

Long known in China for its medicinal and tonic effects, the white jelly fungus or "silver ear" mushroom, *Tremella fuciformis* Berk., has also been used as a special luxury food item. It was very expensive as long as its only source was mushrooms growing on wood logs collected in the wild, but the advent of culture techniques has made it more available, and, although *Tremella* still commands a high price, it is no longer restricted to the very wealthy. Its greatest appeal is to people in China, Japan, and other Asian countries and to Asians residing in other parts of the world.

During the last decades the mushroom has been intensively investigated for medicinal effects in *in vivo* and *in vitro* model systems, and clinical applications were developed for different groups of patients. A wide range of medicinal properties of *Tremella,* including immunostimulating, antiradiating, antidiabetic, anti-inflammatory, hypocholesterolemic, antiallergic effects, and others, was recently reviewed.[12] The medicinal activity of preparations of *Tremella* species is due to the acidic heteropolysaccharide glucuronoxylomannan present in fruiting bodies, pure culture mycelium, or yeastlike budding haploid cultures.[13]

Tremella fuciformis Berk. is one of approximately 40 species, although recently it has been estimated that there are more than 60 species in the genus *Tremella*,[10] a member of the family Tremellaceae, of the order Tremellales, of the class Basidiomycetes. The members of the genus are widely distributed; the distribution is essentially worldwide. *Tremella fuciformis* is found mainly in subtropical regions, but it has also been reported from tropical and temperate regions — and even frigid zones.[6] *Tremella fuciformis* grows on the wood of hardwood trees (Figure 17.1). Chen and Hou[4] found that *T. fuciformis* could be cultivated on trees of 58 species belonging to 18 families, and among these, 28 species were regarded as excellent for bed logs for the cultivation of the fungus. In Taiwan, the mango tree, *Mangifera indica* L., is the most popular for cultivation of the silver ear mushroom.

Cultivation techniques modeled after the wood log method for *Lentinula* have been used for *T. fuciformis* in both Taiwan and China. In Gutian County, Fujian Province, China, wood log cultivation of the white jelly fungus was the only means of cultivation employed in the 1960s, but sawdust cultivation in bottles became common in the 1970s, and in the 1980s, the entire cultivation had been in plastic bags.[8] In 1985 in this county, 2000 metric tons (MT) (dry weight) were produced by the bag culture method. In Taiwan, although bag culture of *Lentinula* and *Pleurotus* is common, wood log cultivation was extensively used for cultivation of *Tremella* through the 1970s. In 1980, 114 MT (dry weight) were produced in southern Taiwan on wood logs;[15] 130,000 MT (fresh weight) were produced in China by bag culture in 1997.[3]

With increased production and a lowering of the price, attention has been given to the uses of the white jelly fungus as a food in ways other than by stewing it in water with sugar and serving it as a tonic or for dessert. Tu[15] has given recipes for using the white jelly mushroom in salads, in

FIGURE 17.1 Fruiting bodies of *Tremella fuciformis*, growing on wood logs. (Courtesy of Dr. K. Mori.)

soups, main dishes, as well as for dessert. Thus, *Tremella*, which was once used mainly for medicine, is now being used mostly for food.

II. BIOLOGICAL CHARACTERISTICS

A. Morphology

The fruiting body of *Tremella fuciformis* is pure white and semitransparent. It consists of from three to ten leaflike, slightly ear-shaped folds, which range in size from 5 to 15 × 4 to 12 cm with a thickness of 0.5 to 0.6 mm. In mass these have an appearance similar to that of a chrysanthemum flower (Figure 17.2). The hymenia occur on both sides of the leaflike folds, the margins of which are lobed and curved. A cross section of the mature fold reveals three layers — two hymenial layers separated by the layer that bears them, known as the trama. The width of the hymenial layer, consisting of basidia and paraphyses, is 145 μm. The trama is 3 to 3.5 mm wide and is made up of dikaryotic hyphae that are 3.2 to 3.5 μm in diameter.

Chen and Hou[5] described two types of fruiting bodies, one or the other of which is formed depending on the conditions of cultivation. The nut-gall type has folds or blades which are larger,

FIGURE 17.2 Mature fruiting bodies of *Tremella fuciformis* with the appearance of chrysanthemum flowers, grown in sawdust substrate in a plastic bag.

thicker, and of greater value than those of the cockscomb type. The size of the fruiting body varies from 1 to 25 cm and the weight from a few grams to a few hundred grams. The base of the fruiting body spreads out into a yellow cartilaginous layer beneath the bark of the wood log. The mushrooms dry to a hard, thin, and friable structure whose color is white to light yellow.

The basidia are egg shaped or almost ball shaped and in size 6 to 11.4×7.2 μm. The basidium is divided longitudinally by septa into four parts. For each part there is an epibasidium approximately 12 to 16×2.5 to 3 μm in size, and a hypobasidium. Sterigmata are located on the epibasidia, and each epibasidium bears one egg/ball-shaped basidiospore of size 4 to 7.5×6.8 to 11.6 μm according to Chen and Hou[5] and 4 to 6×7 to 7.5 μm according to Huang.[9] The basidiospores narrow toward one end and make a white spore print, although the spores are transparent when examined microscopically. Basidiospores can germinate and form mycelia or they can bud to form yeastlike conidia.

The life cycle of *T. fuciformis* is complicated by the fact that basidiospores may germinate in more than one way and that there are several means of asexual reproduction involving both monokaryotic and dikaryotic phases of the life cycle. The sexual phase has been studied by means of mating experiments in which mycelia derived from the germination of single basidiospores were confronted in all possible combinations, and in certain of these, dikaryotic mycelia with clamp connections formed. Analysis of these matings revealed that *T. fuciformis* is heterothallic and tetrapolar.

Dikaryotic mycelium develops into a white, clumping, gelatinized mycelium that forms primordia. With suitable environmental conditions and nutrients, the primordia proliferate to form the leaflike folds that are characteristic of the white fruiting body of *T. fuciformis*. Hymenia located on both sides of the folds are completely exposed to the air, permitting the easy discharge of basidiospores.

The basidiospores may germinate in either of two ways. One type of germination is typical of that of basidiospores of other members of the class in that they form germ tubes, and then the germlings develop into mycelia. The other type of germination of basidiospores involves a budding to form yeastlike conidia. These conidia, under suitable conditions, will germinate to form monokaryotic hyphae and mycelia. By repeated budding, the basidiospore may form a white, yeastlike colony.

Beginning with the studies of Bandoni,[1] which demonstrated tetrapolarity in *Tremella* with conjugation tubes being formed by yeast cells, followed by growth to one another and fusion of conjugation tubes of cells of opposite mating types, a role for diffusible hormones in bringing about the formation of the conjugation tubes and their directed growth toward one another was demonstrated.[2] These hormonal studies were performed with *T. mesentericus* but *T. fuciformis* is tetrapolar and it is likely that future experimental studies with this species will reveal general similarities to the findings with *T. mesentericus*.

Both the primary and secondary hyphae are reported to produce conidiophores and conidia.[6] The conidiophores have the form of a penicillus or brush, which is characteristic of the genus *Penicillium*. Huang[9] reports that both monokaryotic and dikaryotic mycelia can break into oidia on treatment with heat, stirring, or soaking in water. These oidia can germinate and complete the life cycle, as do the conidia derived from basidiospores. It may be that the oidia of Huang[9] are actually the conidia described by Chen and Hou.[6] In the absence of photomicrographs this is difficult to determine. If oidia are formed, it would indicate that *T. fuciformis* certainly has exploited a large number of means of asexual reproduction, which may be of significance not only from the standpoint of survival of the species in nature, but also in cultivation practices, as described below.

B. Natural History

It has already been pointed out the *T. fuciformis* is a basidiomycete of worldwide distribution and in nature grows as a saprophyte on the wood of many hardwood species. Although found mainly

in subtropical regions, it is best characterized as mesophilic in regard to temperature. In regard to moisture, *T. fuciformis* is quite versatile. The mycelium is not easily killed by a long, dry period; i.e., it is quite strongly resistant to drought. The fruiting bodies of this jelly fungus can dry and shrink, and then, in the presence of abundant moisture, take on their characteristic gelatinous texture. The basidiospores will germinate in distilled water or a nutrient solution, or bud to form yeastlike conidia at a temperature of 20 to 25°C. Because of its reputed medicinal benefits, this mushroom was collected growing wild in the woods for centuries before its successful cultivation.

C. Requirements for Mycelial Growth

The mycelium may arise from conidia, from a yeastlike cell, or from the germling that had its beginning as a basidiospore. Mycelia of three types are recognized: (1) uninucleate monokaryotic or primary mycelium; (2) dikaryotic or secondary mycelium; and (3) a fruiting dikaryotic mycelium that is sometimes called tertiary mycelium. The optimal temperature for growth of mycelium is 25 to 28°C with growth occurring within the range of 5 to 38°C. *Tremella fuciformis* is not fastidious regarding growth requirements, and it has reportedly been cultivated on a number of common mycological media. The best pH values for mycelial growth are in the range of 5.2 to 7.2 with optimum at 5.2 to 5.8.

D. Requirements for Fruiting Body Formation

Although fruiting bodies of *T. fuciformis* have been produced in the laboratory on a variety of complex substrates, we are not aware of any detailed studies of nutritional requirements for fruiting. Chen and Hou[5] report the fruiting of *T. fuciformis* on water agar, but this is undoubtedly simply a response to the cessation of vegetative growth by transfer to a non-nutrient medium. Huang[9] reported the development of fruiting bodies between the temperatures of 8 and 23°C, which is at variance with the report of Chen and Hou,[4] which states the optimal temperature for fruiting body formation is in the range of 20 to 28°C with an air humidity between 88 and 90%. Fruiting body development does require some light (50 to 600 foot-candles/m^2) in the laboratory.

III. CULTIVATION METHODS

The early cultivation of *Tremella* was similar to that used for *Lentinula*. Logs were placed in the vicinity of logs on which the fruiting bodies of *Tremella* were present, and inoculation occurred simply by the chance dissemination of spores from the fruiting bodies. Later, the inoculation was not left to the whims of nature, but a type of spawn, referred to by Chen and Hou[5] as a "tissue suspension" was used. This was prepared by grinding up fruiting bodies in water in a mortar. Since the early 1960s, a pure culture spawn method has been used in Taiwan. This pure culture spawn substrate is composed either partly of wood (like the spawn of *Lentinula*) or of sawdust and rice bran.

Modern cultivation of *Tremella* in China began in 1914.[9] In the early 1950s scientists at universities and research institutes succeeded in isolating pure cultures from spore suspensions (yeastlike conidia) for use as spawn for the cultivation of *Tremella*. Around 1959, M.P. Chan first isolated *Tremella* mycelium, as well as a featherlike mycelium that he inoculated as a mixed culture into the wood logs. He obtained fruiting bodies of *Tremella* with this procedure. Starting in 1962, scientists in Shanghai, Zhejiang, and Fujian independently attempted to grow *Tremella* on wood logs using mixed cultures. In 1965, P.R. Hsu verified that the pure culture mycelium of *Tremella*, inoculated on the featherlike mycelium growing in artificial culture medium, can complete the life cycle. In 1968, in Fujian, Canton, and Hubei, large-scale artificial cultivation of *Tremella* was started on wood logs using a mixed culture inoculum. In 1974 in Gutian County, Fujian Province, S.S. Yau improved the method of growing *Tremella* in bottles, and in the same county W.H. Dai further developed cultivation by the bag method, which greatly increased the unit yield. Huang[9] and his

colleagues, using the knowledge gained by the aforementioned researchers, made several studies of the various aspects of *Tremella* cultivation.

There are now two main methods for the cultivation of the white jelly mushroom or silver ear mushroom — wood log culture and plastic bag culture — which are now described.

A. Wood Log Culture

1. Selection of Materials

Tremella fuciformis is a saprophytic fungus that has a preference for growing on the wood of hardwood trees. Studies have been made of those species on which *T. fuciformis* has been found growing in nature and those species, which are most suitable for wood logs.[4]

It has been found that when wood logs are to be used for the cultivation of any edible fungus, the season for felling the trees is very important. For cultivation of *T. fuciformis*, the trees should be felled between late autumn and early spring. This minimizes the peeling of the bark and subsequent contamination by other fungi. It also influences the amount of sugar in the wood logs, which has an effect on the rate of mycelial growth. The water content of the trees also should be considered in the time for cutting into logs and the time of inoculation. Generally, the trees are cut into logs approximately 1.0 to 1.2 m long 1 month after they are felled.

2. Spawn

In Taiwan the spawn consists of 80% sawdust, 20% rice bran or millet, and a suitable amount of water. After the substrate has been sterilized by autoclaving, and cooled, it is inoculated with a mixture of basidiospores, dikaryotic mycelia, or fruiting body tissue. Incubation of the inoculated spawn substrate is at 23 to 26°C for approximately 2 weeks until mycelial running has occurred throughout the substrate. It is stored at 15°C for 2 weeks prior to its use as spawn.

3. Inoculation

Inoculation (spawning) is usually done in the period of January to March. Holes are drilled or punched in the bed logs according to a pattern, as is done with *Lentinula* bed log cultivation. The size of the hole depends on the trunk size, ranging from 1 to 1.5 cm in diameter and a depth of 1.5 to 3 cm. Arrangement of the holes is such that the inoculum sites are evenly spaced, and the number is determined by the surface area of the log. Pieces of spawn are inserted into the holes. The spawn should fit snugly enough so that it does not fall out before the opening is covered with bark and paraffin, but it should not be so tight as to remove all air.

4. Mycelial Running

The inoculated bed logs are placed in a "laying yard." In Taiwan the laying yard is located in a bamboo frame mushroom house in which the conditions maintained are suitable for mycelial growth (mycelial running). That is, the temperature is 20 to 25°C with an optimum of 22°C. To prevent contamination of the logs by weed fungi, many of which grow faster than *Tremella*, excessive moisture should be avoided. The inoculated bed logs are placed in an oblique, upright position against a supporting structure, alternating at the base from opposite sides of the support so that the bed logs make a small V just above the supporting structure. The period for mycelial running in the laying yard is 35 to 45 days.

5. Management for Fruiting

When the mycelium has grown throughout the bed log, it is time to change to conditions that will stimulate fruiting body formation. This operation is called "raising." and one of the conditions

required to stimulate the fruiting is additional moisture. Thus, more frequent watering is required to maintain a relative humidity of 85 to 95% at a temperature of 20 to 27°C.

Fruiting bodies begin to appear about 2 months after inoculation, and they continue to be produced on the wood logs for approximately 7 months. Frequent watering to keep the logs wet during the period of mushroom production is the main requirement in management besides harvesting, which is performed daily to remove the mushrooms when they reach a certain size (10 to 15 cm).

B. Plastic Bag Culture

Sawdust bag cultures using pure cultures of *T. fuciformis* can be used for the cultivation of the white jelly fungus; but, as previously mentioned, the development of a mixed culture technique has been developed in China, which produces better yields.[11] We are emphasizing it in this book because we feel that there is a biological concept involved here that may have applicability to the cultivation of other edible mushrooms. For this reason some careful fundamental research should be performed to determine the details of the contribution of the featherlike mycelium to the fruiting of *Tremella*. For much of the detailed information presented here we are indebted to N.L. Huang,[9,10] who provided his account of the cultivation process using mixed cultures of *T. fuciformis* and *Hypoxylon archeri*.

1. Substrate

Because *T. fuciformis* grows in nature on the wood of a variety of hardwood species and because those woods have been used in wood log cultivation of *Tremella*, sawdust was first used as the principal substrate material in plastic bag cultivation. Other substrate materials for bag culture can also be used, and cottonseed hulls have been found to be superior to sawdust. An account of this is given in Section IV.A, with formulas for the substrate and methods of preparation.

2. Spawn Production

Studies in China over the past several decades by many investigators have finally led to the conclusion that under natural conditions the completion of the life cycle of *Tremella* requires a special condition, which is referred to by Huang[9] as the "biological factor" or as the "friend of the mycelium." Of course, the formation of the fruiting body for completion of the life cycle requires suitable nutrition and conditions of temperature, humidity, light, and pH; however, even when these are at an optimal level, the formation of fruiting bodies is limited in number and size in the absence of the biological factor or friend of the mycelium. This biological factor was found to be an ascomycete that forms a featherlike mycelium.

It has been shown that *Tremella* has only a weak ability to break down wood. For this reason wood with rich nutrients of good solubility, i.e., a wood rich in sapwood, is commonly selected for wood log cultivation. It has also been demonstrated experimentally that *Tremella* lacks the ability to degrade either cellulose or lignin. It is the role of the biological factor or friend of the mycelium to help *Tremella* in the digestion of the wood and to provide some residual nutrition. The featherlike hyphae of the ascomycete lead the way into the wood log, and presumably provide some nutrition to support the growth of *Tremella*. The term **featherlike mycelium** refers to the appearance of the mycelium, while the phrase **leading the way** refers to its role in wood log utilization in association with *Tremella*.

With this indication of a role for another fungus in the fruiting of *Tremella*, it is not surprising that the early workers in China encountered difficulties in producing a reliable spawn. Even in recent years spawns lacked stability, and this frequently caused losses to the growers. It has required 50 years of study and effort by many researchers to arrive at the understanding that the completion of the life cycle under natural conditions requires the special conditions of the biological factor in which the featherlike mycelium of the ascomycete leads the way for the growth of the mycelium

of *Tremella*. By helping *Tremella* in degrading the wood, nutrition is provided, which makes it possible for the spores of *Tremella* to germinate, for the mycelium of *Tremella* to penetrate and colonize the wood, and for the fruiting body of *Tremella* to develop and grow.

The recognition of this important role of the ascomycete as a biological factor for the fruiting of *Tremella* pointed out to those interested in the preparation of spawn that a pure culture spawn of *Tremella* would not, by itself, be reliable for cultivation of mushrooms of the white jelly fungus — especially in bag cultures — because the substrate was sterilized and the bags were closed to prevent contamination from airborne spores. With wood log culture, the spores of the ascomycete serving as the biological factor have a greater chance of being present in or on the wood log and thus of entering the log than they have of entering the plastic bag culture. Thus, a reliable spawn for *Tremella* cultivation by bag culture must be a **mixed culture** consisting of *Tremella* and an ascomycete with featherlike mycelium.

Now that the rationale for using a mixed culture as spawn has been presented, we examine a technique for spawn production that has been described by Huang.[9] First, however, two formulas for substrates that have been used for cultivation of spawn in China are given:

Sawdust and Rice Bran Basic Medium	
Selected broadleaf sawdust	79 kg
Rice or wheat bran	19 kg
Gypsum or lime	1 kg
Sucrose	1 kg
(Add a suitable amount of water)	

Bagasse and Rice Bran Basic Medium	
Bagasse (dry)	79 kg
Rice bran	19 kg
Calcium carbonate	1 kg
Soybean powder	1 kg
(Add a suitable amount of water)	

a. Production Method of the Mother Culture

First, it is necessary to have pure culture of *Tremella* and of the featherlike ascomycetous fungus. The pure culture of *Tremella* can be obtained by isolation of basidiospores, by isolation of tissue, or by isolation from a mushroom log. The basidiospores of *Tremella* have been described as difficult to germinate, and it must be kept in mind that monosporous cultures are homokaryotic, and a dikaryotic mycelium is required for fruiting. Also, the fruiting bodies are gelatinous, and it is easy for contaminating fungi and bacteria to adhere to the surface, so great care must be taken in cleaning the surface of the fruiting body before the basidiospores are collected following discharge from the fruiting body. This contamination problem also makes it difficult to obtain pure cultures from tissue of a fruiting body, and one recommendation [9] is to take the tissue from under the base of the fruiting body where the tissue is not yet gelatinized. Unfortunately, this does not assure success in getting a good pure culture, since the mycelium isolated from the base of the fruiting body is usually slow-growing and not vigorous. Consequently, the main method used in China to obtain a pure culture of *Tremella* that is good for spawn is to use mycelium isolated from a mushroom log.

As described,[9] isolation of a pure culture from a mushroom log requires great care. First, the bark and the base of the mushrooms are removed and the surface of the wood log cleaned with 70% alcohol. Next, the wood is sliced through the place where a mushroom was attached, and a small piece of wood is taken from just under the place where the mushroom was attached. This piece of wood is placed in a test tube containing medium for cultivation at 20 to 28°C. After a few days mycelium will appear around the piece of wood. The test tubes should be observed very carefully daily to distinguish the characteristics for isolating *Tremella* and the featherlike mycelium into pure cultures. The characteristics are as follows:

1. ***Tremella* dikaryotic mycelium:** Between white and yellow, with an erect aerial mycelium and presence of both a surface and a submerged mycelium. The diameter of the hypha is 1.5 to 3.0 μm , and septa-bearing clamp connections are present. The mycelium of *Tremella* grows more slowly than that of most other edible fungi.
2. **Featherlike mycelium:** White with a feather shape, and a long, thin main hypha with featherlike side branches. The old mycelium is light yellow or light brown, and the culture medium gradually changes from light brown to black or very dark green. Aerial hyphae are gray-white and thin. The mycelium has a velvety surface. Usually there are no conidia; but, if they occur, they are yellow-green to grassgreen, are sub-elliptical in shape, and approximately 3 to 5 μm in size. On the surface of the wood log, stroma with perithecia may be formed. Featherlike mycelium has been obtained from two genera and three to four species, one of which is *Hypoxylon archeri*.

Once the pure cultures have been obtained, the mother culture spawn is produced in test tubes by the following procedure:

1. *Tremella* mycelium is subcultured into a number of test tubes, which are incubated at 25°C until the colony grows to a diameter of 1 cm.
2. The featherlike mycelium is then inoculated into each tube.
3. When the two mycelia have grown together, it is ready for use or sale as the mother culture.

b. Production Method of the Primary Spawn

Each test tube of mother culture can be used to inoculate one to four bottles of spawn substrate (e.g., sawdust and rice bran medium or bagasse and rice bran medium). These bottles should be incubated at 25°C until some primordia appear. It then is called the primary spawn.

c. Production Method of Culture Spawn

The growth, weak or vigorous, of the primary spawn, the size of the primordia, and the depth of penetration of the *Tremella* mycelium into the substrate all determine the number of subcultures that can be made from the primary spawn. If the *Tremella* mycelium and the featherlike mycelium in the primary spawn can be evenly mixed, then one bottle of primary spawn can be used to inoculate 100 to 200 bottles for culture spawn; however, to guarantee the quality of the spawn, it is a better practice to keep the subcultures between 40 and 60 bottles.

Production of a good-quality spawn for the cultivation of *Tremella* mushrooms has been found to require careful attention to certain items:[9]

1. As there is a specificity for wood of *Tremella* and the featherlike mycelium, the pure cultures of both *Tremella* mycelium and the featherlike mycelium should be isolated from the same log. It has been shown that failure commonly results when the two cultures used to make a mother culture have been isolated from different places or different logs.
2. The mycelium of *Tremella* grows more slowly than that of the featherlike mycelium, and, consequently, the ratio of the two must be carefully controlled when the two pure cultures are mixed. One way of doing this is to culture *Tremella* first and then introduce the inoculum of the featherlike mycelium into the culture. A ratio of mycelium of approximately 1000 *Tremella*:1 featherlike is usually found to be satisfactory. With a lower ratio, the featherlike mycelium is likely to become dominant, and fruiting body production will not be good. If, instead of mycelium, the yeastlike conidia of *Tremella* are used, the ratio should be greater than 1000:1.
3. Environmental factors must be considered in growing mixed cultures. The mycelia of the two species are able to adapt to the same culture medium and conditions. A temperature of 20 to 28°C (optimum is 23 to 25°C) is suitable for the mixed culture growth.

The moisture content of the primary spawn should be controlled at 65% and that of the culture spawn should be 65 to 70%, because, when the primary spawn is a little bit dry, it is more suitable for the growth of *Tremella* mycelium than for yeastlike conidia. The culture spawn should have a higher moisture content, as this is good for the production of the yeastlike conidia, and these conidia in water are good for penetrating into the substrate and mixing with the featherlike mycelium. This remedies the disadvantage of the *Tremella* mycelium not growing deeply into the substrate. Growth of *Tremella* mycelium deep into the substrate produces a higher frequency of fruiting and a more stable yield.

4. The determination of which bottles of primary spawn can be used for production of culture spawn and how many bottles can be inoculated from a bottle of primary spawn should be determined on the basis of the primordia formed in each bottle of primary spawn. The criteria used to determine if the mycelium is good and suitable for use as spawn are the presence of large primordia, good penetration into the substrate of the mycelium, and the presence of a robust featherlike mycelium.
5. Very strict selection is essential for the production of reliable culture spawn. It is better to avoid use of spawn that has been used for long periods of time, because with time there is apt to be a change in color, loss of vitality, and a slow rate of regeneration. That is, isolation, purification, and regeneration should be conducted frequently. The mother cultures should be stored at 5 to 15°C and subcultured every 1 to 2 months. If the primary spawn is not used immediately, it should be stored at low temperature under dry conditions. After the culture spawn has attained full mycelial growth, it should be used within 45 days.

3. Inoculation

Following sterilization of the plastic bags and contents, the bags are ready for inoculation when the temperature of the substrate drops to 28°C. Inoculation is done in a special room, which has been disinfected. The details of the inoculation procedure are given in Section IV.A.5 of this chapter. In general, the inoculation procedure involves introduction of small pieces of spawn into the substrate through several holes made in the plastic bag and extending into the substrate. After the inoculum has been put into the substrate, the holes are sealed to prevent contamination.

4. Mycelial Running

The inoculated bags are placed in an incubation room at an initial temperature of 28 to 30°C. After a few days, when there is visible growth of the mycelium from the inoculum sites into the substrate, the temperature is lowered to 25 to 28°C, which is the optimal temperature for mycelial running.

5. Management for Fruiting

Gentle currents of air should be supplied in the cultivation room, so that the development of primordia will not be retarded. Development of the fruiting bodies does require some degree of light. Although apparently this has not been examined critically, 50 to 600 foot-candles/m^2 suffice. Details of management for fruiting are given in this chapter in Section IV.A.7. Management for fruiting generally involves controlling aeration, temperature, humidity, and, to a lesser extent, light.

IV. SPECIAL CULTIVATION PRACTICES

A. Cultivation on Cottonseed Hulls in Gutian County, Fujian Province, China

Fujian Province is one of the most advanced provinces in the cultivation of mushrooms in all of China. In Chapter 13 we have described the outdoor plastic bag cultivation technique for growing

Lentinula that has been used so successfully in Gutian County. Here, we present the results of *Tremella* cultivation in Gutian County in Fujian Province and describe the cultivation method employed there. In this presentation we have relied heavily on a publication in Chinese by H.T. Ting.[14]

Although sawdust is generally the first substrate material used in growing a wood-rotting fungus in plastic bag culture, there are other substrates that can be and have been utilized. For example, cottonseed hulls have been widely used in bag culture in China, and cultivation on cottonseed hulls has been found to give higher yields of *Tremella* than does cultivation on sawdust. In Gutian County a yield of 160 to 180 kg of fresh mushrooms can be expected from each 100 kg of cottonseed hulls. This is a biological efficiency of 160 to 180%. In 1986, about one sixth of the farmers in Gutian County were engaged in the production of *Tremella*. In 1985, the production of *Tremella* exceeded 2000 MT (dry weight), which had a value of U.S. $5,380,000. This amounted to 21.4% of the total value of agricultural production in the county. *Tremella* has become a very important cash crop, and its cultivation is a very important agricultural business activity.

Although we have already examined many facets of the cultivation of *Tremella*, we now take a look at the details of a successful production technique for a particular locale, namely, Gutian County, Fujian Province, China.

1. Formulas for Substrate

Both of the formulas below include cottonseed hulls as a basic ingredient. It is important that the cottonseed hulls be fresh and free of mold and insects.

1.	Cottonseed hulls	100 kg
	Wheat bran	20–25 kg
	Gypsum	4 kg
	Magnesium sulfate	0.5 kg
	Water	100–120 kg
2.	Cottonseed hulls	50 kg
	Corncobs	50 kg
	Wheat bran	25 kg
	Gypsum	4 kg
	Urea	0.4 kg
	Water	100–120 kg

2. Preparation of Substrate

For mixing the substrate ingredients, the magnesium sulfate or urea should first be dissolved in water, and at the same time a small amount of water should be added to the corncobs and/or cottonseed hulls. After the corncobs and cottonseed hulls have accepted water and become wetted, the wheat bran and gypsum should be mixed in. Then the remainder of the water should be added and the substrate mixed thoroughly. The pH should be 5.8 to 6.2.

3. Filling the Bags

To avoid fermentation and the formation of acid, which would affect the pH of the substrate, the bags should be filled on the same day that the substrate is mixed. The operations, starting with the beginning of mixing to the final filling of the bags, should be completed within 6 hours. The size of the plastic bag is 12 cm × 50 to 55 cm. Initially, the bag is a plastic tubing, one end of which is tied with string before filling. The bag is then filled by hand or by machine, and, when the bag is full, the other end is tied. The surface of the bag is cleaned by wiping it with cheesecloth, following which four to five holes are punched in the bag with a borer that is 15 mm in diameter. These holes, which will be used for inoculation of the substrate, should be 2 cm deep. Each hole is sealed with a 33 × 33 mm square of medical adhesive tape.

4. Sterilization

The bags are placed in a sterilization chamber at a temperature that is slightly less than 100°C for 8 to 10 hours. An alternative procedure is to sterilize the filled bag first; and, after it has cooled, punch the holes, inoculate, and seal the holes with tape. The steps following sterilization are performed in the inoculating room.

5. Inoculation

The bags are ready for inoculation when the bag temperature drops below 28°C. The inoculation room should be rendered as free as possible from contaminants by wiping the working surfaces with a disinfectant solution and freeing the air of microorganisms by vapors of a germicide.

First, the old mycelium at the top of the spawn bottle should be cut off with a knife and discarded. Then, the spawn that extends 3 cm into the bottle should be crumbled to make a uniform distribution of the spawn mycelium. After removing the tape squares covering the holes, forceps should be used to insert a peanut-sized piece of spawn into each inoculation hole in the bag. The holes should be resealed immediately with the tape squares.

6. Mycelial Running

The inoculated bags should be kept in an incubation room at 28 to 30°C for 1 to 4 days after inoculation. During this period the inoculated bags can be stacked. By 5 to 10 days after inoculation, mycelium will have grown from the inoculum and penetrated into the substrate, and at this time the temperature should be lowered to 26°C and the relative humidity should not be greater than 70%. The bags should now be placed on the beds, separated from one another by 1 to 2 cm.

7. Management for Fruiting Body Formation

Approximately 10 days after inoculation, a round-shaped growth of mycelium will appear from the inoculation site. At this point the tape should be opened slightly for aeration, the room temperature should be dropped to 20 to 25°C, and the relative humidity raised to 80 to 85%.

Every day the windows should be opened four to five times for ventilation, and each time they should be kept open for 15 to 20 minutes. Soon some yellow-reddish droplets will appear at the inoculation sites. These droplets indicate that fruiting bodies will be formed.

Usually, approximately 16 days after inoculation a primordium will appear. The primordium is white and shaped like half a grain of rice. When this happens, the tape squares that cover the inoculation sites should be removed and the bags covered with newspapers. After 1 to 2 days later the holes at the inoculation sites should be enlarged by cutting 1 cm more of the bag around each hole. The enlarged hole permits greater ventilation and facilitates growth of the fruiting bodies. At this time the temperature should be 23 to 25°C. If the room temperature is greater than 25°C, the newspapers covering the plastic bags and empty places in the mushroom house should be sprinkled with water to lower the temperature to 25°C, but care should be taken not to overwater because this can cause damage to the fruiting bodies.

Under the normal management procedures described, mushrooms will appear 18 days after inoculation.

8. Harvesting

The bag cultivation of *T. fuciformis* from spawning to harvesting requires only 35 to 40 days. If the fruiting bodies are ready to be harvested (Figure 17.3) on a rainy day, the harvesting can be delayed for a few days until there is sunshine for drying.

The shape of the mature fruiting body is like that of a chrysanthemum flower, with a diameter reaching 8 to 12 cm. The "petals" are slightly ear shaped. For harvesting, the characteristics of the

FIGURE 17.3 *Tremella* mushrooms grown in synthetic compost in plastic bags. The mushrooms emerge from holes in the bags where the spawn was introduced into the substrate.

mature fruiting body should be known; because, if the mushrooms are harvested too early, the yield will be low. At the other extreme, if the mushrooms are harvested too late, the base of the fruiting body will become blackened, and it may start to decay — affecting adversely the quality of the mushroom.

The fruiting body of *T. fuciformis* should be harvested when:

- The ear-shaped structures are completely open
- The color changes from transparent to white
- The outside part of the fruiting body starts to soften and droop

Based on observed signs of maturity of the fruiting bodies, watering should be stopped the day before harvesting.

A sharp knife should be used to cut the structure attaching the fruiting body to the compost. Any yellowish material in that region should be removed carefully, but cuts should not be made too deeply or all the petals of the fruiting body will fall off.

9. Processing

If the fruiting body is dirty, it should first be washed. The fruiting bodies should be placed in a container for drying in the sunlight, which is the best method for drying. If drying must be done indoors, a temperature of 29°C should be used initially; after the surface of the fruiting body is dry, the temperature should be raised to 48°C. The fruiting body will be completely dry within 24 hours.

An alternative procedure has also been used for rainy-day drying. This involves drying the fruiting bodies initially at a temperature of 30°C and then gradually raising the temperature to 60°C.

It has been reported that 100 kg of sawdust plus supplements will produce 10 to 15 kg (dry weight) of *Tremella* mushrooms, a biological efficiency of approximately 175 to 260% (using a fresh weight/dry weight ratio of 17.5:1, a range of 17 to 18:1 is commonly accepted for *Tremella*).*

* The biological efficiency, defined as fresh weight of mushrooms produced divided by the dry weight of substrate expressed as a percentage, is a useful concept for comparing the effectiveness of different substrates or treatments in the production of mushrooms of a particular species. Because the biological efficiency is measured as the ratio of the fresh weight of mushrooms produced to the dry weight at spawning of the substrate that produced those mushrooms, differences in the proportion of water in fresh mushrooms of different species make a comparison of biological efficiencies of different species meaningless. The high biological efficiency of *Tremella* is a result of the high moisture content of this jelly fungus. Biological efficiency is not a measurement of the dry weight of mushrooms or the amount of protein produced, except in the sense that for a species a higher biological efficiency is indicative of greater dry weight and protein values.

B. Mixed Culture Cultivation of the Golden Ear Mushroom

Tremella aurantia Schw. ex Fr. (*T. aurantialba* Bandoni et Zang), known as the "golden ear" mushroom, has long been used by the Chinese for its medicinal properties, just as its close relative, the silver ear mushroom, *T. fuciformis*. Although the silver ear mushroom is now produced in large quantities and is used as a food, the golden ear mushroom is not cultivated in like quantities, and its uses are as a medicinal tonic. Consequently, it is priced at U.S. $40 to $50 per kg. The golden ear with other relative yellow brain mushrooms, *T. mesenterica* (Retz.:Fr), and *T. cinnabarina* (Mont.) Pat, which have been investigated to date are all pharmacologically active. The medicinal activity is due to the acidic heteropolysaccharide glucuronoxylomannan present in fruiting bodies.[14,13] The medicinal properties include anti-inflammatory, hypocholesterolemic, relief of coughing in elderly individuals, tracheitis and hypertension. It has recently been demonstrated[10,16] that for cultivation of the golden ear mushroom a mixed culture spawn should be used. Starting with ten isolates from tissue of the golden ear mushroom, Yun et al.[17] obtained mother cultures of *T. aurantia* and the associated fungus *Stereum hirsutum* Willd. Fr. From these ten isolates, both pure cultures and mixed cultures were obtained, including yeastlike conidia and mycelium of *Tremella* and cultures of the associated fungus, *S. hirsutum*.

It is interesting to observe that the companion fungus associated with *T. aurantia* is a basidiomycete, whereas the fungi associated with *Tremella fuciformis* are ascomycetes. For the sawdust bag cultivation of the golden ear mushroom, a mixed culture spawn containing *T. aurantia* and *S. hirsutum* is used. The spawn substrate is a sawdust substrate of the following composition:

Sawdust	84%
Wheat bran	12%
Sucrose	1%
Soybean powder	1%
Gypsum	1%
Magnesium sulfate	1%
(Water content should be 70–75%)	

The methods involved in making the culture spawn and for producing golden ear mushrooms in sawdust bag culture (Figure 17.4A) may be similar to those already described for the silver ear mushroom, but details are not available as yet. It can be seen from Figure 17.4B that the golden ear mushroom can fruit at a low temperature.

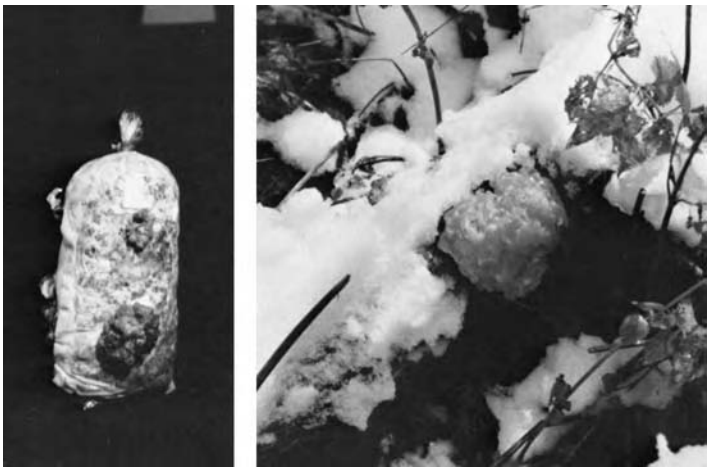


FIGURE 17.4 (Left) Fruiting bodies of *Tremella aurantia* have emerged from sawdust in a plastic bag. (Right) Fruiting body of *Tremella aurantia* grown on wood log surrounded by snow. (Courtesy of Mr. F.S. Yun.)

FIGURE 17.5 (Color figure follows p. 232.) Resembling a bakery, this is the oven/hot air chamber where the *Tremella fuciformis* fruiting bodies are placed for drying.

V. HARVESTING AND PROCESSING

The fruiting body should be harvested when the leaflike blades of the fruiting body are completely open. The mushroom is then 10 to 15 cm in diameter. As stated in the previous section, other indications that the fruiting body should be harvested are a change in color from transparent to white and the softening and drooping of the outside part of the fruiting body. The procedure for harvesting differs in Taiwan and China. In Taiwan, the mushrooms, most of which are produced in wood logs, are generally picked by hand.[15] In China, a knife is used to cut the fruiting body at the base of the leaflike blades and then to cut off the structure attaching the fruiting body to the substrate.[9] After picking, the mushrooms should be washed in water to remove dirt.

The fruiting bodies are next placed in a container for sun drying. In Taiwan, drying is done in an oven heated with charcoal. The oven has special shelves for drying the mushrooms. The drying takes up to 8 hours with the temperature first at 50°C and then gradually reduced to 40°C. In China, sun drying is considered the best method; but, if the weather does not permit this, oven drying at an initial temperature of 29 to 30°C is used. After the surface of the mushroom is dry, the temperature is gradually raised to 48 to 60°C, and the fruiting body will be completely dry in 24 hours. Drying is now commonly done in an oven/hot air chamber with electricity (Figure 17.5). The method used in China takes three times as long as that used in Taiwan. The dry weight of the dried silver ear mushrooms is only about 6 to 8% that of fresh.

The dried mushrooms are put in plastic bags and then stored in sealed boxes to prevent deterioration by insets and molds.

REFERENCES

1. Bandoni, R.J., Conjugation in *Tremella mesenterica, Can. J. Bot*., 41, 467–474, 1963.
2. Bandoni, R.J., Secondary control of conjugation in *Tremella mesenterica, Can. J. Bot*., 43, 627–630, 1965.
3. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China, *Int. J. Med. Mushrooms,* 1, 291–300, 1999.
4. Chen, P.C. and Hou, H.H., Studies on the host range of jelly fungus *Tremella fuciformis* Berk., *Taiwan Mushrooms*, 1(2), 65–70, 1977.

5. Chen, P.C. and Hou, H.H., *Tremella fuciformis*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 629–643, 1978.
6. Chen, P.C. and Hou, H.H., The life cycle of the white jelly mushroom, *Tremella fuciformis*, *Taiwan Mushrooms*, 3(1), 19–27, 1979.
7. Hobbs, C., *Medicinal Mushrooms — An Exploration of Tradition, Healing, and Culture,* Interweave Press, Loveland, CO, 167–170, 1995.
8. Huang, N.L., Cultivation of *Tremella fuciformis* in Fujian, China, *Mushroom Newsl. Trop*., 2(3), 2–4, 1982.
9. Huang, N.L., *Cultivation of Tremella* [in Chinese], Promotion of Science Press, Beijing, 1986.
10. Huang, N.L., *Cultivation of Chinese Tremella fuciformis* Berk., Chinese Agricultural Press, Beijing, 187, 2000.
11. Lin, P.C., Isolation and purification of mycelium of *Tremella*. *Edible Fungi* [in Chinese], 3, 11–13, 1987.
12. Reshetnikov, S.V., Wasser, S.P., Nevo, E., and Duckman, I., Medicinal value of the genus *Tremella* Pers. (Heterobasidiomycetes) (Review), *Int. J. Med. Mushrooms,* 2, 169–193, 2000.
13. Reshetnikov, S.V., Wasser, S.P., Duchman, I., and Tsukor, K., Regulation of growth and biosynthetic activity of the medicinal jelly mushroom *Tremella mesenterica* (Retz.: Fr.) pure culture, *Int. J. Med. Mushrooms*, 3, 45–51, 2001.
14. Ting, H.G., High yield technique for cultivation of *Tremella* in cottonseed hulls in bag culture, *Edible Fungi* [in Chinese], 3, 17–18, 1987.
15. Tu, C.C., Cultivation of *Tremella fuciformis* Berk. in Taiwan with special references to cooking techniques, *Taiwan Mushrooms*, 5(1), 17–27, 1981.
16. Ying, J., Mao, X., Ma, Q., Zong, Y., and Wen, H., *Icons of Medicinal Fungi from China*, Science Press, Beijing, China, 1987.
17. Yun, F.S., Chiu, P.M., Zhang, H.S., and Zhang, K.F., Isolation of *Tremella aurantia* Schw. ex Fr. and its physiological characteristics [in Chinese], Publication of the Shanxi Biological Research Institute, Taiyuan, Shanxi Province, China, 1987.

18 *Dictyophora* — Formerly for the Few

I. INTRODUCTION

One of the common names for *Dictyophora* is "bamboo sprouts," a name derived from the fact that the mushroom can be found in nature pushing up through the soil in bamboo thickets where it grows on the residue and humus of the bamboo plants. Those with an overriding appreciation for the great beauty of the mushroom may prefer to call it the "flower of the fungi" or the "veiled lady mushroom." Those not so aesthetically inclined may disagree because of the odor that emanates from the spore mass of the fruiting body, which is so attractive to insects, and these people may note that it is a relative of the "stinkhorn," *Phallus impudicus*. Persons recognizing the high price that its dried fruiting bodies bring in the marketplace because of its great gastronomic appeal may call it the "king of the dried foods." In China, where it has long been esteemed and where it has been cultivated in synthetic composts since 1979, the name Zhu Sun indicates a preference for the Chinese version of the English common name bamboo sprouts.

It is in China that *Dictyophora* is most famous. Originally collected in the wild from not overly abundant numbers, this delicacy was costly and available only for special occasions such as state banquets.[1] Even in modern times *Dictyophora* has been featured at banquets celebrating events of great historical importance, such as the visit of Henry Kissinger to China to make the arrangements for the reestablishment of diplomatic relations between China and the United States. Kissinger reputedly extolled the meal in glowing terms in which *Dictyophora* played a featured role, but only he knows whether he was speaking as a gourmet or a diplomat or possibly both.

In addition to its good taste, *Dictyophora* is also reported to have beneficial medicinal effects.[9] Although there are numerous scientific studies that have shown the medicinal effects of other edible mushrooms such as *Lentinula* and *Volvariella*, we have not had the opportunity to examine the research reports of such studies with *Dictyophora,* although there are common references in Chinese writings to the long-standing recognition of *Dictyophora* as a healthful mushroom with good medicinal effects that help to maintain the consumer's fitness. Current writings attribute to *Dictyophora* the ability to lower blood pressure, decrease blood cholesterol, and reduce body fat.[6,8] It is also commonly used in China for treatment of dysentery. Now that *Dictyophora* can be cultivated and is more readily available, it is anticipated that more studies will be made to test the medicinal benefits that may be derived from eating *Dictyophora* or substances produced by this mushroom.

Dictyophora commands a high price and is becoming a commercial mushroom of economic importance. In 1982 in Hong Kong, the price reached approximately U.S. $770 per kilogram of dried mushrooms. By 1988, the price was still in the range of U.S. $100 to $200 per kilogram. However, by 2000, due to rapid advances in cultivation techniques, the price dropped to U.S. $10 to $20 per kg.

The two species of *Dictyophora* that have been successfully cultivated in China on a commercial basis are *D. duplicata* (Bosc) Fisch. and *D. indusiata* (Vent. Ex Pers.) Fisch. In the following description of *Dictyophora*, its cultivation, harvesting, and processing, we have drawn heavily from the writings in Chinese of Hu et al.,[2] Huang,[3] and Zhang and Jiang.[10]

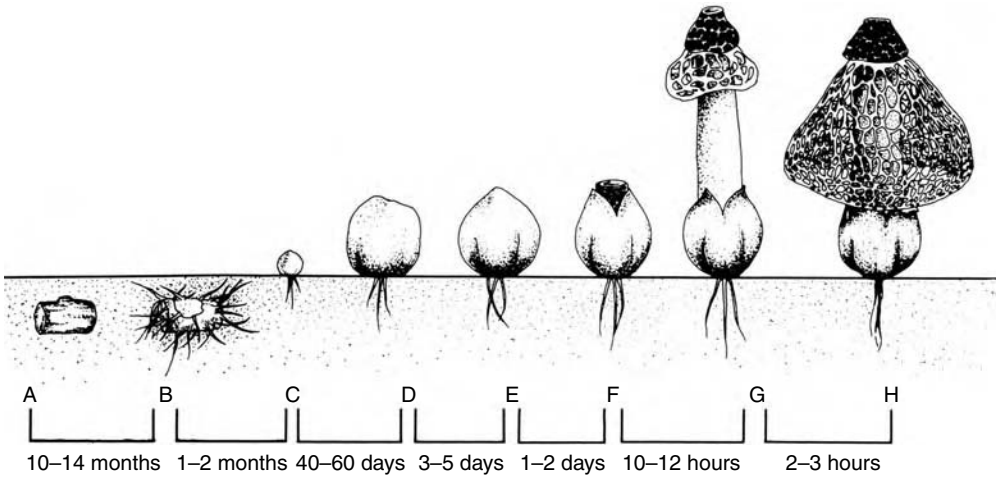


FIGURE 18.1 Different stages in growth and development of a fruiting body of *Dictyophora duplicata*: (A) inoculated log buried in soil, (B) development of rhizomorphs, (C) formation of primordium, (D) mushroom ball formed, (E) apical protrusion, (F) apical breakage and opening, (G) stalk elongation, and (H) mature fruiting body.

II. BIOLOGICAL CHARACTERISTICS

A. Morphology

The fruiting body is a reproductive structure and, of course, as with other mushrooms, it is the structure of economic importance. The fruiting body of *Dictyophora* consists of a pileus, stalk (or stipe), a skirt (the indusium), and a volva. Rhizomorphs, rootlike strands of hyphae, extend from the volva into the soil or substrate (Figure 18.1).

The pileus is 2 to 4 cm high. It is white in the short-skirted form and is off-white in the long-skirted form. The surface of the pileus has an irregular, reticulate surface with circular or ellipsoidal pores. A dark green hymenium is attached to the surface of the pileus. The hymenium is very hygroscopic and becomes moisture laden.

The stalk is cylindrical in shape, hollow, and has a spongy texture. It is very tender, white, and has a length of from 7 to 25 cm. The thickness of the stalk is 0.2 to 0.5 cm.

The skirt flares out under the pileus when the fruiting body is mature. It is 4 to 22 cm long, white, and has openings that are round, ellipsoidal, or polyhedral, of a size ranging from 0.2 to 1.0 cm, which give the skirt a netted appearance. The skirt is very significant in the systematics of the taxon. Within the genus *Dictyophora* there are long- and short-skirted species. For example, *D. indusiata* is long skirted (Figure 18.2), and *D. duplicata* is short skirted (Figure 18.3). At the genus level, the presence or absence of skirts is a distinguishing criterion, e.g., *Phallus impudicus* lacks a skirt.

The volva is formed from the universal veil, which is ruptured by the emergence of the stalk and pileus. The volva consists of two parts — an inner and an outer tissue separating some gelatinous material. Yang and Jong[8] report that the volva of *D. indusiata* is tough and non-edible, but Hu et al.[2] describe the volva as being nutritionally the same as the pileus, stalk, and skirt with 17 different amino acids and having a good taste. They conclude that the volva can be used both as a food and for medicinal purposes and deplore the fact that people customarily discard the volva; however, there is agreement that the pileus should be discarded, as the strong-smelling spores attract insects and the presence of a slimy covering certainly makes the pileus unattractive for eating purposes. Although the volva seems appropriate for use for extraction of substances that are to be used for

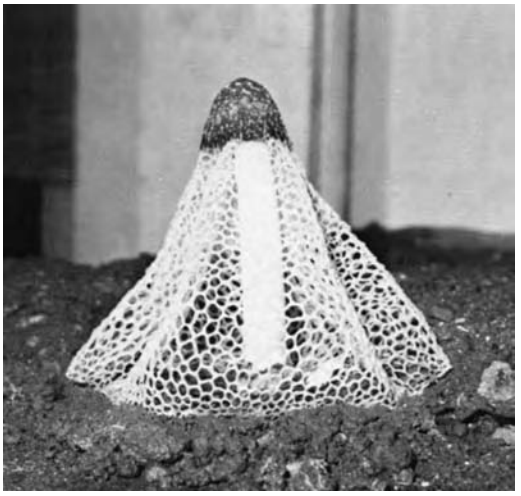


FIGURE 18.2 Long-skirted form of *Dictyophora indusiata*.

FIGURE 18.3 Different stages of *Dictyophora duplicata* cultivated in the mushroom house on a bamboo leaf compost. (Courtesy of Mr. J.N. Lin.)

medicinal purposes, it is probably not suitable for use as food if people find it to be tough, as the texture is one of the outstanding choice features of this mushroom. This point should be determined with a careful survey because the ratio of the volva to stalk plus veil is 2:1, and, if there is a good use for the volva, this should be determined.

The rhizomorph is a differentiated hyphal structure that grows at the bottom of the volva and extends into the soil. In color the rhizomorphs are the same as the volva. There are one or two rhizomorphic strands on the volva of a single fruiting body. The farther the rhizomorphs extend from the volva, the thinner they are. Thus, although the rhizomorphs are relatively thick when close to the volva, as they get farther away, they divide and subdivide and extend into the substrate.

The spores are produced in great numbers on the hymenial layer on the pileus. These basidiospores are the basic unit for propagation of the species. They are colorless, transparent, and have a size of 3.0 to 4.5 × 1.7 to 2.8 μm for *D. duplicata*[2] and 3.9 to 4.8 × 1.9 to 2.6 μm for *D. indusiata.* The spores of *D. indusiata* are smooth, and each has a large, slightly sunken, apical germination pore.[8]

Germination of the spores results in the formation of germlings. The germ tubes emanating from the germination pores develop into uninucleate hyphae, which will, in turn, form primary

mycelia. At an early stage the mycelium is white. Compatible primary mycelia fuse to form dikaryotic mycelium with clamp connections known as the secondary mycelium. When cultured for a longer period of time, the mycelium changes color to pink, light purple, or yellowish brown. The pigmentation becomes more obvious when the mycelium is subjected to changes in temperature, light, mechanical stimulation, or dehydration and is a useful criterion in the identification of *Dictyophora* mycelium. Secondary mycelium is stronger than primary mycelium, and further development of the secondary mycelium leads to the formation of bundles (the primordia of fruiting bodies), which are called tertiary mycelia.

B. Natural History

At one time it was believed that *Dictyophora* was a mycorrhizal fungus, but in recent years reports from China have indicated that this is not the case. *Dictyophora* is a saprophytic fungus, obtaining its nutrients in nature from nonliving plant materials such as the residue from bamboo plants. It has also been found growing on the stumps of dead maple trees, amid decayed needles of red pine trees, and among bryophytes. As there is no specific relationship of *Dictyophora* with a host or host material, and, as the fungus is able to utilize a broad spectrum of nutrients, it is apparent that *Dictyophora* is saprophytic and not a mycorrhizal fungus. It mainly utilizes cellulose and related carbohydrates from wood or straw.

Dissemination of the spores of *Dictyophora* is through the agency of insects. Hu et al.[2] report that three different kinds of bees and five different kinds of flies have been observed on the pileus of *Dictyophora*, attracted there by the odor from a strong-smelling substance from the glebal mass. The short-skirted form (*D. duplicata*) is reported to have a pleasant smell,[5] but the long-skirted form (*D. indusiata*) has an odor characterized as a stench by Hu et al.,[2] or a stink by Yang and Jong.[8] The insects seem to lack our discrimination regarding good and bad odors, and bees, yellow jackets, and flies are attracted to the liquefied part of the fruiting bodies of *D. indusiata* as well as to *D. duplicata*. As a result of contact with this liquefied glebal mass on the pileus, the feet, mouths, and stomachs of the insects carry thousands of spores to be widely disseminated. When the spore becomes located in a place where the environmental conditions are suitable (e.g., temperature and humidity) and a satisfactory substrate is present (e.g., moist, dead wood or compost), the spores will germinate and form a primary (homokaryotic) mycelium. When two compatible primary mycelia come together, a secondary (dikaryotic) mycelium is formed, which can further develop into fruiting bodies. This is the manner in which *Dictyophora* normally completes its life cycle under natural conditions.

C. Requirements for Mycelial Growth

Dictyophora indusiata dikaryotic mycelium grows very slowly. Yang and Jong[8] reported that a dikaryotic stock (ATCC 60890) produced a colony only 20 mm in diameter after 2 weeks growth at 24°C on a medium of potato dextrose agar which had been supplemented with either 1% molasses, or 10% *Agaricus* mushroom extract, or a 10% sawdust spawn of *Tremella fuciformis*. From this report we know neither the effect the supplementation may have had nor the rationale for the use of these particular supplements. It is stated, however, that at a pH value of from 5.0 to 5.5, the mycelial growth is optimal at 22 to 24°C and takes place over the range of 17 to 30°C. An additional piece of information provided by this study is that growth is retarded by continuous exposure to light.

Unfortunately, the published information available to us does not provide experimental data on mycelial growth by strains of monosporous mycelia and stocks of dikaryotic mycelia of *Dictyophora* under various nutritional and environmental conditions. As growth is reported to be slow, it would be interesting, and perhaps useful, to have such data. We do, however, have some reports from China that make a few observations and generalizations about mycelial growth, along with some inferences based upon these observations, that we think are interesting and worthy of inclusion here.

Hu et al.[2] observed that a vigorously growing mycelium of *Dictyophora* is able to penetrate the growing mycelium of many contaminating microorganisms by the process of growing through the junction region where two contaminating mycelia come together. In this situation involving mixed mycelia, the growth of *Dictyophora* is reported to be as good as growth on an uncontaminated substrate. This ability of *Dictyophora* to compete strongly with the mycelia of coexisting fungi is a special characteristic that is not found in the mycelium of other edible fungi. Hu et al.[2] also conjecture that the contaminating microorganisms may produce some substance that favors the growth of *Dictyophora*. Support for this concept is provided by the observation that most mycelia of *Dictyophora*, which usually grow very slowly, grow very rapidly after penetrating the contaminated area.

For mycelial growth the influence of environmental factors has been reported to be as follows:

1. Substrate temperature: Range 5 to 29°C; optimum, 23°C
2. Soil moisture (substrate moisture): 60 to 70% for good growth; less than 30%, mycelium dies; and too high, mycelium dies from lack of oxygen
3. Air: Sufficient oxygen is necessary in the soil or substrate for mycelial growth
4. Light: Not required, but may retard mycelial growth
5. Soil: Not required
6. pH: Slightly acid, pH = 6

D. Requirements for Fruiting Body Formation

Although the general morphology of the fruiting body has been described in Section II.A, details of its development were not given, and the account given here is based primarily upon articles by Hu et al.[2] Under the favorable conditions for mycelial growth, which have just been discussed (Section II.C), the mycelium will reach physical maturity. Strands of hyphae form rhizomorphs, the ends of which expand to form small, white, ball-like structures. These latter structures are the primordia of the fruiting bodies of *Dictyophora*. This primordial structure is the ball or the egg stage of the mushroom.

Several bundles of rhizomorphs are located at the base of the egg, and the small egg and bundles of rhizomorphs expand synchronously until at maturity the egg may be as big as a chicken egg, or even a duck egg. To reach this stage takes approximately 40 to 60 days, depending on the temperature and the compost. Within this egg, the fruiting body develops.

The start of the formation of the egg stage occurs 1 to 2 cm beneath the surface of the compost; and, when it becomes 1 to 2 cm in diameter, it emerges on the surface. Because it is formed beneath the surface, the egg is white, and its formation does not require light. On the surface, however, it receives light, and pigment is developed (Figure 18.4). The pigment on the surface of the egg varies with the strain and may also be affected by light intensity. Thus, the color of the eggs varies and may be pink, red, gray, brown, or off-white. The egg of *D. indusiata* is described as dingy brown by Yang and Jong.[8]

In dry weather some of the surface cells of the egg may die, and, with the cells on the interior still expanding, the surface cracks. This results in a nonsmooth surface of an irregular appearance, similar to the "cracked" appearance of the caps of the donko type of shiitake.

When the egg is sectioned, it can be seen that the center is the white stalk, which is surrounded by the dark green hymenium that grows on the pileus. The skirt grows between the stalk and the hymenium. All of these aforementioned structures are covered over by a very tough internal veil of one layer. The outer layer of the egg is the external veil, and between the internal and external veils is a gelatinous, transparent material.

When the egg reaches physiological maturity, the stalk within gradually elongates, the upper part of the egg forms a very tough protuberance, and the other part of the egg becomes loose, soft, and wrinkled on the surface. Kinugawa[5] has shown that the expansion of the stalk (stipe) is

FIGURE 18.4 Egg or ball stage of *Dictyophora*. These are on the casing soil of the bamboo leaf compost.

accompanied by the breakdown of glycogen and its conversion to sugar. In the unexpanded stalk the cells are folded, but they expand to almost 12 times their original volume as the stalk elongates.

When the ball or egg itself takes on an egg shape, it is indicative that the mature fruiting body will soon be formed. The emergence of the fruiting body from the egg occurs when the egg accepts sufficient water, and the conditions for this are a relative humidity of 85% and an increase in moisture content of the compost to 75%. The egg now breaks at the tip due to elongation and expansion of the stalk. In 10 to 15 hours the mature fruiting body, previously described, is formed.

The apical opening of the pileus is the first structure to emerge from the egg, followed, of course, by the rest of the pileus. From the emergence of the stalk to its fullest elongation requires 10 to 12 hours. From the emergence of the skirt (it is attached at the pileus) until it is completely flared requires only 30 minutes to 1 hour. Thus, the completion of elongation of the stalk and the flaring of the skirt require only 2 to 3 hours.

For fruiting body formation, the influence of environmental factors has been reported as follows:

1. Substrate temperature: Range, 17 to 29°C; optimum, 22°C
2. Soil moisture (substrate moisture): 70 to 75%
3. Air moisture (relative humidity):
   For differentiation and development of egg: 80%
   For development of fruiting body: 85%
   For breaking open of egg and elongation of stalk: 85%
   For flaring of skirt: ≥94%
4. Light:
   Not required for differentiation of the primordium
   Not required for development of primordium to fruiting body
5. Soil: Essential for formation of egg stage
6. pH: 6.0 suitable for the casing

III. CULTIVATION METHODS

Since 1979, *Dictyophora* mushrooms have been produced in China every year from synthetic composts. The cultivation technology is described here. The reader will undoubtedly observe that

there are certain similarities to cultivation methods used with other edible fungi and that there are also some unique features.

A. Cultivation in Forests

The cultivation method in forests is an imitation of the method in which *Dictyophora* grows and forms fruiting bodies in nature. First, a substrate that has been inoculated with a pure culture of dikaryotic mycelium of *Dictyophora* is transferred to a wild region in which *Dictyophora* usually grows. It should be pointed out that this method, which will be described shortly, does require a long growth period; however, there are certain advantages that offset this defect in the method. Its yield is stable, it requires less labor, and the costs are less than growing the mushroom indoors, thus resulting in a high profit.

1. Selection of Place

The place selected should be a forestland on which wild *Dictyophora* normally grows. It should be shaded approximately 80% by various kinds of bamboo and broad-leaf trees.

2. Selection of Materials

The substrate materials that should be selected are the residues (leaves and stems) of bamboo and the branches of deciduous trees such as maple, oak, and cherry. Additional substrate materials that can be used to grow *Dictyophora* include straw, bagasse, and the sawdust and chips from broad-leaf trees. The principal substrate in the method of cultivation in forests is the wood log.

The most suitable trees of the species indicated are those that are 10 to 20 years old and have a diameter of 7 to 20 cm. The time of cutting of the logs is very important; the best period is between leaf fall and the sprouting of buds the following spring. Another feature regarding cutting time is that it should be closely matched with the inoculation (spawning) time by cutting 20 to 60 days before inoculation. As a saprophytic fungus, *Dictyophora* utilizes only the dead material of the tree, and thus a period of time is required for the cells of the log to become dead prior to inoculation. The time that this requires is generally related to the moisture content. The tree with higher moisture content generally dies more slowly than one with lower moisture content. The moisture content is related to such things as the texture of the wood, whether the tree was grown on a high-moisture soil or a sandy soil, and if it was cut after a rainy period or a dry period. In summary, a longer waiting period before inoculation is required for logs from trees that are relatively high in water content. The loss of water from cut logs is related to temperature, with low temperatures requiring longer periods before inoculation, and high temperatures requiring shorter periods.

After felling, the tree is cut into pieces 1 m in length. If some part of the log is damaged, the area that is damaged should be painted with a 5% solution of calcium carbonate or with Bordeaux mixture to prevent the entrance of contaminating fungi at the damaged site. The logs are then stacked in a well-ventilated place for drying.

Materials other than wood logs can be used as substrate for the cultivation of *Dictyophora*. These materials include the leaves and branches of bamboo, wood chips, and bagasse. This type of material must first be dried and then soaked for 1 to 2 days. It should be examined to make certain that the soaking has been sufficient for the water to penetrate into the material. Before the material is inoculated (spawned), however, it should also be confirmed that the surfaces of the material are dry.

3. Spawn

As the mycelium of *Dictyophora* grows slowly, there is a great opportunity for contamination, and it is more difficult to establish pure cultures of *Dictyophora* than it is with many other edible

mushrooms. Thus, pure cultures should be handled by trained technicians at institutes that have good facilities for culture work.[4] The dikaryotic stocks used for making spawn were originally isolated from tissues of fruiting bodies collected in nature. The stock of *D. indusiata* used by Yang and Jong[8] is available from the American Type Culture Collection as ATCC #60890. For cultivation in wood logs, standardized cylindrically shaped wood-pieces that are 1 cm in diameter are inoculated with a dikaryotic stock of *Dictyophora*, and, when the mycelium has grown throughout the wood-pieces, they are used as spawn.

4. Inoculation

The best time for inoculation (spawning) in forest cultivation is, in principle, from October to December. The reason for this is that in January the temperature is lower than in October to December, and, when spawning is done in the spring, the inoculated material must be covered to prevent rain from wetting the casing soil.

The log can be inoculated through notches or holes drilled in the logs. The cutting of notches in the logs for providing entrance of the fungus into the log is an ancient practice that has been used with other edible fungi and was described by Wang Cheng in the *Book of Agriculture* in 1313,[7] as mentioned in Chapter 11. The drilling of holes is a modern practice that is widely used with a number of edible mushrooms that form fruiting bodies on wood.

The diameter of the holes that are drilled in the logs should correspond with that of the wood-piece spawn that is used in inoculation. The hole should not be too big, for then the inoculum could easily fall out, and also moisture would be lost from the log. The hole should not be too small, for then the inoculum could be damaged when the wood-piece spawn is pressed into the hole, thereby interfering with spawn running. Thus, a standardized wood-piece is used for making spawn. The diameter of the wood-piece is 1 cm, and the diameter of the drill used in making the holes is 1.1 cm. The hole is drilled to a depth of 1.5 to 2.0 cm. The holes are drilled in a pattern on the log with the distance between holes along the length of log 5.0 to 7.0 cm, and a distance between holes around the circumference of 4.0 to 5.0 cm. Because the mycelium grows slowly, inoculation sites that are closer together are better than those farther apart.

Cultivation in forests can be accomplished with materials other than wood logs. The substrate material may be bamboo branches, or small blocks of bamboo, or wood. Such materials should be mixed with bamboo leaves (or bamboo sawdust, or wood sawdust) in an amount constituting 20% of the total substrate materials. The bamboo leaves, bamboo sawdust, or wood sawdust should first be mixed with water so that water just comes out when the wetted material is squeezed, but it should not contain so much water that it is dripping. The main substrate material (bamboo branches, bamboo wood, etc.) should be soaked in water for 2 to 3 days before it is used in the compost. In inoculation of this type of compost, one layer of the substrate material and one layer of spawn are used in the ratio of 10 kg of dry substrate material to 1 kg of fresh spawn.

If the substrate material consists of split bamboo, or if the pieces of bamboo are large, then bamboo dust or sawdust must be included along with and closely associated with those substrate materials. A 10:1 ratio of dry substrate material to fresh spawn is used in spawning this substrate material with larger size particles, just as it is for the previous substrate material that is made up of similar materials but of smaller particle sizes.

Spawning in the last two cases described does not need to be standardized as it does with wood logs, and thus branches of bamboo and trees that are of irregular size and shape can be used for making the spawn.

5. Covering the Substrate Materials

As *Dictyophora* is a saprophytic fungus that in nature produces its fruiting bodies in soil, it is not surprising that in cultivation of *Dictyophora* the fruiting bodies will not be differentiated unless the compost is covered with soil. Thus, the cultivation practice is to cover the substrate material (compost).

Recall that there is a preferred time after the cutting of the trees for wood log production of *Dictyophora* that inoculation should take place. There is also a suitable time for covering the substrate that is related to the timing of tree cutting/spawning. For example, if inoculation has been performed earlier than the optimal time, then a longer period of time should elapse before the substrate is covered. Normally, a period of 20 days should elapse after inoculation before covering the substrate. The goal is to maximize the number of dead cells in the log before covering the logs, which also should not be too dry. This situation is difficult to control, but the following guidelines for management of the time for covering may be helpful.[2]

1. Covering should take place when the ends of the logs, as viewed in cross section, show small cracks or splits.
2. Covering should take place when the green layer of bark turns to yellowish brown.
3. If the wood log was of a moisture-containing species, such as sweetgum (*Liquidambar*), and spawning took place in November–December, then the covering should be in March–April. If, on the other hand, spawning was in February–March, then the covering should be postponed to May–June.
4. If the wood log is of small diameter (e.g., 5 to 8 cm), if the texture is loose, and if spawning was in winter, then covering should take place 1 to 2 months after spawning. If, on the other hand, spawning was during a season of higher temperature (autumn or spring), then the wood log must be kept slightly moist for 20 to 30 days after spawning, or else it will be too dry to be covered.

A week before covering is to take place, the selected area should be prepared by clearing away the weeds, removing stones, spreading insecticides, and loosening the surface of the ground. When inoculated wood logs are to be buried, a 5-cm-thick layer of bamboo leaves, small pieces of bamboo or wood, sawdust, or wood chips should first be laid on the area that has been prepared. The logs are then placed on this layer. Logs of the same length are put together. The plot should be 1 m wide, but there is no limit to its length. Between the logs there should be a space filled with bamboo leaves, bamboo wood, or bamboo sawdust. This is called the layer of material and is about 20 to 25 cm thick, and some additional spawn should be placed in it. Next, the layer of material is covered with a layer of humus (the casing soil) to a thickness of 5 to 10 cm, which is in turn covered with bamboo leaves. This casing soil need not be added immediately, but instead the plot can be covered with a plastic sheet until spawn running occurs in the layer of filling material, at which time the casing soil should be added. It is customary to extend the casing soil 30 to 40 cm beyond the logs, because the mycelium usually extends out that far and the eggs of mushrooms may form there. If the casing soil is not so extended, mushrooms might form in the paths surrounding the plot.

A good casing soil for *Dictyophora* is a humus layer from sandy loam soil. The casing soil should not be from a clay soil; nor should a sandy soil, a heavy clay soil, a high-moisture-containing river bottom soil, or a pond-bottom soil be used. The casing soil should be broken up into pieces, and large pieces removed and not used. Also to be removed are stones, grass roots, and humus already infected with contaminating fungi. It should either be sterilized or mixed with suitable amounts of insecticides. The casing soil should then be laid gently on the bed, and it should not be packed down or even heavily pressed. When pieces of substrate material are used rather than logs, these materials should also be covered. Unlike the procedures followed with the wood log method, when pieces of substrate material are used, the spawning and covering can be done immediately and the substrate material can be pressed, although the casing soil should not be pressed.

6. Management

The key to success in *Dictyophora* cultivation involves water management. Because the mycelia are very delicate, they cannot be too dry or too wet. A relatively moist place is required in which

the water content of the substrate and soil should be between 60 and 70%. The mycelium can tolerate a water content of the substrate greater than 75% for only a short period of time, and, if the water content remains that high for a longer period, the mycelium will die from lack of oxygen. If the water content of the substrate is less than 50%, growth of the mycelium will be retarded, and when less than 30%, the mycelium will dry and die.

There are certain precautions that must be taken to prevent damage to the mushrooms. If termites eat the logs, they can destroy whole logs in mushroom farms, and management to prevent this is essential. It is necessary to remove and examine the logs after they have been covered for a couple of months and to apply an antitermite substance if there is an indication of infection. Also, animals stepping on the casing soil will pack it down and make it too hard, resulting in breaks in the mycelium. Thus, management practices to keep animals off the mushroom plot must be employed. If examination of the plot shows that the casing soil is thin in places, supplemental casing should be added to those places.

7. Fruiting

A year after the inoculated logs or other substrate have been covered, the mycelium will have grown out from the inoculum and penetrated the substrate. The mycelium will also have grown into the casing layer and extended to the surface. In May–June of the second year, the eggs will appear on the surface. As previously mentioned, the eggs and rhizomorphs attached to them undergo synchronous expansion. With good rain from the middle of June to the middle of July, the egg will expand very rapidly, and then overnight the mature egg can develop into a fully expanded fruiting body.

Fruiting occurs in a much shorter time if the substrate material consists of pieces of wood or bamboo than if it is a wood log. With pieces of wood or bamboo, a period of only 2 to 3 months is required for vegetative growth, and the formation of fruiting bodies then occurs. Development is even quicker when the substrate material consists of bamboo leaves.

Although fruiting is slow to start with the wood log method, harvests may be obtained for 3 to 4 years; whereas harvests may be taken for only 1 to 2 years with a substrate of chips and pieces, although the fruiting does take place earlier.

Hu et al.[2] reported on some small-plot studies which provide us with some information on the amount of spawn required and the yields on various substrates. The spawn required for 40 kg of logs is two bottles. Harvests were obtained for 3 years: (1) the first year the average harvest for 40 kg of logs was 30 g (dry weight) with a maximum of 120 g; (2) the second year the average was 20 g with a maximum of 80 g; (3) the third year was much lower than the second.

The amount of spawn required for 10 kg of bamboo leaf was one bottle. With this substrate, harvesting can start 2 months after spawning, and within that same year 50 g of dry mushrooms can be harvested.

B. Indoor Cultivation

It was stressed in Section III.A that a high moisture condition is essential for the successful cultivation of *Dictyophora*. This is obviously easier to control in indoor cultivation than in forest cultivation, as are other important factors, such as light, temperature, and protection against pests. The selection of materials (e.g., for substrate, for spawning, and for covering the substrate) is the same for indoor cultivation as for forest cultivation, but there are some features of indoor cultivation for which some additional comments may be helpful in understanding how *Dictyophora* cultivation is performed in China.

1. The Mushroom House

The temperature of the mushroom house should not exceed 30°C for growth of *Dictyophora*, a mesophilic fungus. Therefore, the orientation of the house should be in an east–west direction to

avoid direct sunlight shining on the house, as in summer months this would raise the temperature above 30°C. The structure of the house should include false ceilings as an aid to insulation, and the house also should have low windows to facilitate ventilation.

Within the house, the bedding for support of the substrate materials for mushroom growth should be 1 m wide, 2 m high, and there should be three or four layers with 60 cm between each layer.

2. Containers for Cultivation

The container selected for cultivation of *Dictyophora* should be resistant to rotting, e.g., ceramic pots or plastic boxes such as those used to hold beer bottles, because the mycelium must grow for a long time. A layer of stones, about the size of eggs, should be placed at the bottom of these containers to facilitate drainage, and the bottom of the container should have some holes for the same purpose. A sheet of plastic should be placed in the container so that it covers the bottom and extends up the sides. This plastic sheet, which keeps the small particles of substrate from gravitating to the bottom of the container among the stones, should also have some holes in the bottom for drainage.

Dictyophora can be grown in a tray system similar to that used for the growing of *Agaricus* on compost.

3. Cultivation

The mixed substrate method is used for indoor cultivation. Sawdust alone is not adequate for cultivation, because it does not provide a well-aerated substrate. Therefore, a mixed substrate is used in which sawdust is combined with bamboo or tree branches, or bamboo leaves, or the leaves of broad-leaf trees, or wood chips. Spawning is usually done in the spring and harvesting in the summer. When the temperature rises to 25°C, usually 1 to 3 months after spawning, good vegetative growth takes place. After another 2 to 3 months, this will be sufficient time for subsequent developmental stages to take place.

4. Management

The main consideration in management of indoor cultivation is the control of moisture content. A sprinkler should be used for watering. A wet and dry bulb thermometer should be present in the mushroom house for determination of the relative humidity. The moisture content of the substrate should be determined either by the hand squeeze method or by a soil moisture tester. The windows should be opened once or twice daily for a half hour to permit air exchange. In hot weather the windows should be opened once in the morning and once in the evening. In cold weather the windows should be opened only once, at noon.

IV. HARVESTING AND PROCESSING

A. Harvest Time and Method

Extreme care must be taken to harvest the mushrooms of *Dictyophora* at the proper stage of development of this delicate structure. When the skirt flares, the mushroom should be harvested at once (Figure 18.5). A knife should be used to cut off the mushroom at the base of the volva, because pulling out the fruiting body by hand damages the rhizomorphs that are attached to the base of the volva and extend out into the substrate.

The pileus and volva of the mushroom that has just been harvested should be removed immediately and attention given to the skirt and stalk, which are the commercially important parts of the mushroom of *Dictyophora*. If soil or substrate adheres to the surface of the stalk or skirt, the mushroom must first be cleaned. A soft brush should be used to remove dirt from the body of the

FIGURE 18.5 Fresh mushrooms of *Dictyophora* just harvested (right) from the mushroom house. The dried mushrooms are packed in a plastic bag (left).

mushroom. The brush must be applied very gently to avoid damaging the skirt and stalk, which are then put on a rack and placed in sunlight for drying. This must be done immediately, for if drying is delayed for 2 hours, it may result in a lowering of the quality of the mushroom by one grade.

B. Rapid Drying

If the mushroom has been washed for cleaning, it should be placed in a well-ventilated place for drying and not directly into a dryer while wet, as this tends to make the color darker, which also lowers the grade. The quality of the mushroom that has been sun-dried is good, but there are other effective means of drying, including (1) using infrared lamps or electric heaters; (2) placing the mushrooms on a grid above the smokeless, flameless, red-hot coals of charcoal; and (3) placing the mushrooms in the heated air above a metal plate over a coal fire.

There are certain precautions that should be taken in drying the mushrooms of *Dictyophora*:

1. When drying with charcoal or coal, the mushrooms should not be exposed to smoke, which they strongly absorb. The mushrooms should be dried by heat without smoke, and a baking box or baking room can accomplish this.
2. The drying temperatures should be changed from 40 to 60°C and then back to 40°C, with an exposure of 2 hours at each temperature stage.
3. Removal of moisture from the drying chamber is essential. Thus, at the top of the baking room or baking box there must be a hole to remove the moist air, and at the bottom there must be a hole for the inflow of fresh air. This may require a blower.
4. The baking rack should be clean, dry, and rubbed lightly with grease to prevent the mushrooms from sticking to the rack.
5. Because dry mushrooms break easily, the rack and mushrooms should be removed together from the baking box, very carefully; 20 to 30 minutes after removal, the mushrooms will have softened, and they can be tied in bunches.

C. Grading and Packaging

Grading of *Dictyophora* is done according to size, color, and extent of damage. On these criteria mushrooms are divided into four different grades:

1. **First Grade:** The length of the mushroom is 12 cm, the width is 4 cm, the color is white, and it is without damage.
2. **Second Grade:** The length of the mushroom is 10 to 11 cm, the width is 3 cm, the color is light yellow, and it is without damage.
3. **Third Grade:** The length of the mushroom is 8 to 9 cm, the width is 2 cm, the color is yellowish, and there is slight breakage.
4. **Fourth Grade** (lowest grade): The length of the mushroom is less than 7 cm, the color is dark, and there is some breakage.

Packing is done according to grading. Plastic bags are used as containers. As packaged in China, one bunch of *Dictyophora* mushrooms weighs 20 to 50 g, and it is tied neatly with red strings at both ends. Amounts of 600 g (i.e., 12 to 30 bunches) are put in small bags; two of these small bags are combined in a middle-sized bag (Figure 18.5); and, finally, four middle-sized bags are put into a paper box. Inside the box, one or two layers of moisture-absorbing paper are arranged with the bags of mushrooms, and the box is then sealed with cellophane tape. Even at the lowest price quoted for *Dictyophora* recently, U.S. $100 per kg, this box would have a value of U.S. $480, and, if First Grade (top grade), it should bring a price of twice that amount, U.S. $960. Remember that only the skirt and stalk are included in this packaged material and, as the ratio of volva to skirt plus stalk is 2:1, 2 kg of dry volva is discarded for each kilogram of dry skirt and stalk. Thus, the interest expressed by Hu et al.[2] in utilizing the volva.

Today, *Dictyophora* is not a mushroom in the everyday diet of the man on the street, but with the availability of techniques for cultivation of the mushroom indoors, its numbers are increasing and its price decreasing. One need not dine with heads of state to be served bamboo sprouts. No longer is *Dictyophora* eaten rarely or only by the very few.

REFERENCES

1. Chang, S.T. and Miles, P.G., A new look at cultivated mushrooms, *BioScience*, 34, 358–362, 1984.
2. Hu, N.C., Zou, F.L., Zhou, W., Chan, C.B., and Zhang, K.C., The technique of artificial cultivation of *Dictyophora, Edible Fungi of China,* 3, 26–27, 1986; 4, 20–22, 1986; 5, 20–21, 1986; 6, 24–26, 1986; 1, 25–26, 1987.
3. Huang, Y., *The Basic Principles and Practical Experiences of Mushroom Cultivation,* Xiamen University Press, Xiamen, Fujian, China, 358–376, 1992.
4. Jiang, W. and Wong, W.S., A preliminary study of spawn making of *Dictyophora, Edible Fungi,* 3, 11, 1987.
5. Kinugawa, K., On the growth of *Dictyophora indusiata*, II. Relations between the change in osmotic value of expressed sap and the conversion of glycogen to reducing sugar in tissues during receptaculum elongation, *Bot. Mag*. (Tokyo), 78, 171–176, 1965.
6. Lin, J.N., Zheng, C.R., Zhang, Q.L., and Liu, Q.M., Studies on the artificial cultivation of *Dictyophora duplicata* (Bosc) Fisch., *Mushroom Newsl. Trop*., 2(3), 14–15, 1984.
7. Wang Cheng, *Book of Agriculture*, China, 1313.
8. Yang, Q.Y. and Jong, S.C., Artificial cultivation of the veiled lady mushroom, *Dictyophora indusiata,* in *Proc. Int. Symp. Scientific and Technical Aspects of Cultivating Edible Fungi*, Pennsylvania State University, University Park, July, 437, 1986.
9. Ying, J., Mao, X., Ma, Q., Zong, Y., and Wen, H., *Icons of Medicinal Fungi from China,* Science Press, Beijing, China, 468–471, 1987.
10. Zhang, P. and Jiang, Y., *Domestication and Cultivation of* Dictyophora *in China*, Shanghai General Science Press, Shanghai, 279, 1992.

19 *Ganoderma lucidum* — A Leader of Medicinal Mushrooms

I. INTRODUCTION

Ganoderma lucidum (Curt.: Fr.) P. Karst. (Lingzhi in Chinese; Reishi, Mannentake, or Sachitake in Japanese; and Youngzi in Korean) is a species of the class Basidiomycetes, which belongs to the family Polyporaceae (or Ganodermataceae) of the order Aphyllophorales. Commonly known as a wood-decaying fungus, it causes white rot of a wide variety of trees and can thus be described as a **phytopathogenic fungus**. Because of its perceived health benefits, its fruiting body has gained wide popularity in recent years as a dietary supplement, not only in China and Japan, but also in North America and other parts of the world. The reason it attracts international attention as a valuable Chinese herb is due to the wide variety of its biological activities, such as antitumor, immunomodulatory, cardiovascular, respiratory, antihepatotoxic, and antinociceptive (acting against pain) effects. The diversity in the biological actions of Lingzhi may be attributed to the fact that it is composed of different chemical entities, including alkaloids, amino acids and peptides, inorganic elements, steroids, and fatty and organic acids. The major compounds with significant pharmacological activities appear to be triterpenes and polysaccharides. It is interesting that during the last three decades, more than 150 triterpenes[26] and more than 50 carcinostatic polysaccharides[24] have been isolated and are known to be unique compounds in this mushroom. Therefore, *G. lucidum* products with different triterpenes and polysaccharides or combinations of these two groups are most likely to result in different pharmacological activities.[38]

According to Chinese tradition, Lingzhi is also known as the "miraculous zhi," or "auspicious herb," and it is usually considered to "symbolize happy augury, and to bespeak good fortune, good health and longevity, even immortality."[63]

Ganoderma is now consumed worldwide as a health tonic and as a dietary supplement.[7] Millions of people take it every day to enhance their energy, to improve their digestion, and to sleep better. *Ganoderma* is used also both for the prevention and for the treatment of a number of health problems that require a balanced immunoresponse system and also a healthy cardiovascular system.

Under the influence of Chinese culture, the ideas and convictions associated with Lingzhi spread to Korea and Japan where in the early days they tended to be confined to the literate and intellectual classes. In reality, Lingzhi encompasses several *Ganoderma* species, which are used widely for medicinal purposes: e.g., *G. lucidum, G. luteum* Steyaert, Bull., *G. atrum* Zhao, Xu et Zhang, *G. tsugae* Murr., Bull., *G. applanatum* (Pers. ex Wallr.) Pat., *G. australe* (Fr.) Pat., *G. capense* (Lloyd) Teng, *G. tropicum* (Jungh.) Bres., *G. tenus* Zhao, Xu et Zhang, and *G. sinense* Zhao, Xu et Zhang. According to the two famous Chinese herbal medical books, *Shen Nong Ben Cao Jing* (A.D. 25 to 220, Eastern Han Dynasty) and *Ben Cao Gang Mu* by Li Shi-Zhen (A.D. 1590, Ming Dynasty), there were six known Lingzhi species or varieties in China at that time, whereas now over 90 species have been reported.[66] Worldwide, over 250 *Ganoderma* species have been described.[2,56] However, in therapeutic practices and literature citations, Lingzhi (*Ganoderma)* usually refers to the species *G. lucidum.*

Ganoderma-based products have attracted a great deal of attention during the last decade, not only in Singapore and Malaysia, but also in North America and Europe. The main producers and suppliers of this medicinal mushroom are China, Korea, and Japan. Although definitive figures for *G. lucidum* production worldwide are difficult to obtain, total production of the mushroom in Japan during 1995 was estimated at approximately 500 MT on a dry weight basis.[48] According to Zhou and Gao,[67] estimates for 1997 of the worldwide production of *G. lucidum* were approximately 4300 MT, of which China contributed 3000 MT (dry weight) with about 1500 MT exported, mainly to Korea, Japan, Singapore, and Taiwan. The market value of *Ganoderma* produced worldwide during 1995 was estimated to be U.S. $1628.4 million.[66]

Of particular concern at present is the low reproducibility in terms of production methods and the often poor quality control in some *Ganoderma*-based products. For various reasons (seasonal variations, different soil conditions, stage of fruiting body development) the fruiting body and hence the product quality are very difficult to control. Moreover, manufacturers of *Ganoderma*-based products normally rely on several sources to provide the fruiting bodies used to produce the product. These different sources often show considerable variation with respect to both the quality of the mushroom fruiting bodies and to the processed product. Unfortunately, the perceived future growth of this sector has also resulted in the appearance of an ever-increasing number of less-reputable companies whose activities will inevitably lead to more intensive scrutiny of the sector as a whole. Therefore, it is of paramount importance for the manufacturing industries involved to develop and adopt acceptable and reproducible protocols both for growing the raw materials and for downstream processing to ensure high-quality, standardized, and safe *Ganoderma* products. Such practices are essential for earning and maintaining the enduring public trust, which is vital for securing an expanding market in the future. In summary, it can be said that *G. lucidum* is one of the earliest medicinal mushrooms that has been considered to be of superior grade, i.e., that it is a nontoxic tonic herb without side effects even when taken for a long period. For the last three decades it has been and will continue to be, we believe, one of the most promising and intensively studied mushrooms for medicinal and tonic purposes.

II. BIOLOGICAL CHARACTERISTICS

A. Taxonomic Characteristics

Ganoderma is probably the most morphologically complex genus of polypores. An indication of this problem is the 290 taxonomic names that have been published in the genus.[57] It has been suggested that the reason for this great proliferation of names is partly due to their beautiful and easily preserved basidiocarps and partly due to their medicinal value. The genus, established by Karsten,[25] involves wood-rotting Basidiomycetes with laccate and nonlaccate pilei. Historically, laccate taxa were referred to as the *G. lucidum* complex, and nonlaccate species as the *G. applanatum* complex. The Latin word *lucidus* means "shiny" or "brilliant" and refers to the varnished appearance of the surface of the mushroom. The *G. lucidum* complex is composed of species with annual fruiting bodies having a yellow to reddish laccate cuticle and an upper layer that is smooth or often concentrically zoned and grooved. The surface is sometimes covered with brownish spore powder. The shape is variable: circular to semi-circular or fan shaped or kidney shaped in outline (Figure 19.1). In the *G. applanatum* complex, the fruiting bodies are perennial with a brown to black cuticle with the upper layer of the fruiting bodies composed of a hard surface crust that is usually cracked, furrowed, ridged, and/or lumpy or knobby in age, but not varnished. In Latin, the word *applanatum* means flattened.

The taxonomy of *Ganoderma* species usually has been based on classical descriptive criteria. As a result, the concept of a species in the genus is neither well established nor universally accepted. In recent years, several alternative taxonomic approaches have been proposed. These include biochemical tests, sequence analyses of ribosomal genes and spacers,[51,52] and rDNA analyses.[15]

FIGURE 19.1 Two fruiting bodies of *Ganoderma lucidum* showing its common kidney shape.

These studies have helped to clarify the distribution of the different species' complexes in the genus; however, our knowledge of the physiology and DNA variation of *Ganoderma* species is still only partial. Clearly, more sequence data and an increased taxonomic sampling are needed and a detailed classical analysis of morphological data in a total evidence framework would also help to untangle this problematic genus.[15] In medical studies and therapeutic practices, *G. lucidum* is far more important than any one of the others.

B. Morphological Characteristics

Fruiting bodies are stipitate, dimidiate, or reniform and rarely suborbicular. They are thick, corky, and yellowish in the growing margins and then turn to brownish in the matured part with shining laccate on the surface. The margin of fruiting bodies is usually thin or truncate and often slightly incurved. The stipe is lateral and rarely eccentric. It is thick, dark, and then turns a purple-brown. The stipe's laccate is more conspicuously shiny than that of the pileus. Pores are white at first and become light brown at a later stage. Spores are brown, ovoid, and truncate at one end. The epispores are smooth and the endospores are rough with a large central gutta (or drop).[65]

C. Growth Parameters

Ganoderma lucidum is found more frequently in subtropical regions than in the temperate zones. It is an annual mushroom, growing on a wide variety of dead and dying trees. The growth parameters[8,41,58] are summarized as follows:

1. **Temperature:** Temperature for mycelial growth ranges from 15 to 35°C and the optimum temperature is 24 to 25°C; for primordia initiation it ranges from 18 to 25°C; and for fruiting body development the range is 20 to 25°C.
2. **Water Content in Substrate:** Should be maintained at 60 to 65%.
3. **Relative Humidity:** Mycelial running in the range of 60 to 70%; primordia initiation, 85 to 90%; fruiting development, 70 to 85%.
4. **Air:** During the fructification period, good ventilation is necessary.
5. **pH Value:** The optimum for mycelial running is 5.0 to 5.5.
6. **Light:** During primordia initiation, light is required at about 500 to 1000 lux; and for fruiting body development, 750 to 1500 lux.

III. CULTIVATION OF *GANODERMA LUCIDUM*

Although the medicinal value of *G. lucidum* has been treasured in China for more than 2000 years, the mushroom was found infrequently in nature. This lack of availability was largely responsible for the mushroom being so highly cherished and expensive. During ancient times in China, any person who picked the mushroom from the natural environment and presented it to a high-ranking official was usually well rewarded. Even in the early 1950s, this custom continued and the mushroom was presented to Chinese leaders, both in Mainland China and in Taiwan, following its occasional discovery in the wild. In nature, Lingzhi is distributed in temperate, subtropical, and tropical regions and in China grows mainly in the southeastern regions. As the supply of the wild mushroom is limited and as there is great difficulty to properly control the quality of its fruiting bodies in nature, it has become increasingly popular to use Lingzhi that has been grown in a controlled environment.

Artificial cultivation of this valuable mushroom was successfully achieved in the early 1970s,[49] and, since 1980 particularly in China, production of *G. lucidum* has developed rapidly. Similarly, as for other cultivated edible mushrooms, the process for producing *G. lucidum* fruiting bodies can be divided into two major stages. The first stage involves the preparation of the fruiting culture, stock culture, mother spawn, and planting spawn, and the second stage entails the preparation of the growth substrates for mushroom cultivation. Currently, the methods most widely adopted for commercial production are the wood log, short wood segment, tree stump, sawdust bag, and bottle procedures.[8,22,23,45,49,58]

Examples of cultivation substrates, using plastic bags or bottles as containers, include the following (note that these examples are for reference purposes only and can be modified according to the strains selected and the materials available in different localities):

1. Sawdust 78%, wheat bran 20%, gypsum 1%, and soybean powder 1%
2. Bagasse 75%, wheat bran 22%, cane sugar 1%, gypsum 1%, and soybean powder 1%
3. Cottonseed hull 88%, wheat bran 10%, cane sugar 1%, and gypsum 1%
4. Sawdust 70%, corncob powder 14%, wheat bran 14%, gypsum 1%, and cereal straw ash 1%
5. Corncob powder 78%, wheat/rice bran 20%, gypsum 1%, and straw ash 1%

After sterilization, the plastic bags can be laid horizontally on beds or on the ground for fruiting.

Log cultivation methods include the use of natural logs and tree stumps, which are inoculated with spawn directly under natural conditions. A third alternative technique involves the use of sterilized short logs about 12 cm in diameter and approximately 15 cm long, which allow for good mycelial running. The wood logs should be prepared from broad-leaf trees, preferably from oak. Felling of the trees is usually carried out during the dormant period, which is after defoliation in autumn and prior to the emergence of new leaves the following spring. The optimum moisture content of the log is about 45 to 55%. The sequence for the short-log cultivation method is as follows:

1. Selection and felling of the tree
2. Sawing/cutting the log into short segments
3. Transfer of segments to plastic bags
4. Sterilization
5. Inoculation
6. Spawn running
7. Burial of the log in soil
8. Tending the fruiting bodies during development from the pinhead stage to maturity
9. Harvesting of the fruiting bodies
10. Drying of the fruiting bodies by electrical driers
11. Packaging

FIGURE 19.2 (Color figure follows p. 232.) Fruiting bodies of *Ganoderma lucidum* grown on the short wood segments, which are buried in soil. The fruiting bodies are still actively growing as indicated by the yellowish color at the margins.

It should be noted that the prepared logs or segments are usually buried in soil inside a greenhouse or plastic shed. The soil should allow optimum conditions of drainage, air permeability, and water retention, but excessive humidity should be avoided. This method provides for a short growing cycle, higher biological efficiency, good quality of fruiting bodies (Figure 19.2) and, consequently, superior economic benefit. However, this production procedure is clearly more complex, and the production costs much higher than natural log and tree stump methods.

IV. TRADITIONAL USES

Lingzhi has played an important role in Chinese traditional medicine solely or in combination with other herbal medicines. Uses in ancient folk medicine include treatment for a "tight chest," to improve intellectual capacity and memory, to promote agility, to lengthen life span, and to relieve hepatopathy, nephritis, hyperlipemia, arthritis, asthma, gastric ulcer, arteriosclerosis, leukopenia, diabetes, and anorexia.[4,24] Contemporary traditional Chinese medicine (TCM) using cultivated *Ganoderma* mushrooms[5] includes treatment for neurasthenia, debility from prolonged illness, insomnia, anorexia, dizziness, chronic hepatitis, hypercholesterolemia, mushroom poisoning, coronary heart disease, hypertension, altitude sickness, carcinoma, bronchial cough in elderly individuals,[65] and inhibition of cholesterol synthesis.[27] For a long time in China, *Ganoderma* has been known as a kind of panacea in the folklore, curing all kinds of diseases.[43] All ancient Chinese Materia Medica have treated Lingzhi as of superior grade, which as indicated previously means that it is a nontoxic tonic herb without side effects even when taken for a long period at a high dosage.

According to oriental tradition, some *Ganoderma* products are treated as forms of herbal medicine, and others as a "health food." Actually, *G. lucidum* should not be classified as an edible mushroom since its fruiting bodies are always thick, corky, and tough, and do not have the fleshy texture characteristic of true edible mushrooms, e.g., *Agaricus bisporus* and *Lentinula edodes* (Berk. Pegler). However, its mycelial products or the extracts from fruiting bodies can be used as additives to food or drinks, which are then marketed as health food or "health drinks." In most cases, the mushroom products are crude extracts or refined or partially refined extractives, which cannot be termed pharmaceuticals. It is generally understood that conventional drugs contain only one active ingredient and that this single compound has been extensively tested for safety and efficacy through the use of comprehensive animal studies and clinical trials on actual patients. Therefore, in terms of their sale as drugs or pharmaceuticals is concerned, *Ganoderma* products still face difficulties

because they do not meet the requirements of pharmacological and government drug control regulations. However, observational experience of the long-term therapeutic effects of *Ganoderma* and the recognition of the pharmacological properties of the mushroom based on systematic animal studies conducted over the past 20 years clearly show that some *Ganoderma* components possess important bioactivity. This applies particularly to those bioactive compounds extractable with hot water (polysaccharides) or alcohol (triterpenes), which show great potential as superior dietary supplements or "nutriceuticals" for enhancing human immune responses. It is also noteworthy that *Ganoderma* products have repeatedly been reported to be lacking significant toxicity and side effects. Extractable *Ganoderma* products may be better and more readily accepted as nutriceuticals than as pharmaceuticals.

V. BIOLOGICAL COMPOUNDS

Recent application of modern analytical techniques has, in a number of cases, provided a scientific basis for these earlier empirical observations. *Ganoderma* has been reported to have multiple beneficial values and concerted medicinal effects on various diseases. The diversity in the beneficial values and medicinal effects may be attributed to the fact that Lingzhi is composed of a vast number of bioactive compounds. The major compounds with significant pharmacological activities appear to be triterpenes and polysaccharides[42] although bioactive proteins, nucleic acids, and other substances have also been identified. Of particular interest are a group of fungal immunomodulatory proteins (FIPs), which have been isolated from *G. lucidum, G. tsugae,* and other mushrooms.[30,32,33] Those FIPs have been classified into a distinct family of proteins on the basis of similarities in their amino acid sequences and immunological effects. During the last two decades, more than 200 substances have been isolated from *Ganoderma* fruiting bodies, spores, cultivated mycelium, and culture broth, and some of their chemical and physical structures are known. However, only some of these triterpenes and polysaccharides are treated in this chapter.

A. Triterpenes–Triterpenoids

Kubota et al.[34] were the first to isolate the triterpene compounds, ganodermeric acids A and B, from *G. lucidum*. Since then, the physiochemical properties of more than 150 lanostane-type triterpenoids found in *G. lucidum* have been identified and divided into ten groups according to their structural similarities and the known biological and medicinal activities.[26] These compounds have been isolated from fruiting bodies, spores, as well as mycelia. Their chemical structure is based on lanosterol, which is an important intermediate in the biosynthetic pathway for steroids and triterpenes in microorganisms and animals. Triterpenes have cytotoxic, hepatoprotective, and hypolipidemic properties. They influence platelet aggregation, and inhibit angiotensin-converting enzymes and histamine release.[26,41,42,50] The different triterpenes appear to have different bioactive properties. Some of these triterpenes have shown the following activities:

1. Bitterness

Ganoderma lucidum has a remarkably strong bitterness, which has not been found in any other mushroom, and the bitterness varies in strength depending on the place of production, cultivation conditions, strain used, etc. It has been observed that the stipe has a stronger bitterness than the pileus. However, such bitterness has not been found in cultured mycelia or in the culture broth. Although the bitterness has not been found to be related to any pharmacological effects, the bitterness attracts attention as a marker substance for pharmacological evaluation and chemical quality judgment. From a marketing point of view, some customers, e.g., Koreans, are only interested in the *Ganoderma* products with bitterness. The mushroom produces bitterness during the course of fruiting and the bitterness of fruiting bodies is contributed to by substances, such as ganoderic acids

A, C, I, and J; lucidenic acids A, D, and I; and lucidones A and C. These triterpenoids are generally classified as at least two types: one is a C_{30} ganoderic acid type and the other is a C_{27} lucidenic type.[54]

2. Cytotoxicity

Cytotoxic triterpenes are potential anticancer agents and some cytotoxic compounds have been isolated from the mycelial and fruiting body extracts. It is reported that ganoderic acids Z, Y, X, W, V, and T from the mycelia demonstrated cytotoxic activities *in vitro* on hepatoma cells, and several lanostanoids isolated from fruiting bodies exhibited potent inhibition of tumor cells *in vitro*.[26]

3. Platelet Aggregation Inhibition

Platelets in the blood are normally nonreactive to intact vascular endothelium. However, they respond quickly when vascular damage occurs by adhesion, aggregation, and coagulation. Inhibitors of platelet aggregation have potential for the treatment of apoplexy. Ganoderic acid S showed an amphipathic effect on the platelet aggregation.[59,61]

4. Antihypertension

Morigiwa et al.[53] found that ganoderic acid F had the strongest antihypertension activity, and other terpenoids such as ganoderic acids B, D, H, and Y had weaker effects.

5. Hepatoprotective Activity

In Chinese folk medicine, the fruiting bodies of *G. lucidum* have been used for the treatment of chronic hepatitis. Hirotani et al.[20] found that ganoderic acids R and S from the cultured mycelia showed strong antihepatotoxic activity in the galactosamine-induced cytotoxic test with primary-cultured rat hepatocytes. Another hepatoprotective compound, ganosporeric acid A, was isolated from the ether soluble fraction of the spores of this mushroom by Chen and Yu.[9,10]

6. Anti-HIV

Human immunodeficiency virus (HIV) was isolated as an etiological agent of acquired immunodeficiency disease syndrome (AIDS).[1,14] Anti-HIV activities were reported in a water-soluble extract of *G. lucidum*.[17,28] More recently, el-Mekkawy et al.[12] isolated the anti-HIV compounds and reported them as Ganoderiol F and ganodermanontriol. Min et al.[47] also isolated anti-HIV components as ganoderic acid β, ganodermanondiol, ganodermanontriol, ganolucidic acid A, and lucidumol B.

7. Hypoglycemic Effects

Several triterpenes, e.g., ganoderans A, B, and C, isolated from *G. lucidum* fruit bodies have been shown to have a strong hypoglycemic effect.[19] The side effect of the drugs for diabetes could be minimized with complementary *Ganoderma* products. Patients with diabetes should also be concerned about other serious diseases, which could occur concurrently, e.g., coronary artery disease due to a thickening and hardening of medium-size and large arteries with narrowing of the arterial lumen by atherosclerotic plaques, hypertension, weakening of the immune system, eye diseases, etc. The major components of *Ganoderma* products are reportedly good for preventing or alleviating these concurrently occurring diseases.

B. Polysaccharide

Some years ago, Lee et al.[35] reported that water-soluble extracts of *G. lucidum* inhibited the growth of Sarcoma 180 and a fibrosarcoma in mice. Subsequently, a polysaccharide moiety present

TABLE 19.1
Bioactive Polysaccharides Isolated from Different Sources of *G. lucidum*

Source	Major Active Sites or Compounds	Pharmacological Activity	Ref.
Fruiting body	β-(1→3)-D-Glucan	Antitumor	50
Mycelia	β-(1→3)-D Glucan with β-(1→6) branches	Antitumor	50
Culture broth	Glucoprotein	Swimming endurance capacity	64
Spore	Aqueous extract	Myotonia and polymyositis	—

predominantly in the ethanol-precipitable fraction of the aqueous extract was found to increase the life-span of tumor-implanted mice, to exhibit antitumor effects against fibrosarcoma in C3H mice, and to inhibit metastasis of the tumor in the lung.[13,18,36] Since these initial reports, more than 50 carcinostatic polysaccharides have been isolated from the basidiocarps and mycelium of *G. lucidum*.[24] Many polysaccharides were extracted with hot water, ammonium oxalate solution, alkali solution, dimethyl sulfoxide solution, and supercritical fluid CO_2, and separated by various chromatographic methods. Strong antitumor activities were found in various hetero-β-D-glucans having a β-(1→3)-D-glucan branch as the active site, such as β-D-glucan, glucurono-β-D-glucan, arabinoxylo-β-D-glucan, xylo-β-D-glucan, manno-β-D-glucan, and xylomanno-β-D-glucan, as well as being present in their protein complexes.[50] These compounds have also been ascribed hypoglycemic properties.[41,42,50] Recently, a glycoprotein (containing 82.8% carbohydrate and 17.2% protein) obtained from the mycelium culture broth of *G. lucidum* exhibited an increase in the swimming endurance capacity of mice. However, the fruiting body and mycelium of the mushroom did not show any positive response in this regard.[64] The extracts from different sources (fruiting body, mycelium, and culture broth) have different properties with different functions (Table 19.1). It has been widely reported that the antitumor and anticancer effects of the polysaccharides are based on the enhancement of the host's immune system rather than direct cytocidal effects.[11,37,62,68] The various components of the immune system shown to be affected include activated macrophages, natural killer cells, and cytotoxic T cells together with their secretory products, such as tumor necrosis factor, reactive nitrogen and oxygen intermediates, and interleukins.[55] These extractable polysaccharide compounds may increase the expression level of M-CSF (macrophage-colony stimulating factor) in both splenocytes and peritoneal exudate cells of the mouse. The expression level of TNF-α (tumor necrosis factor-α), one of the antitumor molecules that has direct antitumor activity, was also up-regulated. Therefore, the overall antitumor effect of *G. lucidum* compounds may be due to the up-regulation of certain cytokines such as M-CSF and TNF-α. It should be noted that immune responses are complex reactions involving several types of cells such as macrophages and lymphocytes. The killing mediated by cytotoxic T lymphocytes and natural killer cells represents an important mechanism in immune defense against tumors, virus-infected cells, parasites, and other foreign invaders.

C. Fungal Immunomodulatory Protein

More recently, various mushroom species have been reported as the source of a new group of fungal immunomodulatory proteins (FIPs). These include LZ-8 (Ling Zhi-8) from *G. lucidum*,[30] FIP-fve from *Flammulina velutipes* (Gr.) Sing.,[32] FIP-vvo from *Volvariella volvacea* (Bull.: Fr.) Sing.,[21] and FIP-gts from *G. tsugae* Murr.[40] Definition of this group of proteins, which have been classified into a distinct family by Ko et al.,[32] is based on similarities in their amino acid sequences and their effects on components of the immunological responses. LZ-8, isolated and purified from the mycelium of *G. lucidum*, showed optimal stimulatory activity of blast-formation toward mouse spleen cells at a concentration of 3.13 μg/ml.[30] LZ-8 is not a lectin as it lacks hemagglutination activity toward human red blood cells, but it possesses mitogenic activity toward mouse spleen

cells and human peripheral blood leucocytes. In addition, this FIP can suppress autoimmune diabetes in young female non-obese diabetic mice[31] and also has a significant effect on cellular immunity as demonstrated by its effect in delaying the process of rejection of transplanted allogeneic pancreatic rat islets and allografted mouse skin.[60]

D. Steroids

Ganoderma lucidum also contains a high amount of steroids. There are two categories of steroids, ergosterol and cholesterol. Approximately 20 different steroids have been isolated from this mushroom.[16,44] These steroids appear to account for the anti-atherosclerotic and lipid-lowering effects of *G. lucidum*.[29]

VI. CONTEMPORARY USES

There is intense industrial interest in a novel class of compounds extractable from either the mycelium or fruiting body of *Ganoderma lucidum*. These compounds mentioned above exhibit either medicinal or tonic qualities and have immense potential as dietary supplements for use in the prevention and treatment of various human diseases. Should those *Ganoderma* products be categorized as health foods, nutriceuticals (which are also called dietary supplements or complementary medicines), or pharmaceuticals?

Unfortunately, many people confuse nutriceuticals with **functional foods**, which are consumed as part of the normal diet and may have been modified or enriched in some way to provide health-giving benefits. A **nutriceutical**,[5] on the other hand, is a refined or partially refined food extractive, which is consumed in the form of capsules or tablets as a dietary supplement or complementary medicine (not a food), and which has potential therapeutic applications. A regular intake can enhance the immune response of the human body, thereby increasing resistance to disease and, in some cases, cause regression of a diseased state. As *Ganoderma* nutriceuticals are extracted from a very well-known mushroom species, there is again little likelihood of toxicity and overdose. Although some degree of control by the regulatory agencies is both necessary and desirable, accessibility would be comparable with over-the-counter medicines. Nor are nutriceuticals to be confused with **pharmaceuticals**, which are almost invariably a defined chemical preparation, the specifications of which are listed in a pharmacopoeia. Pharmaceuticals possess medicinal properties and are mainly used therapeutically for the treatment of specific medical conditions. Because many pharmaceuticals have a high potency, there is often a considerable potential for toxicity and, for this and other reasons (e.g., purity and sterility), their production and usage is subject to close control by regulatory agencies. There is a wide range of availability from over-the-counter medicines to strictly controlled dangerous drugs. The main features of functional foods, nutriceuticals, and pharmaceuticals are summarized in Table 19.2.

Because the *Ganoderma* products are hot water extractable complex compounds rather than single-chemical components, they should be considered a kind of nutriceutical or dietary supplement or complementary medicine, not as a health food or as a pharmaceutical. Scientific evidence is increasingly suggesting that the various compounds occurring in *Ganoderma* products generate an immense immune-response enhancement through concerted effects. However, it should be emphasized that good, honest products obtained through an acceptable protocol for growing the materials and processing the products are of paramount importance in earning enduring public credibility and securing an expanding market in the future.

VII. PRODUCTS OF *GANODERMA LUCIDUM*

According to Chinese tradition, *G. lucidum* fruiting bodies were either boiled in hot water or soaked in Chinese liquor when used for medicinal purposes. These traditional methods for demonstrating

TABLE 19.2
The Main Features of Functional Foods, Nutriceuticals, and Pharmaceuticals

	Composition	Usage/Activity	Mode of Delivery	Degree of Control by Regulatory Agencies	Accessibility
Nutraceutical	Modified or enriched food material	Maintenance of healthy diet Low potency	Consumed as part of normal diet Little if any danger of overdose or toxicity	Low	Readily availability from general retail outlets
Nutriceutical	Refined or partially defined food extractives	Dietary supplement Medicinal properties Use primarily prophylactically but with potential for therapeutic value	Capsule/tablet Low toxicity Little danger for overdose	Medium	Comparable with over-the-counter medicines
Pharmaceutical	Almost invariably a defined chemical preparation with medicinal properties Specifications listed in a pharmacopoeia	Specific medical treatment Mostly therapeutic usage	Administered orally, inhalation, topically, injection High potency Toxic potential	High production, purity, and usage subject to high level of control	Ranges from over-the-counter medicines to strictly controlled dangerous drugs

Source: Data from Chang, S.T. and Buswell, J.A., *World J. Microb. Biotech.*, 12, 473, 1996.

the medicinal effects of the mushroom closely coincide with the modern techniques used to extract the polysaccharide (hot water) and the triterpene (alcohol) components, which constitute the two major groups of medicinal compounds in *G. lucidum.* The extracts or powders can be processed into different forms for administration, such as tablet, capsule, granule, and injection. The most popular form is the capsule.

There are several types of *G. lucidum* products currently available on the market. These include:

1. Whole fruiting bodies ground into a powder and then processed into capsule or tablet form.
2. Dried and pulverized mycelium harvested from submerged liquid cultures grown in fermentation tanks.
3. Dried and pulverized preparation of the combined substrate, mycelium, and mushroom primordia following inoculation of a semisolid medium with fungal mycelium and incubation until the primordia appear.
4. Hot water extracts of mycelium harvested from submerged liquid cultures grown in fermentation tanks, which have been evaporated to dryness and made up into capsule or tablet form.
5. Hot water or alcohol extracts of fruiting bodies (for extraction of the polysaccharide and triterpene components, respectively), which have been evaporated to dryness and made up into capsules or tablets either separately or integrated together in designated proportions.
6. Extract powder of fruiting bodies by supercritical fluid CO_2 extraction technologies, which contains a large spectrum of substances due to the low temperature used in processing.
7. Capsules of pure spore powder, which have been promoted forcefully in recent years with the medicinal effects still controversial. The topic of whether the walls of spores should be broken or removed also has been much debated. The walls could be removed or broken by mechanical methods; however, the answer is not yet clear whether the medicinal effects would actually be increased or enhanced by the processing. Lin[39] reported that unbroken spores contain higher crude fats and higher total sugars than the broken spores; however, broken spores contain more crude proteins and water-soluble polysaccharides than the unbroken spores. From this report, it can be seen that both types of samples have their pros and cons; therefore, it is difficult to come to any solid conclusions at this time. Furthermore, the fats of the broken spores are oxidized easily and produce some odd odors, thereby affecting the storage life and the quality of the products. However, extract of the spores was shown to be efficacious for hepatic protection and used clinically for the treatment of atrophic muscle rigidity.[10]
8. There are also other forms of *Ganoderma* products prepared as mixtures with other medicinal herbs. Occasionally, *Ganoderma* tea, *Ganoderma* beer, and *Ganoderma* hair tonic, and even *Ganoderma* flower pots for ornamental symbols of happiness, good fortune, and good health (Figure 19.3) have appeared in the market.

VIII. MARKET VALUE OF *GANODERMA LUCIDUM* PRODUCTS

Although there are no published figures relating to the market value of *G. lucidum* products worldwide, the market value of *Ganoderma*-based natural health-care products in Taiwan in 1995 was estimated at U.S. $215 million (*Commercial Week,* 7(15), 80–82, 1996). In the same year, the market values of mushroom products were roughly estimated by different commercial sources to be U.S. $350 million in China, U.S. $600 million in Korea, U.S. $300 million in Japan, U.S. $91.2 million in Malaysia, U.S. $60 million in Hong Kong, U.S. $2.2 million in Singapore, and U.S. $10 million in other countries, giving an estimated total world market value for *G. lucidum* products in 1995 of U.S. $1628.4 million.[6]

FIGURE 19.3 An ornamental *Ganoderma lucidum* flowerpot serves as a symbol of happiness, good fortune, and good health.

IX. A PROTOCOL FOR QUALITY MUSHROOM NUTRICEUTICALS

It is apparent that *Ganoderma*-based products can serve as superior dietary supplements or nutriceuticals. The problem is that the products are so diverse, and there is currently a lack of standard protocols for guaranteeing a reproducible high-quality product. Therefore, some of these products have no enduring public credibility. Consequently, there is serious need for improved quality control, which is essential both to increase and maintain consumer confidence, and to meet current and future standards set by the regulatory authorities.

It is essential that mushroom products be of good quality and free from potentially harmful substances. The following five guidelines are suggested as a protocol for obtaining quality products:

1. **GLP (Good Laboratory Practice):** A known mushroom strain must be used; the source and nature of the strain culture should be properly maintained and preserved without contamination and degeneration.
2. **GAP (Good Agriculture Practice):** Growth conditions must be known; the substrate should be free of heavy metals and have consistent levels of ingredients; the water should be free of contamination. The environmental conditions should include unpolluted air, a good sanitary growth area, and optimal temperature and humidity for growth and fruiting of the strain used. Finally, fruiting bodies should be harvested at optimal maturity and free of molds and insects.
3. **GMP (Good Manufacturing Practice):** The parameters for processing must be known and maintained. The temperature, duration, and percentage of solvents used in extraction should be constantly monitored.
4. **GPP (Good Production Practice):** The following tests must be conducted: laboratory tests should determine dosages effective for a particular health problem; a chemical analysis of the products to determine organic components and heavy metal contents; microbial analysis to determine if the type and level of microorganisms present is within safe limits; and standardization of the formulation of the products.
5. **GCP (Good Clinical Practice):** Medical practitioners must conduct high-quality clinical trials including double-blind studies.

Again, good-quality and honest products are of paramount importance in earning enduring public credibility and securing an expanding market in the future.[3,46]

X. CONCLUSION

Although Lingzhi (*Ganoderma lucidum*) has been used for more than 2000 years in China as a herbal remedy, the general public still remains cautious about the therapeutic effects of these medicinal mushroom products. Recent experimental studies have helped substantiate the long-standing traditional claims and uses of Lingzhi. However, the problem is that these products are so diverse because the bioactive composition of the different sources of the fruiting bodies is highly variable depending on the species, the growing environment, the cultivation methods, as well as the extraction procedures. There are currently no regulations assuring standard protocols for guaranteeing reproducible high product quality. If these Lingzhi products can be manufactured according to the proposed protocol in Section IX, then *Ganoderma*-based products should be able to serve as superior dietary supplements or nutriceuticals. Obviously, there is a serious need for improved quality control. This improved quality control is essential both to increase and maintain consumer confidence, and to meet current and future standards set by the regulatory authorities.

Because the active ingredient of *G. lucidum* products is not a single, chemically defined compound as used in conventional drug treatments, these products do not fulfill the requirement to be classified as pharmaceuticals. Their fruiting bodies are usually thick, corky, and tough, and, in this form, are not suitable as a dietary food. Therefore, *G. lucidum* products should be considered as a type of nutriceutical or as a dietary supplement as described by Chang and Buswell.[5]

REFERENCES

1. Barre-Sinoussi, F., Chermann, L.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Daugust, C., Axler-Blin, C., Vezinet-Brun, F., Rousioux, C., Rozenbaum, W., and Montagnier, L., Isolation of T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS), *Science*, 220, 868–871, 1983.
2. Buchanan, P.K., Hseu, R.S., and Monclavo, J.M., *Ganoderma — Systematics, Phytopathology and Pharmacology*, Applied Microbiology Laboratory, National Taiwan University, Taipei, 1995.
3. Chan, K., Linking chemical and biological characteristics in assuring the quality of Chinese medicinal materials and OTC products, in *Ganoderma*: *Genetics, Chemistry*, *Pharmacology and Therapeutics*, Lin, Z.B., Ed., Beijing Medical University Press, Beijing, 24–34, 2002.
4. Chang, H.M. and But, R.P., Eds., *Pharmacology and Applications of Chinese Materia Medica,* World Scientific Press, Singapore, 642–653, 1986.
5. Chang, S.T. and Buswell, J.A., Mushroom nutriceuticals, *World J. Microb. Biotechnol*., 12, 473–476, 1996.
6. Chang, S.T. and Buswell, J.A., *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Aphyllophoromycetideae) — a mushrooming medicinal mushroom, *Int. J. Med. Mushrooms*, 1, 139–146, 1999.
7. Chang, S.T. and Mshigeni, K.E., Mushrooms and human health: their growing significance as potent dietary supplements, University of Namibia, Windhoek, Namibia, 79, 2001.
8. Chen, A.W., Cultivation of the medicinal mushroom *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Reishi) in North America. *Int. J. Med. Mushrooms*, 1, 207–215, 1999.
9. Chen, R.Y. and Yu, D.Q., Studies on the triterpenoid constituents of the spores from *Ganoderma lucidum*, *Acta Pharm. Sin.,* 26, 267–273, 1991.
10. Chen, R.Y. and Yu, D.Q., Studies on the triterpenoid constituents of the spores of *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Aphyllophromycetideae), *Int. J. Med. Mushrooms*, 1, 147–152, 1999.
11. Chen, W.C., Hau, D.M., Wang, C.C., Lin, I.H., and Lee, S.S., Effects of *Ganoderma lucidum* and krestin on subset T-cell in spleen of gamma-irradiated mice, *Am. J. Chin. Med.*, 23, 289–298, 1995.

12. El-Mekkawy, S., Meselhy, M.R., Nakamura, N., Tesuka, Y., Hattori, M., Kakiuchi, N., Shimotohno, K., Kawahata, T., and Otake, T., Anti-HIV and anti-HIV-protease substances from *Ganoderma lucidum*, *Phytochemistry,* 49, 1651–1657, 1998.
13. Furusawa, E., Chou, S.C., Furusawa, S., Hirazumi, A., and Dang, Y., Antitumour activity of *Ganoderma lucidum*, an edible mushroom, on intraperitoneally implanted Lewis lung carcinoma in synergenic mice, *Phytother. Res*., 6, 300–304, 1992.
14. Gallo, R.C., Sarin, P.S., Gelmann, E.P., Rober-Guroff, M., Richardson, E., Kalyanaraman, V.S., Mann, D., Sidhu, G.D., Stahl, R.E., Zolla-Pazner, S., Leibowitch, J., and Povic, M., Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS), *Science,* 220, 865–867, 1983.
15. Gottlieb, A.M., Ferrer, E., and Wright, J.E., rDNA analyses as an aid to the taxonomy of species of *Ganoderma*, *Mycol. Res*., 104, 1033–1045, 2000.
16. Ha, T.B., Gerhauser, C., Zhang, W.D., Ho-Chong-Line, N., and Fouraste, I., New lanostanoids from *Ganoderma lucidum* that induce NAD(P) H: quinone oxidoreductase in cultured hepatic 7 murine hepatoma cells, *Planta Med.,* 66, 681–684, 2000.
17. Hattori, M., El-Mekkawy, S., and Meselhy, R., Inhibitory effects of components from *Ganoderma lucidum* on the growth of human immunodeficiency virus (HIV) and the protease activity, in *Proceedings of the 1st International Symposium on Ganoderma lucidum in Japan*, Mizuno T., Ide, N., and Hasegawa, Y., Eds., 128–135, 1997.
18. He, Y.Q. and Li, R.Z., Studies of active polysaccharides of *Ganoderma lucidum*. *J. Beijing Med. Univ*., 21, 225–227, 1989.
19. Hikino, H., Konno, C., Mirin, Y., and Hayashi, T., Isolation and hypoglycaemic activity of *Ganoderma* A and B, glycans of *Ganoderma lucidum* fruit bodies, *Planta Med,* 4, 339–340, 1985.
20. Hirotani, M., Ino, C., Fyruya, T., and Shiro, M., Ganoderic acids T, S, and R, new triterpenoids from the cultured mycelia of *Ganoderma lucidum*, *Chem. Pharm. Bull*., 34, 2282–2285, 1986.
21. Hsu, H.C., Hsu, C.I., Lin, R.H., Kao, C.L., and Lin, J.Y., Fip-vvo: a new fungal immunomodulatory protein isolated from *Volvariella volvacea, Biochem. J*., 323, 557–565, 1997.
22. Hsu, Z.C., *New Technology for Cultivation of Ganoderma lucidum* [in Chinese], Chaoyang Edible Fungi Research Institute, Liaoning, China, 1994.
23. Hung, Z., Artificial cultivation of *Ganoderma lucidum* [in Chinese], in *Modern Research on Ganoderma lucidum* [in Chinese], Lin, Z.B., Ed., Beijing Medical University Press, Beijing, China, 61–87, 1996.
24. Jong, S.C. and Birmingham, J.M., Medicinal benefits of the mushroom *Ganoderma*, *Adv. Appl. Microb.*, 37, 101–134, 1992.
25. Karsten, P.A., Enumertio boletinanum et polyporarum fenicarum systemate novo dispositorum, *Rev. Mycol.,* 3, 16–19, 1881.
26. Kim, H.W. and Kim, B.K., Recent advances on the biologically active triterpenoids of *Ganoderma lucidum*, in *Ganoderma: Genetics, Chemistry, Pharmacology and Therapeutics*, Lin, Z.B., Ed., Beijing Medical University Press, Beijing, 10–19, 2002.
27. Kim, H.W., Shim, M.J., and Kim, B.K., *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae) inhibits proliferation of human peripheral blood lymphocytes by blocking interleukin-2 secretion, *Int. J. Med. Mushrooms*, 2, 313–321, 2000.
28. Kim, H.W., Shim, M.J., Choi, E.C., and Kim, B.K., Inhibition of cytopathic effect of human immunodeficiency virus-1 by water-soluble extract of *Ganoderma lucidum*, *Arch. Pharmacol. Res*., 20, 425–431, 1997.
29. Kimura, Y., Okuda, H., and Arichi, S., Effects of the extracts of *Ganoderma lucidum* on blood glucose level in rats, *Planta Med.,* 54, 290–294, 1988.
30. Kino, K., Yamashita, A., Yamaoka, K., Watanabe, J., Tanaka, S., Ko, K., Shimizu, K., and Tsunoo, H., Isolation and characterization of a new immunomodulatory protein, Ling Zhi-8, from *Ganoderma lucidum, J. Biol. Chem*., 264, 472–478, 1989.
31. Kino, K., Mizumoto, K., Sone, T., Yamazi, T., Watanabe, J., Yamashita, A., Yamaoka, K., Shimizu, K., Ko, K., and Tsunoo, H., An immunomodulatory protein, Ling Zhi-8 (LZ-8) prevents insulitis in nonobese diabetic mice, *Diabetologia*, 33, 713–718, 1990.
32. Ko, J.L., Hsu, C.I., Lin, R.H., Kao, C.L., and Lin, J.Y., A new fungal immunomodulatory protein, FIP-fve isolated from the edible mushroom, *Flammulina velutipes* and its complete amino acid sequence, *Eur. J. Biochem*., 228, 244–249, 1995.

33. Ko, J.L., Lin, S.J., Hsu, C.I., Kao, C.L., and Lin, J.Y., Molecular cloning and expression of a fungal immunomodulatory protein, FIP-fve, from *Flammulina velutipes*, *J. Formosan Med. Assoc*., 96, 517–524, 1997.
34. Kubota, T., Asawa, Y., Miura, I., and Mori, H., Structures of ganodermeric acid A and B, two new lanostane type bitter triterpenes from *Ganoderma lucidum* (Fr.) Karst., *Helv. Chim. Acta*, 65, 611–619, 1982
35. Lee, S.S., Chen, F.D., Chang, S.C., Wei, Y.H., Liu, I., Chen, C.F., Wei, R.D., Chen, K.Y., and Han, P.W., *In vivo* antitumor effect of crude extracts from the mycelium of *Ganoderma lucidum*, *J. Chin. Oncol. Soc*., 5, 22–28, 1984.
36. Lee, S.S., Wei, Y.H., Chen, C.F., Wang, S.Y., and Chen, K.Y., Antitumor effects of *Ganoderma lucidum*., *J. Chin. Med.,* 6, 1–12, 1995.
37. Lieu, C.W., Lee, S.S., and Wang, S.Y., The effect of *Ganoderma lucidum* on induction of differentiation in leukemic U937 cells, *Anticancer Res.*, 12, 1211–1215, 1992.
38. Leung, S.W.S., Yeung, K.Y., Ricky, Y.L.S., and Man, Y.K., Lingzhi (*Ganoderma*) research — the past, present and future perspectives, in *Ganoderma: Genetics, Chemistry, Pharmacology and Therapeutics*, Lin, Z.B., Ed., Beijing Medical University Press, Beijing, 1–9, 2002.
39. Lin, D.X., Discussion on *Ganoderma lucidum* as a supplementary curing of cancer, *Edible Fungi China*, 19, 3–5, 2000.
40. Lin, W.H., Hung, C.H., Hsu, C.I., and Lin, J.Y., Dimerization of the N-terminal amphipathic β-helix domain of the fungal immunomodulatory protein from *Ganoderma tsugae* (FIP-gts) defined by a yeast two-hybrid system and site-directed mutagenesis, *J. Biol. Chem*., 272, 20044–20048, 1997.
41. Lin, Z.B., Physiological effects of *Ganoderma lucidum*., in *Modern Research on Ganoderma lucidum* [in Chinese], Lin, Z.B., Ed., Beijing Medical University, Beijing, China, 148–177, 1996.
42. Lindequist, U., Structure and biological activity of triterpenes, polysaccharides and other constituents of *Ganoderma lucidum*, in *Recent Advances in Ganoderma lucidum Research*, Kim, B.K., Kim, I.H., and Kim, Y.S., Eds., Pharmaceutical Society of Korea, Seoul, 61–91, 1995.
43. Liu, G.T., Recent advances in research of pharmacology and clinical applications of *Ganoderma* P. Karst. species (Aphyllophoromycetideae) in China, *Int. J. Med. Mushrooms*, 1, 63–67, 1999.
44. Ma, J., Ye, Q., Hua, Y., Zhang, D., Cooper, R., Chang, M.N., Chang, J.Y., and Sun, H.H., New lanostanoids from the mushroom *Ganoderma lucidum*, *J. Nat. Prod*., 65, 72–75, 2002.
45. Mayzumi, F., Okamoto, H., and Mizuno, T., Cultivation of Reishi (*Ganoderma lucidum)*, *Food Rev. Int*., 13, 365–382, 1997.
46. Miles, P.G. and Chang, S.T., *Mushroom Biology — Concise Basics and Current Developments*, World Scientific Press, Singapore, 1997.
47. Min, C.N., Nakamura, N., Miyashiro, H., Bae, K.W., and Hattori, M., Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-1 protease, *Chem. Pharm. Bull*., 46, 1607–1612, 1998.
48. Mizuno, T., Studies of bioactive substances and medical effects of the *Ganoderma lucidum,* in *Recent Progress in Ganoderma lucidum Research*, Kim, B.K., Moon, C.K., and Kim, T.S., Eds., Pharmaceutical Society of Korea, Seoul, 69–89, 1997.
49. Mizuno, T., Naoi, Y., Mayuzumi, F., Ogino, M., and Okamoto, H., Artificial cultivation of *Ganoderma lucidum* in Japan, in *Ganoderma lucidum,* Mizuno, T., Ed., Il-Yang Pharm. Co., Ltd., Seoul, Korea, 1996.
50. Mizuno, T., Wang, G.Y., Zhang, J., Kawagihi, H., Nishitoba, T., and Li, J.X., Reishi, *Ganoderma lucidum* and *Ganoderma tsugae*: bioactive substances and medicinal effects, *Food Rev. Int*., 11, 151–166, 1995.
51. Moncalvo, J.M., Wang, H.F., Wang, H.H., and Hseu, R.S., Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences, *Mycologia*, 87, 223–238, 1995.
52. Moncalvo, J.M., Wang, H.F., Wang, H.H., and Hseu, R.S., Gene phylogeny of the *Ganoderma lucidum* complex — comparison with traditional taxonomic characters, *Mycol. Res.*, 99, 1489–1499, 1995.
53. Morigiwa, A., Kitabatake, K., Fujimori, Y., and Ikekawa, N., Angiotensin converting enzyme-inhibitory triterpenes from *Ganoderma lucidum*, *Chem. Pharm. Bull*., 34, 3025–3028, 1996.
54. Nishitoba, T., Bitter terpenoides, in *Ganoderma lucidum*, Mizuno, T., Ed., Il-Yang Pharm. Co., Ltd., Seoul, Korea, 256–260, 1996.

55. Ooi, L.S.M., Ooi, V.E.C., and Fung, M.C., Induction of gene expression of immunomodulatory cytokines in the mouse by a polysaccharide from *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Aphyllophoromycetideae), *Int. J. Med. Mushrooms,* 4, 27–35, 2002.
56. Ryvarden, L., Can we trust morphology in *Ganoderma*? in *Ganoderma: Systematics, Phytopathology and Pharmacology*, Buchanan, P.K., Hseu, R. S., and Moncalvo, J. M., Eds., Department of Agricultural Chemistry, National Taiwan University, Taipei, China, 19–24, 1994.
57. Ryvarden, L., Studies in neotropical polypores. 2: Preliminary key to neotropical species of *Ganoderma* with a laccate pileus, *Mycologia*, 92, 180–191, 2000.
58. Stamets, P., *Growing Gourmet and Medicinal Mushrooms*, 3rd ed., Ten Speed Press, Berkeley, CA, 352–366, 2000.
59. Su, C.Y., Shiao, M.S., and Wang, C.T., Predominant inhibition of ganodermic acid S on the thromboxane A2-dependent pathway in human platelets response to collagen, *Biochim. Biophys. Acta,* 1437, 223–234, 1999.
60. van der Heun, L.G., van der Vleit, J.A., Bocken, C.F.M., Kino, K., Hoitsman, A.J., and Tax, W.J.M., Ling Zhi-8: studies of a new immunomodulatory agent, *Transplantation*, 60, 438–443, 1995.
61. Wang, C.N., Chen, J.C., Shiao, M.S., and Wang, C.T., The inhibition of human platelet by ganodermic acids, *Biochem. J.,* 277, 189–197, 1997.
62. Wang, S.Y., Hsu, M.L., Hsu, H.C., Tseng, C.H., Lee, S.S., Shiao, M.S., and Ho, C.K., The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes, *Int. J. Cancer,* 70, 669–705, 1997.
63. Wasson, R.G., *Soma-Divine Mushroom of Immortality*, Harcourt, Brace & World, New York, 381, 1968.
64. Yang, B.K., Jeong, S.C., Park, J.B., Cho, S.P., Lee, H.J., Das, S., Yun, J.W., Lim, W.J., and Song, C.H., Swimming endurance capacity of mice after administration of exo-polymer produced from submerged mycelial culture of *Ganoderma lucidum, J. Microbiol. Biotechnol*, 11, 902–905, 2001.
65. Ying, J.Z., Mao, X.L., Ma, Q.M., Zong, Y.C., and Wen, H.A., *Icons of Medicinal Fungi from China*, Science Press, Beijing, China, 144–145, 1987.
66. Zhao, J.D. and Zhang, X.Q., Resources and taxonomy of Ling Zhi (*Ganoderma*) in China, in *Program and Abstracts, 94 Int. Symp. on Ganoderma Research*, Lin, Z.B., Ed., Beijing Medical University, Beijing, China, 44–47, 1994.
67. Zhou, S.F. and Gao, Y.H., The immunomodulating effects of *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Liung Zhi, Reishi mushroom) (Aphyllophoromycetideae), *Int. J. Med. Mushrooms*, 4, 1–11, 2002.
68. Zhu, S.N. and Mori, M., *The Research on Ganoderma lucidum* (Part one), Shanghai Medical University Press, Shanghai, 346, 1993.

20 *Agaricus blazei* and *Grifola frondosa* — Two Important Medicinal Mushrooms

I. INTRODUCTION

In the previous chapter we examined the most well known of the medicinal mushrooms, *Ganoderma lucidum*, which has been treasured in China for thousands of years as an herbal drug, medicinal food, and health-producing tonic. In this chapter we consider two other important medicinal mushrooms, *Agaricus blazei* (Murrill) ss. Heinem. and *Grifola frondosa* (Dicks.:Fr.) S.F. Gray, which recently have become the most promising and most intensively studied species. A wide range of medicinal traits have been attributed to them for treatment of tumor, hypertension, diabetes, obesity, and hepatitis.[13,22–24]

II. BIOLOGICAL CHARACTERISTICS OF *AGARICUS BLAZEI*

A. History

Agaricus blazei Murrill, or *Agaricus blazei* (Murrill) ss. Heinem, was first recognized as a new species by an American mycologist, W.A. Murrill, who found it on the lawn of Mr. R.A. Blaze in Gainesville, Florida.[25]

This currently widely cultivated species of *Agaricus* originated in Brazil. Wasser et al.[30] reported recently that the cultivated species of this mushroom is not *A. blazei*, but a species almost identical, *A. blazei* Murrill ss. Heinem. From critical analysis, they suggested that the North American endemic species, *A. blazei* ss. Murrill, and the widely cultivated edible and medicinal *A. blazei* ss. Heinem are two different species. *Agaricus blazei* ss. Heinem is described as a new species — *A. brasiliensis* sp. The main natural habitat of *A. blazei* ss. Heinem is the mountainous district of Piedade, situated 130 km from the capital of São Paulo in southern Brazil, where the mushroom serves the people as a part of their diet. In the past this region of Piedade was known for its wild horses. A special kind of soil, rich in dung, which was located there combined with the type of climate, made the region especially suitable for the growth of this unique *Agaricus* medicinal mushroom, which may have originated in this particular area.

The residents of the region enjoy good health, a low incidence of cancer, and great longevity. These facts attracted the attention of two California researchers, Shinden and Runbert, who traveled to the region to study the environment, water quality, and lifestyle of the inhabitants. After some time, they discovered that the local population consumed a certain kind of mushroom with regularity. These results were reported to the scientific community in 1965. That same year, this well-known mushroom, called *Cogmelo de deus* (mushroom of God) in Brazil, was taken to Japan by Takatoshi Furumoto, a Japanese immigrant, who commercially grew *A. bisporus* and *Lentinula edodes* on his small farm in the Piedade municipality.

In 1976, some cultures of this mushroom were sent from Japan to Dr. Heinemann, a Belgian fungal taxonomist, who classified it as *A. blazei* Murrill ("himematsutake" or "kawariharatake" in

FIGURE 20.1 (Color figure follows p. 232.) Fruiting body of *Agaricus blazei* grown on compost bed.

Japanese) belonging to the Agaricaceae family. Studies on the artificial culture and cultivation of this mushroom were started at the Iwade Mushroom Institute, and an attempt to produce it commercially was made in 1978.[18] In 1980 in Japan, it was discovered that this species produces several polysaccharides, among them β-glucan, that are able to inhibit the growth of malignant tumors in test animals. Immediately afterward Dr. Iwade patented this specimen with the name, Himematsutake (princess mushroom). Since 1988, this novel mushroom, of Brazilian origin, has been cultivated in Japan, China, Brazil, Taiwan, Thailand, Vietnam, and Indonesia. Commercial cultivation in the United States has just recently begun.[28]

B. Morphology

The pileus ranges from 6 to 11 cm and 7 to 25 cm in diameter depending on the strain. They are fleshy, broad, convex at first, soon hemispheric, then broadly convex, and eventually flatten out. In color they are white, cream, pale brown, and darker toward the center. The texture is smooth, fibrillose, and the pileal surface often disrupts into adpressed fibrillose, dark brown or ochraceous brown adpressed scales on a whitish cream background. The stipe is 5 to 11 × 1.5 to 3.5 cm, centrally positioned, cylindrical, and occasionally slightly twisted. It sometimes enlarges toward the base, and is white or whitish. On handling it becomes yellow or ochraceous. It is solid, later becomes fistulose in the center, and is smooth.[28] This mushroom grows singly or in clusters that arise from sites of dense rhizomorphs that lead to and often are attached to the stem bases (Figure 20.1).

C. Requirements for Growth

Agaricus blazei is a saprophyte and prefers a warm temperature and high humidity, that is, temperatures of 25°C during the day and 20°C at night with 90 to 95% humidity. The general requirements for growth are summarized below.[11]

1. **Temperature:** The range for mycelial growth is 10 to 37°C and the optimum temperature is 23 to 27°C; for the development of fruiting bodies it is 20 to 33°C with an optimum of 22 to 25°C.
2. **Air:** It is an aerophilic mushroom and prefers growing when fresh air exchanges are greater than in other cultivated mushrooms, such as *A. bisporus.* During both mycelial running and fruiting development phases, gentle fresh air exchanges are essential.

3. **Light:** During the phase of spawn running, light is not required, and direct light, which can inhibit the growth of mycelium, should be avoided. In Brazil, this mushroom is also called the "sunlight mushroom," because it prefers to have scattered, refractive light during the fruiting development phase. The light intensity can directly influence the color and quality of the mushroom. When optimum light is given during fructification, a better quality of mushroom with darker color and firmer texture can be produced.
4. **pH Value:** The mycelium grows well in the range of pH 4.5 to 8.0, with an optimum of pH 7. The pH value of the compost at spawning should be about 7.5 and the casing layer should be around 7.5 to 8.0.
5. **Water:** The optimum water content of the compost at spawning is 55 to 60%, and that of the casing soil is 60 to 65%. The relative humidity in the mushroom house should be 75 to 85%.

III. BIOLOGICAL CHARACTERISTICS OF *GRIFOLA FRONDOSA*

A. History

Grifola frondosa is called "maitake" in Japanese, which means "dancing mushroom." In China it is called the "gray tree flower" or "chestnut mushroom," and in North America the "hen of the woods" or "sheep's head mushroom." In Japan there is a legend that people who found the mushroom in the deep mountains danced with joy since it was rarely found and the people knew of its delicious taste and health benefits. There are three homologues of *G. frondosa*:

1. *Grifola albicans* is similar to *G. frondosa* but has a white to pale yellow color and a brittle, less crispy texture; and in nature its fruiting bodies appear about 1 week earlier than *G. frondosa.*
2. *Grifola gigantea* has caps that are comparatively larger than those of *G. frondosa*, 10 to 20 cm in diameter; it is edible and is used for medicinal purposes in China.
3. *Grifola umbellate* is also edible and is a similar species with whitish to gray to smoky brown circular caps with central stems; its fruiting bodies arise from sclerotia, which have been used by the Chinese as an immune system stimulant; and, finally, it is not common in nature.

Grifola frondosa has only been available by artificial cultivation since the mid-1980s in Japan. Mass production was started about 5 years later following the elucidation of its cultural biology.[15] At present, this cultivated mushroom is on the market in large amounts. In 1999, Japanese cultivators produced nearly 40,000 MT and in 2001 China produced 14,600 MT of *G. frondosa*. Both in Japan and China, the annual production has constantly increased because of its excellent taste and valuable medicinal properties.

B. Morphology

Grifola frondosa is a large, fleshy polypore. Its fruiting body has a size of 15 to 60 cm or more and consists of a mass of numerous small, overlapping caps, arising from a common, fleshy, repeatedly branched base (Figure 20.2). Caps range from 2 to 7 cm in width and are spoon shaped, tongue shaped, or fan shaped and flattened. The surface of the fruiting body is dry, smooth or rough to fibrillose, gray to brown or grayish brown, and the margin is often wavy. Stalks (branches) are smooth, fleshy, but tough, white or pale grayish, and are attached off-center, or more often laterally attached to the sides of caps.[1] The mushrooms are dark grayish brown when young becoming lighter gray with age. Some varieties fade to a light yellow at maturity.[28]

FIGURE 20.2 (Color figure follows p. 232.) Fruiting bodies of *Grifola frondosa*.

In natural habitats, clusters of large fruiting bodies are found on the ground at or near the base of stumps of tree trunks of dead, dying, or aging hardwoods, such as oaks, elms, maples, black gums, beech, and chestnut. Fruiting bodies in a cluster of 37 kg or more in weight have been recorded both in the United States and China.[4] Most of the clusters of mushrooms found, however, come in the range of 1.87 to 3.37 kg. Because they have numerous overlapping, spoon-shaped caps, which are reminiscent of a fluffed-up hen, this group of mushrooms is one of the safest and most easily recognizable of all edible mushrooms.[1]

C. Requirements for Growth

Grifola frondosa grows in temperate climates and prefers good light and fair air conditions. It is a saprophytic "white rot" fungus that grows on stumps or at the base of dead or dying deciduous hardwoods, especially oaks, chestnuts, and elms. In nature, it has been found in northeastern Japan, the United States, and in the temperate regions of China and Europe. The general requirements for growth are summarized as follows (Table 20.1):

1. **Temperature:** The range of temperatures for mycelial growth is relatively wide from 5 to 32°C and optimum temperature is 20 to 25°C. The temperature for formation of primordia is 18 to 22°C. The temperatures for fruiting body development range from 10 to 25°C and the optimum is 18 to 22°C. It is the same as with other cultivated edible mushrooms in that fruiting bodies develop slowly at lower temperatures and grow faster at higher temperature.
2. **Humidity:** The water content in the substrate should be maintained in the range of 60 to 63%. During the mycelial running stage, the relative humidity of the air in the culture room should not be too high to avoid development of mold. The relative humidity of 60 to 65% is proper. However, during the stage of fruiting body development, the relative humidity in the culture room should be higher, that is, maintained at 85% or above. The optimum is 85 to 95% and when lower than 80%, the fruiting bodies dry out easily, particularly at the pinhead stage.
3. **Light:** During fructification good illumination of light in the culture room is absolutely necessary for development of the normal size and color of the mushrooms. If the light is inadequate during this stage, it is difficult for the vegetative mycelium to differentiate

TABLE 20.1
Growth Parameters of *G. frondosa*

	Mycelial Growth		Formation of	Fruiting Development	
	Range	Optimum	Primordium	Range	Optimum
Temp. °C	5–32	20–25	18–22	10–25	15–20
RH%	60–70		80–90	80–95	85–95
Light (lux)	50		200	200–500	
pH Value	5.5–6.5				

Source: Data from Wu, J.L. et al., in *Cultivation of Eighteen Precious and Delicious Edible Mushrooms* [in Chinese], Huang, N.L., Ed., Chinese Agricultural Press, Beijing, China, 130–145, 1997.

into the reproductive primordium. Even though the fruiting bodies are formed, they often become abnormal in shape and color, light or white.

4. **Air:** *Grifola frondosa* is like *Agaricus blazei* and is an aerobic (aerophilic) mushroom similar to *A. blazei* in its aeration requirements. In the culture room, an exchange of air should be made at least five to six times every day. In poor aeration conditions, abnormal mushrooms are formed.
5. **pH:** *Grifola frondosa* prefers growing on a slightly acid medium with a pH at 5.5 to 6.5.

IV. CULTIVATION METHODS

A. *Agaricus blazei*

The technique used in cultivation of *A. blazei* is basically the same technology as for *A. bisporus.* However, the former is a mushroom of the tropics and subtropics and requires warmer temperature and higher humidity than the latter, which is a common edible mushroom of temperate climate. In Brazil, sugar cane bagasse solely, or mixed with rice straw, was found to be the best culture bed material and the cultivation of this mushroom is carried out mainly outdoors. In Japan, this mushroom is cultivated both in greenhouses and in outdoor systems. Although this mushroom was first introduced to Japan from Brazil in 1965, the mushroom in dry form and the raw materials for powders or tablets are now mainly imported to Japan either from Brazil or from China. This novel mushroom for both edible and medicinal uses was introduced from Japan to China in 1992. The Plant Protection Research Institute of the Agriculture Academia Sinica at Fujian Province conducted the experimental trials first. After 2 years of small-scale trials, the method with compost made of bagasse or rice straw or a mixture of wheat or rice bran, soybean powder, superphosphate of lime, and added water was found in these trials to be practicable. In 1994, the cultivation of this mushroom was extended to other areas of Fujian Province. Now it is cultivated in many parts of China during the warm summer months on relatively small-scale farms. In 2000, it was estimated that 100 MT of dry mushrooms were produced in China. In recent years, this mushroom has also been cultivated in Korea, Thailand, Indonesia, and the United States.[11,19,28,32]

B. *Grifola frondosa*

Artificial cultivation of *G. frondosa* has a short history of about 20 years. Cultivation was first started in Japan, and then later the techniques spread to China and Korea. Now cultivation has gradually expanded to other parts of the world including the United States. Three cultivation methods, bottle culture, bag culture, and outdoor bed culture, have been described in detail by Mayuzumi and Mizuno.[15]

1. **Bottle cultivation:** This method is suitable for year-round cultivation and can be mechanized with minimum labor requirements. The mushrooms are usually small because of the limited nutrients available in the bottles.
2. **Bag cultivation:** This method is used most frequently in Japan. Hardwood sawdust is mixed with rice and wheat bran as basal ingredients of the culture substrate, which is packed in a polypropylene bag that is molded into a square shape.
3. **Outdoor bed cultivation:** This method was first attempted in Japan under natural climatic conditions. This method requires more time (about 6 months) from spawning to fruiting body formation.

Chen[3] reported a practical guide for synthetic-log cultivation of this mushroom. Details are given for the process of cultivation, including selection of strains, types of spawn, where to obtain the spawn, where to obtain bags for mushroom cultivation, and substrate formulation. Guidelines on how to regulate growth parameters (temperature, relative humidity, light, and ventilation in terms of CO_2 vs. O_2) for each growth stage are constructed in tabular form, readily accessible for application. Additional valuable information for practical cultivation can also be found.[29,31]

Because the optimum temperature for fructification of this mushroom is rather narrow, 15 to 20°C, the best time for cultivation in the outdoors depends on the local climatic conditions, and the optimum season must be selected to grow the mushroom. If the cultivation bag or bed is indoors, close attention should be given to regulate temperature, relative humidity, light, and air, with air exchange adjusted by ventilation. Based on normal growth, this mushroom requires more air than other common mushrooms, with the exception of *Agaricus blazei*. The particulate size of the substrate ingredients must be selected with care because they can be too fine and too compact for good air exchange. It has been found that a mixture of hardwood sawdust (fine:coarse = 3:1), or a mixture of hardwood sawdust and woodchips is good, and sometimes cotton seed hulls are added to the mixture to provide good air exchange during mycelial running.

V. NUTRITIONAL CONTENT AND MEDICINAL PROPERTIES

A. *AGARICUS BLAZEI*

The fresh fruiting bodies of this mushroom contain 85 to 87% water. On a dry weight basis, crude protein content can range from 38 to 45%, making this species one of the most protein-rich of all the cultivated edible mushrooms, and it is more than twice that of *Lentinula edodes*. In addition, the mushroom contains on an average 41.5% carbohydrate, 6 to 8% fiber, and 3 to 4% crude lipid.[18,28,32]

In recent years, *A. blazei* has become one of the most promising of the new edible and medicinal mushrooms. It is a species native to Brazil, where it has received attention as a folk medicine due to its claimed medicinal values, and it is often consumed as food and prepared as tea. It is popularly used to combat physical and emotional stress, stimulate immunity, improve life quality of people suffering from diabetes, reduce cholesterol, combat diseases such as osteoporosis and gastric ulcer, and it is also used as an antioxidant and anticarcinogen.[16] The scientific knowledge regarding these claims is still insufficient. However, a protective effect of *A. blazei* teas was demonstrated *in vivo* against the clastogenicity (having capability of breaking chromosomes) induced by cyclophosphamide.[16] Mice pretreated with three different teas of the mushroom, obtained at 4, 21, and 60°C, showed a significant reduction in the frequencies of micronuclei in erythrocytes that do not stain uniformly (polychromatic erythrocytes) and in reticulocytes, after 25 and 50 mg/kg of cyclophosphamide. (It should be noted that the extracts with such low temperatures could be lectins — glycopeptides). Furthermore, the results reported[7] did not indicate any significant influence of those three different temperatures at which the solution was prepared on the antimutagenic activity. Linoleic acid (an unsaturated fatty acid that represents 70 to 78% of the total lipids of this fruiting

body) was identified as the main substance with antimutagenic activity.[16] The aqueous extracts also exert a hepatoprotective effect on both liver toxicity and the hepatocarcinogenesis process induced by a moderately toxic dose of diethylnitrosamine.[2]

Considerable efforts have been made to isolate polysaccharides and protein-bound polysaccharides from this mushroom by hot water (95 to 100°C). Such compounds have shown antitumor activity in tumor-bearing mice.[12,14,18,21] Various bioactive substances extracted from medicinal mushrooms are known, the majority composed of chains of D-glucans in β-(1→3)-linkages. However, the main feature of *A. blazei* is a unique D-glucan content consisting solely of an unsubstituted β-(1→6)-D-glucan-linked backbone.[2,22,23] The biggest group of *A. blazei* active substances is composed of polysaccharides, which are obtained mostly from fruiting bodies. Cultured mycelia and liquid medium filtrate from a submerged cultivation of *A. blazei* were also shown to be sources of antitumor substances. The main ingredients of their polysaccharides are, however, different from those that are isolated from fruiting bodies. It has been reported[8,9] that the unique polysaccharides of this mushroom promote natural killer cells that are selectively cytotoxic on tumor cells, enhance apoptosis in cancerous cells, but not in healthy cells, and also trigger an immune response.

It would be worthwhile to pursue a further comparative study to determine which temperature is best to extract the bioactive compounds in this important medicinal mushroom. Several companies in Brazil have used raw mushroom powder, without extraction, for capsules. One company in Brazil marketed tea product tablets, which were made by soaking the ground mushroom powder in 60°C warm water for 15 minutes and then adding other ingredients. These are the best ways to distribute the bioactive compounds for medicinal or nutriceutical purposes. In another method the fruiting bodies can be boiled at 95 to 100°C and the bioactive β-glucan compounds extracted.

B. *Grifola frondosa*

The fresh fruiting bodies of *G. frondosa* at harvesting contain 91% moisture. On a dry weight basis, crude protein content is 19.7% and pure protein is 13.1%. The ratio of total essential amino acid content with total amino acids is 40.00, and the ratio of total essential amino acids with total nonessential amino acids is 66.67.[33] In addition, the mushrooms contain on an average 61.06% carbohydrates, 9.7% fiber, and 3.2% crude lipid.[31] Vitamins B_1 and B_2 are present, but neither vitamin A nor vitamin C is found. Maitake contains ergosterol (provitamin D), which is a common component of mushrooms.[20] The maitake fruiting body is also rich in potassium and phosphorus, and contains lesser amounts of magnesium, calcium, sodium, and zinc.

Grifola frondosa has been prized by traditional Chinese and Japanese herbalists as a precious and delicious food and also as a medicinal food. It has been frequently used in traditional Chinese medicine to treat spleen and stomach ailments and to lessen anxiety. Since the mid-1980s, the biological activities of *G. frondosa* have been evaluated in detail because the artificially cultivated technology was developed at that time, and a large quantity of the mushrooms was available as material for research. Both basic research and clinical experience have shown that this mushroom possesses the ability to produce antitumor effects, immunological enhancement, and also has anti-HIV, antihypertension, antidiabetes, antihyperlipemia, and antiobesity properties.[34] In recent years, it has emerged in the United States as well as in Europe and received great attention for its potential as a complementary medicine.

The first drug developed from medicinal mushrooms was lentinan, an antitumor polysaccharide, which was isolated and purified from a hot water extract of *Lentinula edodes* fruiting bodies.[5,6] About 20 years later, an antitumor glucan was isolated from the fruiting bodies of *G. frondosa* by Nanba et al.[26] The structure of lentinan was reported as a β-(1→3)-linked D-glucan with β-(1→6) branches.[5,27] However, the glucan of *G. frondosa* is composed of a backbone of β-(1→6)-linked glucose residues with side chains of β-(1→3)-glucose residues.[26] Minato et al.[17] revealed that both glucans from *L. edodes* and *G. frondosa* are a type of biological response modifier and do not show any direct cytotoxicity against tumor cells. Their antitumor action is considered host mediated.

It has been reported that the glucan isolated from *G. frondosa* possesses an immunomodulating effect, which is seen in the activation of a variety of macrophage functions, for example, production of some cytokines and superoxide anions, phagocytosis, and cytotoxicity.[24] It has also been reported that macrophages secreted tumor necrosis factor-α (TNF-α) through the stimulation of a glucan of *G. frondosa.*[24,34] TNF-α is recognized as the primary cytokine produced mainly by activated macrophages; it is an important host defense molecule that affects tumor cells. Hoffman et al.[10] observed that TNF-α was released from macrophages through a β-glucan-mediated mechanism.

It has also been reported[17,34] that β-(1→3)-glucan (grifolan) from liquid-cultured mycelium showed antitumor activity through host-mediated immunity. It is considered that this β-(1→3)-glucan from mycelium stimulates macrophages to augment this antitumor activity. It should be noted that β-(1→6)-glucan was isolated from fruiting bodies of the same mushroom as described above.

REFERENCES

1. Arora, D., *Mushrooms Demystified*, Ten Speed Press, Berkeley, CA, 564–565, 1986.
2. Barbisan, L.F., Miyamoto, M., Scolastici, C., Salvadori, D.M.F., Ribeiro, L.R., Eira, A.F., and de Camargo, J.L.V., Influence of aqueous extract of *Agaricus blazei* on rat liver toxicity induced by different doses of diethylnitrosamine, *J. Ethnopharmacol.*, 83, 25–32, 2002.
3. Chen, A.W., A practical guide for synthetic-log cultivation of medicinal mushroom *Grifola frondosa* (Dicks.: Fr.) S.F. Gray (maitake), *Int. J. Med. Mushrooms*,1, 153–167, 1999.
4. Chen, A.W., Stamets, P., Cooper, R.B., Huang, N.L., and Han, S.H., Ecology, morphology, and morphogenesis in nature of edible and medicinal mushroom *Grifola frondosa* (Dicks.: Fr.) S.F. Gray — maitake (Aphyllophoromycetideae), *Int. J. Med. Mushrooms*, 2, 221–228, 2000.
5. Chihara, G., Hamuro, J., Maeda, Y.Y., Arai, Y., and Fukuoka, F., Fractionation and purification of polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) Sing. (an edible mushroom), *Cancer Res.,* 30, 2776–2781, 1970.
6. Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T., and Fukuoka, F., Inhibition of mouse Sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing., *Nature*, 222, 687–688, 1969.
7. Delmanto, R.D., Lima, P.L.A., Sugui, M.M., Eira, A.F., Salvadon, D.M.F., Speit, G., and Ribeiro, L.R., Antimutagenic effect of *Agaricus blazei* Murrill mushroom on the genotoxicity induced by cyclophosphamide, *Mutat. Res*., 496, 15–21, 2001.
8. Fujimiya, Y., Suzuki, Y., Katahura, R., and Ebina, T., Tumor-specific cytocidal and immunopotentiating effects of relatively low molecular weight products derived from the basidiomycete, *Agaricus blazei* Murrill., *Anti-Cancer Res*., 19, 113–118, 1999.
9. Fujimiya, Y., Suzuki, Y., Oshiman, K., Kobori, H., Moriguchi, K., Nakashima, H., Matsumoto, Y., Takahara, S., Ebina, T., and Katakura, R., Selective tumoricidal effect of soluble proteoglucan extracted from basidiomycete, *Agaricus blazei* Murrill, mediated via natural killer cell activation and apoptosis, *Cancer Immunol. Immunother.*, 46, 147–159, 1998.
10. Hoffman, O.A., Standing, J.E., and Limper, A.H., *Pneumocystis carinii* stimulates tumor necrosis factor-α release from alveolar macrophages through a β-glucan-mediated mechanism, *J. Immunol.*, 150, 3932–3940, 1993.
11. Huang, N.L., *Agaricus blazei*, in *Cultivation of Eighteen Precious and Delicious Edible Mushrooms*, Huang, N.L., Ed., Chinese Agricultural Press, Beijing, China, 95–101, 1997.
12. Kawagishi, H., Inagaki, R., Kanao, T., Mizuno, T., Shimura, K., Ito, H., Hagiwara, T., and Nakamura, T., Fractionation and antitumor activity of the water-insoluble residue of *Agaricus blazei* fruiting bodies, *Carbohydr. Res*., 186, 267–273, 1989.
13. Kubo, K. and Nanba, H., Anti-diabetic mechanism of Maitake (*Grifola frondosa),* in *Mushroom Biology and Mushroom Products*, Royse, D.J., Ed., Pennsylvania State University, University Park, 215–222, 1996.
14. Lin, Y.X., Ye, Z.Q., Huang, Y.Y., and Xie, H.L., Fractionation and characterization of water-soluble polysaccharides from medicinal mushroom *Agaricus blazei* Murrill fruit body, *Int. J. Med. Mushrooms*, 4, 2002.

15. Mayuzumi, Y. and Mizuno, T., Cultivation methods of maitake (*Grifola frondosa*), *Food Rev. Int*., 13, 357–364, 1997.
16. Menoli, R.C.R., Mantovani, M.S., Ribeiro, L.R., Speit, G., and Jordao, B.Q., Research antimutagenic effects of the mushroom *Agaricus blazei* Murrill extracts on V79 cells, *Mutat. Res.,* 496, 5–13, 2001.
17. Minato, K.I., Mizuno, M., Kawakami, S., Tatsuoka, S., Denpo, Y., Tokimoto, K., and Tsuchida, H., Change in immunomodulating activities and content of antitumor polysaccharides during the growth of two medicinal mushrooms, *Lentinus edodes* (Berk.) Sing. and *Grifola frondosa* (Dicks.:Fr.) S. F. Gray, *Int. J. Med. Mushrooms*, 3, 1–7, 2001.
18. Mizuno, T., Kawariharatake, *Agaricus blazei* Murrill: medicinal and dietary effects, *Food Rev. Int*., 11, 167–172, 1995.
19. Mizuno, T., Cultivation of the medicinal mushroom Royal Sun Agaricus — *Agaricus blazei* Murrill (Agaricomycetideae), *Int. J. Med. Mushrooms*, 2, 215–220, 2000.
20. Mizuno, T and Zhuang, C., Maitake, *Grifola frondosa*: pharmacological effects, *Food Rev. Int*., 11, 135–149, 1995.
21. Mizuno, T., Hagiwara, T., Nakamura, T., Ito, H., Shimura, K., Sumiya, T., and Asakura, A., Antitumor activity and some properties of water-soluble polysaccharides from "himematsutake," the fruiting body of *Agaricus blazei* Murrill, *Agric. Biol. Chem.,* 54, 2889–2896, 1990.
22. Mizuno, T., Morimoto, M., Minato, K.I., and Tsuchida, H., Polysaccharides from *Agaricus blazei* Murrill stimulate T-cell subsets in mice*, Biosci. Biotechnol. Biochem.*, 62, 434–437, 1998.
23. Mizuno, M., Minato, K., Ito, H., Kawade, M., Terai, H., and Tsuchida, H., Anti-tumor polysaccharide from the mycelium of liquid-cultured *Agaricus blazei* Murrill, *Biochem. Mol. Biol.*, 47, 707–714, 1999.
24. Mizuno, M., Yamakawa, A., Minato, K., Kawakami, S., Tatsuoka, S., Terai, H., and Tsuchida, H., Preparation of antibody against antitumor β-glucan in *Grifola frondosa* and its application, *Food Sci. Technol. Res*., 5, 398–401, 1999.
25. Murrill, W.A., New Florida fungi, *J. Fla. Acad. Sci.*, 8, 175–198, 1945.
26. Nanba, H., Hamaguchi, A., and Kuroda, H., The chemical structure of an antitumor polysaccharide in fruit bodies of *Grifola frondosa* (maitake), *Chem. Pharm. Bull.*, 35, 2453–2458, 1987.
27. Sasaki, T. and Takasuka, N., Further study of the structure of Lentinan, an antitumor polysaccharide from *Lentinus edodes, Carbohydr. Res*., 47, 99–104, 1976.
28. Stamets, P., Techniques for the cultivation of the medicinal mushroom royal sun *Agaricus — Agaricus blazei* Murr. (Agaricomycetideae), *Int. J. Med. Mushrooms*, 2, 151–160, 2000.
29. Stamets, P., *Growing Gourmet and Medicinal Mushrooms*, Ten Speed Press, Berkeley, CA, 367–376, 2000.
30. Wasser, S.P., Didukh, M.Y., Amazonas, M.A.L.A., Neno, E., Stamets, P., and Eira, A.F., Is a widely cultivated culinary-medicinal royal sun *Agaricus* (the Himematsutake mushroom) indeed *Agaricus blazei* Murrill? *Int. J. Med. Mushrooms,* 4, 267–290, 2002.
31. Wu, J.L., Lin, J.C., Fu, M.C., and Wei, S.T., *Grifola frondosa*, in *Cultivation of Eighteen Precious and Delicious Edible Mushrooms* [in Chinese], Huang, N.L., Ed., Chinese Agricultural Press, Beijing, China, 130–145, 1997.
32. Yu, C.B., *New Techniques for Cultivation of Agaricus blazei* [in Chinese], Biyang Research Institute of Fungi, Henan, China, 2001.
33. Zhou, C.Y., Guo, Q., Bai, Y.Q., Yang, Y., Jia, W., and Zhang, J.S., Nutrition composition analysis in fruitbodies and submerged mycelia of *Grifola frondosa* [in Chinese], *Acta Edulis Fungi*, 8, 10–14, 2001.
34. Zhuang, C. and Mizuno, T., Biological responses from *Grifola frondosa* (Dicks.: Fr.) S.F. Gray — Maitake (Aphyllophoromycetideae), *Int. J. Med. Mushrooms*, 1, 317–324, 1999.

21 Other Cultivated Mushrooms — Their Number Grows

I. INTRODUCTION

Although the intentional growing of mushrooms began approximately 1000 years ago and the wild species that have been used as food number in the hundreds, the species that have been extensively cultivated are few. Even today in North America and Western Europe, almost 90% of the cultivated mushrooms are of one species, *Agaricus bisporus*. However, both regions are now considered to be in a saturated market, and people are willing to pay more for new varieties of mushrooms.[19] There has been a gradual increase in consumption of other mushrooms, such as *Pleurotus* spp., *Lentinula edodes,* and *Hypsizygus marmoreus*. On the other hand, in the worldwide market, the percentage of production of *A. bisporus* has declined from 71.6% in 1981 to 31.8% in 1997.[2] We have shown in the previous chapters that a number of other species are cultivated: some, e.g., *Lentinula, Auricularia*, *Flammulina*, and *Pleurotus*, in fairly large amounts; and others, e.g., *Pholiota*, *Tremella*, and *Dictyophora*, in increasing amounts. The five most popularly cultivated edible mushrooms are *A. bisporus, L. edodes, Pleurotus* spp., *Auricularia* spp., and *F. velutipes*, which accounted for 83.9% of total world production in 1997. The top five in China are *Pleurotus* spp., *L. edodes, Auricularia* spp., *A. bisporus*, and *F. velutipes,* which made up 88.5% of the national production in 2001. In Japan, the top five are *L. edodes, F. velutipes, H. marmoreus, Grifola frondosa,* and *Pholiota nameko,* which contributed up to 96.0% in 1999. There are still others, for which cultivation techniques are available that are expected to be produced in increased amounts, some that are very popular but for which only semicultivation techniques are available, and some which are very popular in some regions but are less well known or less appreciated elsewhere.

Two of the edible mushrooms that we anticipate will be cultivated in increasing amounts are *Auricularia*, known as the wood ear mushroom, and *Hericium*, with the common name of either monkey-head or bear's-head mushroom. Brief accounts of these mushrooms are given in this chapter. Mushrooms that are very popular but are as yet produced only by semicultivation techniques because they are mycorrhizal fungi are *Tuber,* which is the truffle famous in French cuisine, and *Tricholoma matsutake,* the pine mushroom that is exalted by the Japanese. These and *Stropharia rugosoannulata*, a mushroom popular in Germany and Eastern Europe, are considered even more briefly.

The introduction of the bag culture technique not only increased the amount and place of production of some species of edible mushrooms, but also increased the number of species that could be conveniently cultivated. Those species formerly cultivated on wood logs are now capable of being grown in bag culture on substrates composed not only of sawdust but also of other agricultural and industrial wastes, e.g., cottonseed hulls, ground-up corncobs, sunflower seed hulls, waste paper, etc. The bag culture technique offers the opportunity of more controlled experimentation on various aspects of the cultivation process.

Edible wild mushrooms are found in the markets in many countries of Europe and Asia. People there also enjoy excursions to the countryside and woodlands to gather wild mushrooms, and this is also a popular pastime in America. Furthermore, gourmets appreciate the fine flavor and texture of many wild mushrooms. Consequently, there are many mushrooms that would be welcomed by

the public if they were available in greater amounts and not just in the season in which the mushrooms fruit in nature. The demand for these wild edible mushrooms has been responsible for the establishment of businesses to supply them. The largest of these in 1987 was that of Helmut Lingens of Germany, whose organization imports edible mushrooms from collectors all over the world. These collectors have been given instructions for the proper collection and processing of the specific species that are desired.

As an example of the availability of and interest in wild edible mushrooms, we cite a survey made in China,[18] which reported that the edible species in China number more than 900. Of these 900 species, only about 60 species are either already being cultivated or are in the process of being domesticated. The number of cultivated species increases to 90 if it includes the species for mycelial production in liquid media. This means that only about 10% of known mushroom species in China are utilized. Thus, a vast majority of the edible species are available only in the wild. It was suggested[12] that if farmers were organized to collect those wild mushrooms available at the time of the farmers' slack season, the supply of mushrooms in the local markets would be supplemented, and also the wild mushrooms could be exported after processing.

II. *AURICULARIA*

Auricularia spp. are members of the class Basidiomycetes, subclass Phragmobasidiomycetidae, order Auriculariales, and family Auriculariaceae. The fruiting bodies of members of the genus *Auricularia* are waxy and cartilaginous, and the color ranges from purplish brown to black, especially when they are dried. The genus name *Auricularia* is derived from the Greek word *auricula*, meaning ear. Therefore, this group of mushrooms is commonly called wood ear, Jew's ear, or simply ear fungus because of the shape of their fruiting bodies. The fruiting bodies are not only shaped like a human ear (Figure 21.1), but also feel like ears, due to their rubbery, gelatinous state when fresh.[10,22]

Among the ten species identified by Lowy[17] from his worldwide collection, *A. auricula* (Hook.) Underw. and *A. polytricha* (Mont.) Sacc. are the two most popular edible species. The former is usually collected from its natural habitat but it has been massively produced by plastic bag methods, and the latter is commonly cultivated on logs of various trees or on sawdust with different supplements. They are saprophytic fungi.

The *Auricularia* mushroom has been reported[3] to be the first mushroom cultivated — in China around A.D. 600. Since then the mushroom has continued to be one of the most popular of the

FIGURE 21.1 Fruiting bodies of *Auricularia polytricha* grown on sawdust substrate in a bottle.

edible mushrooms in China. Dr. Peter Buchanan, mycologist of the Plant Diseases Division of the Department of Scientific and Industrial Research in Auckland, New Zealand, kindly provided us with information[6] concerning shipment of *A. polytricha* from New Zealand to China and Hong Kong. Records indicate that this trade occurred from 1872 through 1914, and in some years amounted to 200 to 300 metric tons (MT) (dry weight). Over 100 years ago, Chinese working in New Zealand clearing forestlands observed *Auricularia* growing on trees in large numbers. They harvested and dried the fruiting bodies, and soon the mushrooms were being shipped to China and Hong Kong from New Zealand in considerable amounts as an export item.

Because of the increasing importance of this mushroom due to its nutrient contents and general appreciation as a table delicacy, the biological nature[4,9,21,22,26] and cultivation methods[4,16,25] have been investigated recently. In 1986, the world production of *Auricularia* mushrooms was estimated to be 5700 MT (dry weight).[14] In1997, the world production of the mushroom was estimated to be 485,300 MT (fresh weight), which made up 8.0% of the total world production of cultivated edible mushrooms.[2]

The basic principles and techniques for cultivation of *Auricularia* mushrooms are similar to those of *Lentinula* (see Chapter 13) and *Tremella* (see Chapter 17) in that they can be grown both on wood logs and on media of sawdust plus supplements in plastic bags. As is the case for both *Lentinula* and *Tremella*, cultivation of *Auricularia* in China has been rapidly increasing in recent years; e.g., in 2001, the production of this mushroom increased to 1124 thousand MT. *Auricularia* is also being cultivated in more countries than ever before.

III. *HERICIUM*

Hericium erinaceus (Bull. Ex Fr.) Pers. is now cultivated in China. It is called Houtou (monkey-head mushroom) as its fruiting body looks like the head of a baby monkey. Elsewhere it is sometimes referred to as the bear's-head mushroom. *Hericium* is a member of the class Basidiomycetes, subclass Holobasidiomycetidae, order Aphyllophorales, and family Hericiaceae. The fruiting body is 5 to 30 cm in size in nature, with a rudimentary stipe. It is white but tends to yellow, and its outstanding feature is that it is covered by teeth 3 to 6 cm long. The spores are white, subglobose in shape, smooth and 6 to 7 μm in size. It is a famous edible fungus in China, and it has been described by some as delicious and highly prized, by others as tasty. It is also reputed to have good medicinal properties. A Chinese traditional drug prepared by drying fruiting bodies is called "Houtou" for treating chronic stomach diseases. Recently, it has been reported that polysaccharides contained in the *H. erinaceus* fruiting body have carcinostatic effects on gastric, esophageal, hepatic, and skin cancer, based on the regulation of immunological functions.[20] Prior to 1959–1960 it was available only in the wild, and it was fairly difficult to collect because its habitat is in the deep forest where it grows on old and dead wood.

Hericium was domesticated by scientists at the Shanghai Agricultural Academy of Science, and from there the cultivation technique spread to other places. It is now grown in plastic bag cultures on a variety of substrates: sugarcane bagasse, sawdust, cottonseed hulls, corncobs, and chopped up paddy straw — each supplemented with rice or wheat bran, sucrose, gypsum, and sometimes additional ingredients. The cottonseed hull substrate is considered by some[15] to be the best material for cultivating the monkey-head fungus, because (1) its cost is low, (2) it is easy to prepare, and (3) it gives a high yield. Thus, a cottonseed hull substrate is recommended for cultivating *Hericium* in any cotton-growing region. At the present time, it is mainly produced in China. The annual production was estimated to be 2800 MT in 1998 and 9547 MT in 2001.

A spawn substrate of the following composition has been used:

Sawdust	78 kg	Gypsum	1 kg
Rice bran	20 kg	(Water added to give moisture content of 65%)	
Sucrose	1 kg		

The bottles are filled with this substrate, sterilized, cooled, and then inoculated with a pure culture of *H. erinaceus* that may be obtained by tissue culture or by spore isolation. Mycelial growth was found to be best at 25°C and to stop at 35°C. As is the case with most mushrooms, the formation of fruiting bodies requires a slightly lower temperature (20°C is the optimum), with fruiting body development slowed, or even inhibited, at 25°C. In the other direction, pinhead formation is difficult below 14°C, and, even if pinheads do form at these low temperatures, mushrooms do not develop.[5]

For cultivation in bottles, the substrate should fill the bottle, and the mycelium should have run to the bottom by 20 days' incubation at 25 to 28°C. When this has happened, the bottle should be transferred to a place where the temperature is lower, 15 to 24°C. At this temperature, the mycelium will continue to grow and accumulate nutrients until primordia form at the mouth of the bottle. When primordia appear, the plug should be removed, the humidity should be between 85 and 90%, and little light is required (strong light inhibits the development of fruiting bodies). These are the general conditions that will lead to mushrooms at the stage of spore discharge, about 10 days after the formation of the primordia.

When the fruiting bodies have formed spines (Figure 21.2) and start to discharge spores, it is time for the mushroom to be harvested. A knife should be used to cut the stalk inside the bottle. The stump of the stalk should not be over 0.8 to 1.5 cm in length, because a long stump leads to easy contamination of the substrate, nullifying the opportunity for subsequent flushes. After harvesting, the surface of the substrate should be raked gently to provide sufficient air and as a stimulation for the next flush, and it should be watered for 6 days. The primordia will emerge again in about 8 to 10 days. Generally, each bottle can be harvested three to four times, but only one mushroom is produced at each flush. Each mushroom weighs 60 to 70 g, with large ones reaching 90 to 100 g, and the mushroom of the first flush is larger than those of subsequent flushes.

The harvested mushrooms can be placed on newspapers or bamboo mats for drying (Figure 21.3) either in shade or in sunlight. Another method that is sometimes used is to thread them onto a string extended between poles in such a way that they are facing the sun and also receiving good ventilation. In rainy weather drying should be done by an electric oven or hot air convection system of some sort. In the drying process, care should be taken not to damage the spines. An initial temperature of 40 to 50°C is gradually raised to 60°C for drying. When the mushrooms are dry, they are packed in a plastic bag, which is then sealed.

FIGURE 21.2 *Hericium erinaceus,* the monkey-head mushroom (also known as the bear's head mushroom), growing in bottles on sawdust substrate (Left) Bottle oriented to show attachment of mushroom to the substrate. (Right) The spines project downward as shown.

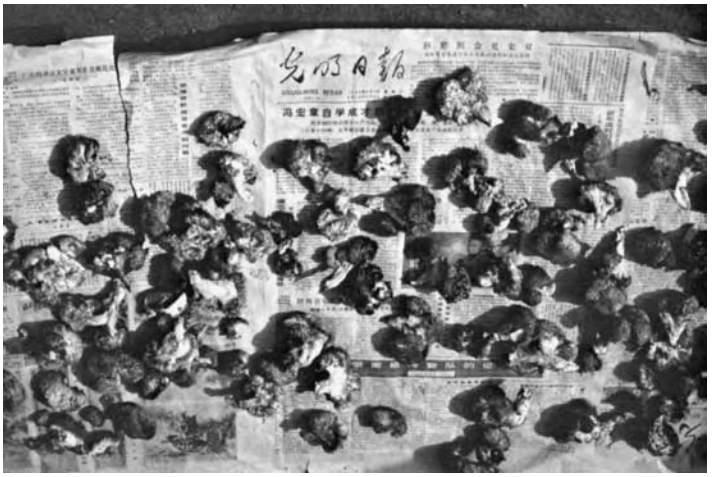


FIGURE 21.3 After harvesting, monkey-head mushrooms can be sun-dried on newspapers.

Another method of preservation is to soak the mushrooms in brine. The stalk is first removed, because it has a bitter taste, and then the mushrooms are washed with clean water. They are then boiled in a 1/1000 solution of citric acid for 10 min. Next, the mushrooms are placed in cold water for cooling, and then they are put into a container, and 25% salt (according to mushroom weight) is added. The procedure is to put one layer of mushrooms into the container, then a layer of salt, and to repeat this process. Finally, the mushrooms are pressed down to make sure that they are immersed in the salt water. When preparing mushrooms preserved in a brine solution for the table, they should first be rinsed in warm water several times to remove the salt.

The causes of formation of abnormal mushrooms, which are of lower value, have been studied.[11] The abnormalities include an altered morphology of the mushroom brought about by irregular branching, an absence of spines, and an abnormal color. These abnormalities have been found to be due to such things as unsuitable nutrition, a high carbon dioxide concentration, improper conditions of temperature and humidity, and improper water management. Thus, management to provide proper aeration, temperature, humidity, and substrate moisture content is essential.

The relative ease of growing the monkey-head mushroom on a variety of agricultural wastes and the fact that it is used for medicinal purposes as well as for food make it very likely that *H. erinaceus* will be cultivated in increased amounts in the future.

IV. OTHER TYPES OF INTEREST

A. Mycorrhizal Mushrooms

To date, there has been a singular lack of success in fruiting any of the mycorrhizal fungi in culture. Many of the best of the edible fungi require mycorrhizal association, and many attempts have been made to fruit such mycorrhizal fungi as the truffle (*Tuber*), the matsutake or pine mushroom (*Tricholoma*), the chanterelle (*Cantharellus*), and the cepe (*Boletus*), especially the first two. There is little reason to doubt that the close symbiotic relationship of these fungi with the roots of plants has evolved into a close dependency of the fungus on the plant for the conditions that must be fulfilled to trigger the shift from vegetative growth to such a complex structure as the fruiting body. Even though the fungus may be grown vegetatively in pure culture, the growth is frequently slow. For example, mycelial growth of *Tricholoma matustake* (S. Ito et Imai) Sing. is relatively slow in culture on a variety of agar media. Attempts to induce fruiting by incorporation in the media of

various nutritional ingredients in combination with different physical conditions have not produced the sought-for goal — fruiting bodies formed under controlled conditions.

The mushroom scientist frequently takes the approach of mimicking the conditions of nature in order to get the fungus to do under controlled conditions what it does in nature. Thus, the ecology of the mushroom is studied, and this is the approach that has been taken in France in studying *Tuber*[7] and in Japan in studying *Tricholoma matsutake*.[24] These ecological studies have been responsible to a large extent for the development of semicultivation techniques, which have made possible good yields of these mushrooms by a type of farming.

The golden chanterelle (*Cantharellus cibarius* Fr.) and allied species are highly appreciated edible mushrooms and usually considered to be mycorrhizal fungi. There have been many efforts to cultivate this species, and Danell and Camacho[8] have reported the first successful fruiting body formation in the greenhouse hosted by pine seedlings only 16 months old.

It is to be anticipated that further studies along ecological lines with other mycorrhizal fungi will lead to a greater production of those other highly prized mushrooms by semicultivation methods. Such studies will provide the necessary nutritional, physiological, genetic, and morphogenetic information for the eventual understanding of the events that lead to the fruiting of these mycorrhizal mushrooms, and with this understanding will surely follow the ability to duplicate the process in the laboratory and mushroom house. Since in the past decade there has been a great impetus in research on mycorrhizal associations, we can expect that the synthesis of some of the information gained will lead to the development of concepts that will be of value in helping to interpret the phenomenon of fruiting of mycorrhizal fungi.

B. Species with Regional Appeal

There are a number of edible mushrooms that have strong appeal in certain regions, usually stemming originally from their availability in nature. *Termitomyces*, famous as the mushroom gardened by termites, is collected in the wild and marketed in countries of Central Africa and Southeast Asia. The cultivation by termites of *Termitomyces* has been presented in an interesting article by Bels and Patarageteit.[1] To date, humans have not been able to match the skill of the termites in cultivation of this mushroom.

Stropharia rugoso-annulata is a mushroom of great popularity in Germany, Poland, Hungary, Czechoslovakia, and currently in regions spreading out from this center. This is interesting because the species was first described when found in the United States, and only later was it found in Europe, but it is not known whether the species was introduced into Europe from America or had existed in Europe earlier.[23]

About 1970, thousands of people in the countries previously mentioned undertook cultivation of this mushroom in their gardens, as some simple and easy procedures had been worked out and published for general distribution. The fact that a single fruiting body of *S. rugosa-annulata* can have a pileus 5 to 40 cm in diameter and may weigh 60 g probably adds to the fascination that it holds for some people. This is reflected by the common name "Gartenriese" (giant of the garden) given to it. *Stropharia rugoso-annulata* is a heterothallic, tetrapolar basidiomycete. The dikaryotic mycelium bears clamp connections. A saprophytic fungus, *S. rugoso-annulata* is easily cultivated on straw. As with other edible species, the yield depends on the skill and knowledge of the grower. With so many people involved in growing this mushroom, it is not surprising to find that there is a great range in yield, with 3 to 30 kg/m^2 reported.[23] Although this mushroom has been grown by a large number of individuals, the state of the art in its cultivation has not yet reached the stage where it will be cultivated on a large scale with highly mechanized techniques by large growers. There are certain drawbacks, including a fairly long period of cultivation, poor storage qualities, and fragility of the mushrooms. Other mushrooms have greater potential for large-scale cultivation.

We selected *S. rugoso-annulata* to illustrate how regional popularity of a mushroom species can come about and how quickly this can spread to adjacent areas. There are other examples that

could have been cited, and the next few years will likely add to the list of mushrooms that have centers of cultivation in specific regions.

China was first in successfully cultivating *Auricularia, Lentinula, Tremella, Ganoderma, Hericium, Volvariella* and other edible and medicinal mushrooms. In the past 10 years, several economically important edible mushrooms have been commercially cultivated. Huang[13] describes in detail the cultivation of 18 new commercially cultivated edible mushrooms, which are (1) *Pleurotus eryngii* (DC. ex Fr.) Quel., (2) *Pleurotus ferulae* Lenzi, (3) *P. eryngii* var. *nebrodensis* (= *P. nebrodensis*), (4) *P. cystidiosus* O.K. Miller, (5) *P. abalonus* Han, Cheng et Cheng, (6) *P. djamor* (Fr.) Boedjin *sensu* Lato, (7) *P. tuber-regium* (Fr.) Sing., (8) *Agrocybe aegerita* (Brig.) Sing., (9) *Hypsizygus marmoreus* (Peck) Bigelow, (10) *Armillariella mellea* (Vahl. Ex Fr.) Karst., (11) *Stropharia rugoso-annulata* Farlow and Murrill, (12) *Agaricus blazei* Murrill, (13) *Oudemansiella radicata* (Relh. ex Fr.) Sing., (14) *Coprinus comatus* (Mull. ex Fr.) S.F. Gray, (15) *Macrolepiota procera* (Scop. ex Fr.) Sing., (16) *Clitocybe maxima* (Gartn et Mey. ex Fr.) Quel., (17) *Grifola frondosa* (Fr.) S.F. Gray, and (18) *Fistulina hepatica* (Schaeff.) Fr. There is no doubt that more and more new mushroom species will be domesticated and commercially cultivated not only in China but also in other parts of the world in the future.

REFERENCES

1. Bels, P.J. and Pataragetvit, S., Edible mushrooms in Thailand, cultivated by termites, in *Tropical Mushrooms*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 445–461, 1982.
2. Chang, S.T., World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing., in China, *Int. J. Med. Mushrooms,* 1, 291–300, 1999.
3. Chang, S.T. and Miles, P.G., Historical record of the early cultivation of *Lentinus* in China, *Mushroom J. Trop*., 7, 47–52, 1987.
4. Cheng, S. and Tu, C.C., *Auricularia* spp., in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 605–625, 1978.
5. Chiu, R.S., Growth conditions for *Hericium*, *Edible Fungi*, 1, 24, 1981.
6. Church, A.H., An edible fungus of New Zealand, *N.Z. J. Sci*., New Issue 1(2), 55–58, 1891.
7. Delmas, J., *Tuber* spp., in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 645–681, 1978.
8. Danell, E. and Camacho, F., Successful cultivation of the golden chanterelle, *Nature*, 385, 303, 1997.
9. Duncan, E.G., Population diversity and speciation in *Auricularia polytricha*, in *Tropical Mushrooms*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 409–425, 1982.
10. Findlay, W.P.K., *Wayside and Woodland Fungi*, Frederick Warne, London, 1967.
11. Ho, C.C., The types of abnormal monkey head mushrooms, *Edible Fungi*, 4, 42, 1981.
12. Huang, N.L., Collection, processing, and exporting of wild mushrooms, *Edible Fungi*, 4, 46–47, 1987.
13. Huang, N.L., *Cultivation of Eighteen Precious and Delicious Edible Mushrooms*, Chinese Agricultural Press, Beijing, China, 1997.
14. Kong, Y.C., *Cultivation Methods of Edible Fungi*, China Food Press, Beijing, China, 1986.
15. Liu, C.Y., Technique of cultivation of monkeyhead mushroom, *Edible Fungi,* 4, 33–34, 1981.
16. Lou, L.H., Cultivation of *Auricularia* on logs in China, in *Tropical Mushrooms*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 437–441, 1982.
17. Lowy, B., A morphological basis of classifying the species of *Auricularia*, *Mycologia*, 43, 315–358, 1951.
18. Mao, X., Edible mushroom resources and researches in China, *Mushroom Market*, 8, 8, 2002.
19. Megens, H., Western fresh mushroom market — key drivers and impact on value chain, *Mushrooms Int.*, 81, 4–6, 2000.
20. Mizuno, T., Bioactive substances in *Hericium erinaceus* (Bull.:Fr.) Pers. (Yamabushitake), and its medicinal utilization, *Int. J. Med. Mushrooms*, 1, 105–119, 1999.
21. Quimio, T.H., Physiological considerations of *Auricularia* spp., in *Tropical Mushrooms*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 397–408, 1982.

22. Quimio, T.H. and de Guzman, R., Taxonomy and basidiocarp development of *Auricularia* mushrooms, in *Tropical Mushrooms*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 383–395, 1982.
23. Szudyga, K., *Stropharia rugoso-annulata*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 559–571, 1978.
24. Tominaga, Y., *Tricholoma matsutake*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 683–697, 1978.
25. Vilela, L.C. and Silverio, C.M., Cultivation of *Auricularia* on composted sawdust in the Philippines, in *Tropical Mushrooms*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 427–435, 1982.
26. Wang, C.W., Cellulolytic activity during the sporocarp development in *Auricularia polytricha*, *Mushroom Newsl. Trop*., 1(2), 2–4, 1980.

22 Technology and Mushrooms

I. INTRODUCTION

Despite the expanding and improving role played by modern technology every day in human civilization, humans still face, and will continue to face, three basic problems: (1) food shortage, (2) environmental pollution, and (3) a reduction in the quality of human health because of the continuous increase in world population. The 20th century began with a world populated by 1.6 billion and ended with 6 billion inhabitants, with most of the growth occurring in developing countries. The population of the world is increasing with an annual growth rate of 1.7%, and by the year 2050 there will be over 9 billion persons on Earth, placing further demands on already inadequate or diminishing resources such as food and energy. We are all very much aware of the famines caused by food shortages in various parts of the world, of the crises produced by inadequate supplies of energy, of the pollution problems imposed by populations concentrated within small areas, and by the waste products of our modern industrialized society. Even so, it may come as a shock to the reader to learn that one third of the world's population has too little protein in their diet. The problem of providing an adequate diet for the world's population is an old one that is still with us today, and this will become even more serious in the future in that larger numbers of individuals will be affected by it. At present, approximately 800 million people in the world are living at the poverty level.

The search for solutions to problems of these dimensions should involve many approaches because they are not simple problems. Different approaches may provide partial solutions that will contribute to the alleviation of the problem. Our concern in this book is with the production of edible and medicinal mushrooms, which, as you are aware by now, are relatively high in protein and which may be grown on the organic wastes of industry, agriculture, and households. Mushrooms will certainly not provide the complete solution to the problems of insufficient food, environmental pollution, and reduced quality of human health for an expanding population, but increased production of both edible and medicinal mushrooms can contribute to the solution of these problems. An interesting and useful aspect of mushroom cultivation is that the organic wastes used as nutrients for the cultivation of mushrooms are abundant, and they are frequently detrimental to the environment in that they contribute to the pollution problem.

Misconceptions about mushroom culture are exceedingly common, especially in the developing countries. Although it is thought to be very simple, we have tried to show in this book that mushroom cultivation is, in fact, a complicated business. It involves a number of different operations including: preparation of a fruiting culture, spawn, and compost/substrate, as well as crop management and marketing. Although it can be treated as a very primitive type of farming, as in the cultivation of the straw mushroom, *Volvariella volvacea*, in the countries of Southeast Asia,[4,5,30,50] it can also be a highly industrialized agricultural enterprise with a considerable capital outlay, as in the cultivation of the *Agaricus* mushroom.[50] In any case, it is the aim of mushroom growers and researchers to try to increase the yield from a given surface area, to shorten the cropping period, and to have many more flushes with a high yield each time. In this connection, a thorough understanding of the starting materials for the synthesis and the proper preparation of the substrate, the selection of suitable media for spawn making, the breeding of high-quality strains, as well as the improvement

of bed management and the prevention of the development of pests and mushroom diseases must all be given careful consideration.

Mushroom science was defined in Chapter 1 as "the discipline that is concerned with mushroom cultivation." It was also noted that, like any branch of science, it needs systematic investigation to establish facts and principles for future development. Moreover, constant production of successful crops requires both practical experience and scientific knowledge. Practical experience can be obtained through a period of personal participation that includes training in and observation of the practices of mushroom cultivation. The scientific knowledge basically comes from three fields: microbiology, fermentation, and environmental engineering.

II. MICROBIAL BIOTECHNOLOGY

A. What Is Biotechnology?

The biotechnological exploitation of microbes has become more and more important both for industrial purposes and for the treatment and utilization of solid organic waste materials. Most agricultural, industrial, and household solid waste materials are rich in organic matter containing mainly cellulose, hemicellulose, and lignin. These components of the wastes are the most resistant materials to biological degradation that occur widely in nature, and they have little or no food value in their unaltered form. Furthermore, the disposal of some of these waste materials can be a major source of environmental pollution. However, when modified through proper treatment with microorganisms, these improved wastes can serve more effectively as growth substrates for cattle and other ruminants or for organisms used directly by humans as food, such as mushrooms, which are rich in food protein.

Biotechnology defies a simple, accurate, useful definition, but it can be described. Biotechnology denotes those processes that produce commercial quantities of useful substances through the use of microorganisms, plant cells, animal cells, or parts of cells such as enzymes. Biotechnology is also the construction of microorganisms, cells, plants, and animals that have useful traits by recombinant DNA techniques, cell fusion, or other methods besides traditional breeding techniques. The application of molecular biology to understanding how cells and organisms work so that the activities of cells and organisms can be altered or repaired is another aspect of biotechnology.[43]

B. Lignocellulose Degradation and Utilization

Estimates of total solar energy that impinges on the Earth annually, the total energy that is fixed each year in biomass, and the annual energy requirements in the world are given in Table 22.1. The yield per year in the world of the most abundant recyclable biomass components available to humankind — cellulose, hemicellulose, and lignin — are given in Table 22.2. Because such a large amount of energy is present in lignocellulosic materials, they constitute principal objects of conversion to useful products by human activities.

TABLE 22.1
Estimated Energy per Year

Estimated needed in world	7.2×10^{16}
Solar energy free on Earth[a]	7.2×10^{20}
Solar energy fixed in biomass	7.2×10^{17}

[a] Total energy the Earth receives a year.

Source: Data from Pack, M.Y., Korea Advanced Institute of Science, personal communication, 1986.

TABLE 22.2
Biomass Yield in World per Year

Biomass on Earth	1.64×10^{11}
Biomass on land	1.09×10^{11}
Cellulose	4.91×10^{10}
Hemicellulose	2.18×10^{10}
Lignin	2.18×10^{10}

Note: Values are tons, dry.

Source: Data from Pack, M.Y., Korea Advanced Institute of Science, personal communication, 1986.

Lignocellulose is a major component of wood and other plant materials, and lignocellulosic compounds are complex and insoluble. Lignin is made up of aromatic building blocks that are very resistant to breakdown. The lignocellulosic materials can be treated by various chemical methods, e.g., with dilute hydrochloric acid and calcium chloride, to increase the digestibility and nutritional qualities and even to form sugars to serve as carbon sources. However, these chemical methods are tedious and costly. Furthermore, treatments to eliminate adverse side effects of the chemicals are also very complex. In contrast, microbiological techniques have become popular in recent years to improve nutritional quality and to upgrade the economic value of the solid organic wastes. Some examples are described below.

1. Isolation of Actinomycete Strains

Isolates of actinomycetes with the ability to break down lignin from wheat lignocellulose and capable of fast solubilization of up to 40% of the lignin substrate have been isolated.[20] The actinomycetes offer advantages over fungi: (1) their initial attack is faster; (2) their degradative system is more robust in that it is not adversely affected by low oxygen tensions; and (3) recombinant DNA technology as a method of improvement is feasible with actinomycetes but not, as yet, with higher fungi.

2. Selection of Suitable Species of White-Rot Fungi

It has been known for a long time that white-rot fungi can degrade lignin and lignocellulosic materials, thereby increasing the digestibility of these wastes. Sugar beet pulp (SBP) fermented in the presence of *Sporotrichum thermophile* and *Thielavia terrestris* gave a protein content of 14.6 and 13.4%, respectively, after 3 days in culture, which was a net increase of 48 and 36%, respectively, over the protein content of the unfermented SBP, a by-product of the sugar industry.[3] Because of its low protein content, unfermented SBP must be supplemented with other protein sources before it can be used effectively in animal feeding. Under favorable conditions, these fungi preferentially degrade lignin while preserving cellulose. Other white-rot fungi, *Sporotrichum pulverulentum, Dichomitus squalens*,[60] *Eucryphia cordifolia, Laurelia philippiana*,[61] and *Stropharia rugosoanulata,*[25] can also degrade lignin and increase the digestibility of lignocellulosic substances like wood and wheat straw. The highest digestibility of white-rot-decomposed wood for ruminants was 77%, and the average digestibility was 30 to 60%. The digestibility of underdecomposed wood was maximally 3% (Table 22.3).

Pleurotus ostreatus, an edible mushroom, was used for increasing the feed value of wheat straw by solid substrate fermentation — a cheap and simple technology compared with the conventional submerged fermentation.[24] In addition to increasing the crude protein content, digestibility was upgraded because of the fungal breakdown of lignin and cellulose.

TABLE 22.3
Lignin Content and *in Vitro* Ruminant Digestibility of Nondecomposed Wood and Wood Decomposed by a White-Rot Fungus, *Ganoderma applanatum*

Wood	Fungus	Lignin (%)	*In Vitro* Digestibility (%)
Eucryphia cordifolia Cav.	—	26.2	0.0
Laurelia philippiana Looser	—	26.7	3.1
E. cordifolia	*G. applanatum*	1–23	77.7–30
L. philippiana	*G. applanatum*	21–27	34–26

Source: Data from Zadrazil, F. et al., *Eur. J. Appl. Microbiol. Biotechnol*., 15, 167–171, 1982.

3. Isolation of Hypercellulolytic Mutants

The enzymatic hydrolysis of cellulose to glucose involves the cooperative functioning of at least three enzymes, namely, an exoglucanase, endoglucanases, and a β-glucosidase. Several fungi are known to secrete these enzymes and, among them, the best known is *Trichoderma reesei*. When lignocellulosic materials are hydrolyzed using cellulase from *T. reesei*, cellobiose accumulates as a result of a deficiency in activity of the enzyme β-glucosidase, which is necessary for cellulose hydrolysis. The isolation of hypercellulolytic mutants of *T. reesei* has greatly improved the potential for conversion of lignocellulosic materials to monomer sugars. Cellulase produced through mixed cultures of *T. reesei* and *Aspergillus phoenicis*[12] and *T. harzianum* and *A. ustus*[31] also showed increased β-glucosidase activity and greatly improved hydrolytic potential.

4. Cultivation of Mushrooms

In the development and upgrading of utilization of organic wastes, mushroom production is one of the few proven examples of microbial biotechnology. Edible fungi are at present the only organisms that can effectively convert lignocellulose waste materials directly into human food or animal feed. In many parts of the world organic wastes are used to grow varieties of mushrooms that are an acceptable and nutritious food. The technique of mushroom cultivation can be primitive, as in rural farming for *Volvariella* and *Pleurotus* mushrooms and even for *Lentinula* and *Agaricus* mushrooms in developing countries; or it can be highly industrialized as in the *Agaricus* industry in Western countries that use modern technology and sophisticated equipment.[6] Biological efficiency, i.e., the yield of fresh mushrooms in proportion to the weight of compost at spawning, can reach 100% for *A. bisporus* in experimental tests, with 65 to 75% as a good average value over a year. For *P. sajor-caju*, 200% biological efficiency has been recorded, with over 80% as an average. The biological efficiency of *V. volvacea*, on the other hand, varies from 2.5 to 65%.[7] After harvesting the mushrooms, the organic wastes modified by the mushroom mycelia can make a nutritious feed for cattle.

The composting process required for the cultivation of mushrooms is aimed at achieving nonsterile but selective growth media, which favor the mycelial growth of mushrooms against a wide range of competitor microorganisms present in the compost. During the composting process, the microbial flora builds up rapidly. Species of mesophilic, thermotolerant, and true thermophilic bacteria, actinomycetes, and fungi have been isolated (Table 22.4).[54] Their attack on the lignocellulosic straw materials included tunneling, cavity formation, fiber release, and other types of wall erosion.

Microbial activity converts the basic raw materials of straw and other organic wastes into mushroom compost. The total crop of these microorganisms (both living and dead) in compost has

TABLE 22.4
Major Groups of Microorganisms Isolated from Compost during Substrate Preparation

Bacteria	Actinomycetes	Fungi
Mesophiles		
Flavobacterium spp.	*Streptomyces* spp.	*Mucor* spp.
Pseudomonas spp.	*Nocardia* spp.	*Aspergillus* spp.
Serratia macescens	*Micropolyspora* spp.	*Penicillium* spp.
Thermotolerants		
Pseudomonas spp.		*Aspergillus fumigatus*
Bacillus licheniformis		
Thermophiles		
Bacillus coagulans	*Thermoactinomyces* spp.	*Torula thermophile*
Bacillus stearothermophilus	*Thermomonospora* spp.	*Humicola insolens*
Bacillus subtilis		*Rhizomucor pusillus*
		Talaromyces lanuginosa

Source: Data from Wood, D.A., *J. Chem. Tech. Biotechnol.*, 34B, 232–240, 1984.

been termed the **microbial biomass**.[18] This microbial biomass build-up during composting may serve as a concentrated source of nitrogen, carbon, and minerals for mushrooms; e.g., the extracellular enzymes and their products produced by the *A. bisporus* mycelium are shown in Table 22.5. Thus, mushrooms can produce a wide range of hydrolytic and oxidative enzymes, which can degrade all of the major fractions from the composted straw. Lignin, cellulose, and hemicelluloses are all utilized in the period from inoculation to the end of cropping (Table 22.6). In addition to degrading the major components of composted straw, the mushroom mycelium also degrades the microbial flora built up during composting. Microbial biomass in compost (prior to spawning) was estimated to be about 2% of the compost dry weight, assuming that microbial biomass contains 50% w/w carbon.[18] The ratio of fungal plus actinomycete to bacterial biomass in compost at spawning was nearly 3:1.

TABLE 22.5
Extracellular Enzymes of *Agaricus bisporus*[55]

Enzyme	Nutrient in Growth Substrate (Compost)	Production of Enzyme Activity for Assimilation by Fungus
Laccase	Lignin or phenols	?
Endocellulase	Cellulose and cellobiose	Cellobiose and glucose
Exocellulase	Cellulose and cellobiose	Cellobiose and glucose
β-Glucosidase	Cellulose and cellobiose	Cellobiose and glucose
Xylanase	Xylan	Xylose
Proteases	Protein (plant and microbial)	Amino acids
Phosphatase	Phosphate esters	Phosphate
β-*N*-Acetyl-muramidase (lysozyme)	Bacterial cell walls	?
β-*N*-Acetyl-glucosaminidase	Microbial cell walls	?
Laminarinase	β-(1→3)-Glucans	Glucose
DNAase	Microbial DNA	Sugars and nucleotides
RNAase	Microbial RNA	Sugars and nucleotides
Lipase	Plant or microbial lipids	Fatty acids

TABLE 22.6
Nutrient Removal during Mushroom Life Cycle

Component	At Inoculation	After Cropping
Total	100.00	82.40
Ether solubles	1.26	0.48
Water solubles	3.21	18.70
Hemicelluloses	6.80	2.64
Cellulose	12.51	7.51
Lignin	22.91	7.77
Ash	45.20	56.90
Total nitrogen	1.56	1.77
Water-soluble nitrogen	0.14	0.88

Source: Data from Wood, D.A. and Fermor, T.R., *Mushroom J.,* 114, 194–197, 1982.

Basic research using genetic engineering techniques on the extracellular enzymes that break down lignocellulose may lead to increased activity of the extracellular enzymes produced by the microorganisms. This can increase the productivity of mushrooms, because it makes the lignocellulose substrate more suitable for mushroom growth. The genetic improvement of current commercial strains of mushrooms by increasing yields of extracellular enzymes is also a potentially useful strategy. Such strains can increase substrate bioconversion.

The application to soil of the spent compost, consisting of degraded cellulose and lignin following mushroom production, is important for improvement of soil conditions. In addition to providing a balanced nitrogen (Table 22.7) and carbon source for microbial growth, the spent compost will be further degraded in the soil to form a fine black acidic material known as **humus**.

TABLE 22.7
Chemical Analysis of Cotton-Waste Composts

Constituents	Fresh Compost before Spawning	Used Compost after Growing *V. volvacea*	Reused Compost after Growing *V. volvacea* and Then *Pleurotus florida*	Decomposted Compost after Growing *V. volvacea* and Decomposed for 3 months
Moisture, %	N.D.	66.80	74.13	67.40
pH value	7.53	8.87	8.98	8.50
Total N	0.63	1.54	1.99	7.21
Total C	53.64	43.91	30.40	40.96
C:N ratio	85.15	28.51	5.28	5.68
Ash	3.44	12.41	11.63	26.31
Ethanol extract	2.25	2.10	1.97	1.59
Hot water extract	9.39	6.97	7.79	7.75
Hemicellulose	8.06	6.58	9.67	23.78
Cellulose	73.15	38.70	34.98	39.79
Lignin	5.85	16.78	18.58	29.44
Organic matter	92.48	75.70	52.41	70.62

Note: Values are g/100 g dry sample, except for moisture content, pH, and C/N ratio. N.D.: not determined.

Source: Data from Chang, S.T. and Hayes, W.A., *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, 1978.

This is very important in maintaining soil structure, good aeration, and water-holding capacity. Actually, the spent mushroom compost after 2 to 3 months of further decomposition and leaching can be used either as garden manure or to grow vegetables.[8,29,51] Some spent composts can also be used as growth substrate for other mushrooms.

5. A New Cloning Strategy for Filamentous Fungi

A new cloning strategy for *Aspergillus nidulans* has been devised and successfully used. The method screens for chromosomal aberrations sufficiently large that changes in restriction patterns of genomic DNA can be easily detected in Southern blots probed with cloned DNA that overlaps the aberration. This method has already enabled several genes of interest to be cloned.[2]

III. BIOTECHNOLOGY IN THE MUSHROOM INDUSTRY

A. General Review of Mushroom Production

The world production of cultivated mushrooms in 2000–2001 was estimated by some authorities to have exceeded 9 million metric tons (MT). An unofficial 2001 national production of cultivated mushrooms in China was estimated to be 7,818,000 MT. It should be recognized, however, that exact production figures are unavailable from some countries, including some in which production has increased dramatically in recent years. In some countries, consumers now have the opportunity of selecting species that formerly were not available to them. As an example of this, the shiitake mushroom, *Lentinula edodes*, is now being grown in the United States and some European countries, and it is thus available fresh as well as dried. The technique of fruiting *Lentinula* on sawdust in bag culture has altered the production method and shortened the time between inoculation and fruiting.

Ecological studies of the mycorrhizal fungus *Tricholoma matsutake* have made it possible to improve the farming conditions for this highly prized and highly priced mushroom, even though it cannot be fruited in culture for commercial needs. In 1984, South Korea produced 869 MT of *T. matsutake*, which had a value of U.S. $24,000,000. In the same year a production of 19,400 MT of *Agaricus bisporus* returned U.S. $10,293,000. *Lentinula edodes* with 779 MT produced, of which 465 MT were exported, returned U.S. $9,015,000. Production of *Pleurotus ostreatus* approximated 2400 MT and had a value of U.S. $5,280,000. Thus, the value of the *T. matsutake* crop was just slightly less that 50% of the value of the total mushroom production in South Korea in that year.

In 1983, Japan produced 158,855 MT (fresh equivalent weight) of the shiitake mushroom, *L. edodes*. Of this total production, 84,175 MT, or about 53%, were processed to yield 12,025 MT of dried shiitake. In the same year, Japan exported 2795 MT of dried shiitake, which amounted to 23.2% of the total dried shiitake produced.

Hong Kong in 1983 imported 2458 MT of dried shiitake, worth about H.K. $405 million (U.S. $52.1 million) with an average price of approximately H.K. $165 (U.S. $21.20) per kg. Among the 2458 MT of shiitake imported, 1638 MT were from Japan; these were valued at H.K. $378 million (U.S. $48.6 million) for an average price of H.K. $231 (U.S. $29.70) per kg. This means that 66.6% of the dried shiitake imported by Hong Kong came from Japan, with the remainder coming from China and South Korea. It should be noted, however, that the Japanese dried shiitake amounted to about 93% of the total market value, because the mushrooms from Japan were of better quality and brought higher prices. The 1638 MT of Japanese dried shiitake imported by Hong Kong in 1983 comprised 58.5% of the total dried shiitake exported by Japan.

B. Major Steps of Mushroom Technology

An overall summary of the major steps in mushroom technology is shown in Figure 22.1. Details of each process have been described in Chapter 1.

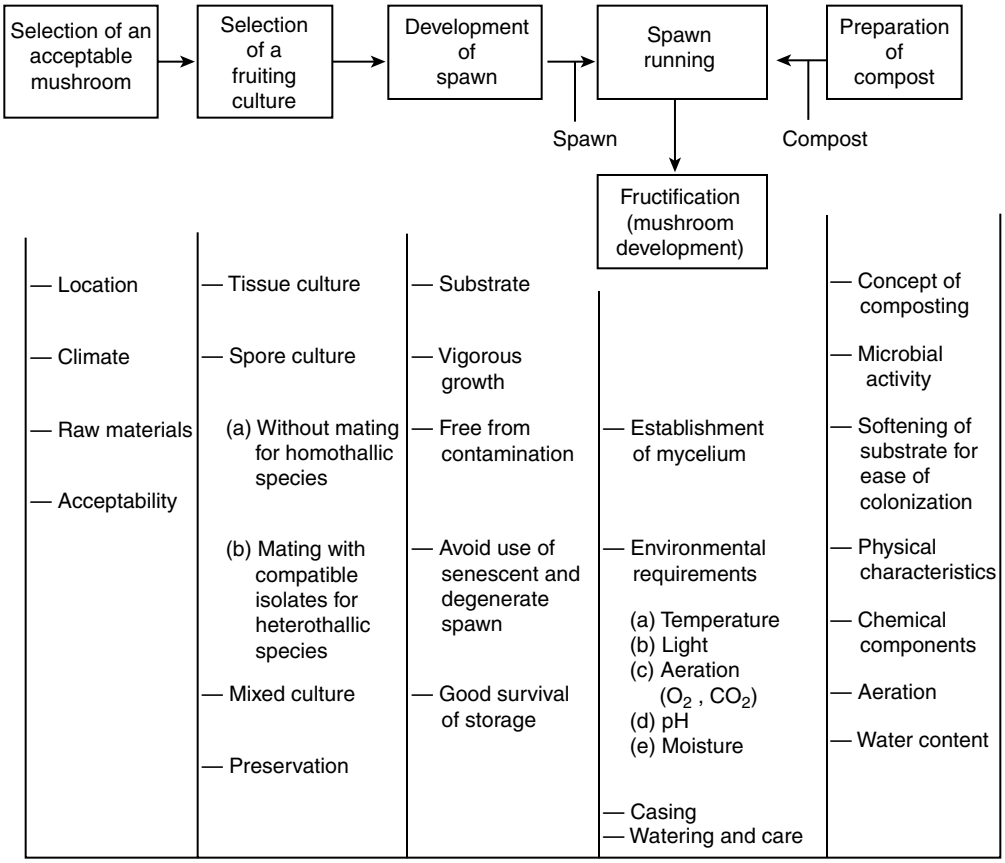


FIGURE 22.1 Major steps in mushroom cultivation.

IV. SOME FUNGAL GENETIC TECHNIQUES AND THEIR POSSIBLE APPLICATIONS

The method of breeding edible fungi may develop along three different strategies: (1) long-term, (2) mid-term, and (3) short-term lines. These three lines cannot be sharply separated, and adjustments and changes may be made at any time. That is, the lines may be flexible if requirements dictate flexibility rather than rigidity.

1. **Long-term strategy** starts with the domestication of wild species. China is rich in resources for edible fungi. There are estimated to be more than 1500 species, and among these there are 980 species known. More than 60 of these have already been domesticated, but from domestication of a new mushroom to cultivation requires a long time. At the same time, attention must be paid to the quality and marketing potential, for if these are not good, there cannot be successful large-scale cultivation. Nevertheless, from a long-term point of view, domestication of good-quality wild species is very important.
2. **Mid-term strategy** involves the selection and breeding of good-quality strains or stocks. Starting with the present species that are cultivated on a large scale, such as *Agaricus, Lentinula, Volvariella,* and *Pleurotus sajor-caju*, the goal is to select, by breeding, strains that are suitable for cultivation in different places, for high yield, and for good quality. To accomplish these objectives, the breeding method may include the following:
   a. Selection of single-spore isolates (suitable for *A. bisporus* and *V. volvacea*).

b. Breeding by hybridization of compatible single-spore isolates, which are mated to form a dikaryotic stock.
c. Utilization of induced biochemical markers and drug-resistant markers in a well-planned breeding program.
d. Utilization of protoplast fusion techniques for breeding new strains.
e. Gene transformation systems using strong gene expression in vectors.

3. **Short-term strategy** begins with the importation of desirable strains. From different sources good strains that are already under cultivation can be imported, but these imported strains must undergo careful experimental tests to verify their good qualities and suitability for the local conditions. The edible fungi are living organisms with strong local adaptation tendencies for such characteristics as stability, yield, and quality. A good strain at one place for the climate, compost material, etc. may not be at all suitable at another place. It is for this reason that an imported strain must be put through the experimental procedures to check its suitability. Several cases are known in which imported strains were used for large-scale cultivation without first testing their suitability, and serious losses occurred. Evaluation of cultures prepared from different parts of a mushroom fruiting body showed an improvement in yield from mycelium, which originated from the pileus tissue when compared with the yield from stipe and gills.

A. Protoplast Fusion for Genetic Manipulation

Protoplasts have been utilized in several areas of fungal research, namely, in the study of enzyme localization, for the propagation of cell membrane organelles, ultrastructure, and cell wall regeneration. More recently, the study of protoplast fusion has led to a new approach to genetic manipulation of fungi.[17,38,39] It has been successfully used to produce heterokaryons between strains that are incompatible when conventional methods are used.

The release of protoplasts is dependent on three major factors: the lytic enzymes used, the osmotic stabilizer, and the physiological status of the organisms.[9] However, complex interactions exist among these variables. To establish a set of conditions for making protoplasts for any new organism is, at first, largely a matter of trial and error.

As indicated by research reports, it is clear that protoplast production and protoplast reversion are possible in all edible fungi tested.[9,22,27,35,42,57,59] The efficiency of protoplast release, however, differs greatly. It is known that there is cell wall diversity in both composition and architecture among species. Because of this diversity, the mycelium lytic enzymes used are not uniformly effective in digesting the hyphal walls of *Lentinula edodes, Agaricus bisporus*, and *Volvariella bombycina*. The range of testing conditions employed may not have been appropriate. The possibility of membrane instability in these fungal protoplasts also exists. Any or all of these possibilities may be responsible for the variation in the efficiency of protoplast release.

The percentage of reversion was obtained by viable count determinations following plating of protoplasts on osmotically stabilized agar media. The frequency of reversion can be quite variable for any single species and has never been reported to be 100%. The proportion of nucleated and non-nucleated protoplasts in the preparation should be determined. This is an important consideration, because the inability of protoplasts to revert may be due to the absence of nuclei. In the filamentous fungi, another significant factor may be the origin of protoplasts. Protoplasts are released from all parts of hyphae, and it is possible that some protoplasts are devoid of some necessary cytoplasmic organelles, as organelles are known to have different locations in the fungal hypha.

In recent years several studies have shown that the technique of protoplast fusion can be used quite successfully to produce somatic hybrids in filamentous fungi. Hybrids have been produced between species of *Aspergillus*,[26] between species of *Penicillium*,[1] and between species of *Pleurotus*.[48,58] These species would not hybridize by natural means using conventional techniques. However, the somatic hybrids produced by protoplast fusion are, in many cases, abnormal in morphology

and of unstable behavior. The degree of this abnormality and instability depends to a large extent on how closely the parental species are related.[11] Although there are some difficulties and problems with the resultant fusants, the technique of protoplast fusion, by by-passing the sexual cycle, has the potential for crossing strains of a particular species, or even different species or genera, that would not normally mate. However, more selectable markers should be developed and carefully scored for fusion incompatibility.

B. Di-Mon Matings and Sporeless Mutants

The value of sporeless mutants for the cultivation of *Pleurotus* has been discussed. Species of *Pleurotus* do not have a veil covering the gills, so spores are shed early when the fruiting body is small, for a long period, and in great abundance. These spores may do any or all of the following:

1. Contaminate mushroom beds with unwanted strains. This probably is of minor importance, as most cultivation is being done by bag cultivation.
2. Serve as a vehicle for transmission of viruses that attack the mushroom beds. This has the potential of being harmful in the same manner as the virus infections of *Agaricus*; however, to the best of our knowledge, it has not yet been demonstrated that transmission of disease-causing viruses by basidiospores of tetrapolar species is a serious problem.
3. Cause allergenic reactions in humans. There is considerable evidence that mushroom workers have suffered respiratory problems and allergic reactions to spores of *Pleurotus* and, to a lesser extent, *Lentinula*. With an increased amount of cultivation of *Lentinula* in bag cultures in mushroom growing houses, it is conceivable that this problem may become more serious in *Lentinula* cultivation, but it is already serious in *Pleurotus* cultivation.
4. Cause sanitation problems in mushroom houses with spores covering all surfaces.

For the reasons just cited, a sporeless mutant, whose fruiting body has qualities equivalent to those of commercial spore-forming strains in yield, nutrient value, flavor, texture, time to fruiting, etc., has great appeal to growers and potential growers. A commercial supplier of cultures and spawn now offers two sporeless strains of *Pleurotus* that were developed by the late Dr. Gerlind Eger-Hummel in Germany.

Reports of sporeless or spore-deficient mutants of three species of *Pleurotus* appear in the literature: (1) the report of a sporeless stock of *P. ostreatus* by Eger et al.,[14] (2) Ohira's account of a sporulation-deficient mutant in *P. pulmonarius*,[36] and (3) the description of a sporeless mutant of *P. florida* by Chang et al.[10]

These sporeless mutants may arise spontaneously or be induced. The sporeless character is expressed only in the dikaryon in the examples given for *Pleurotus*, but, *a priori,* there is no reason sporelessness should not be a trait that could be incorporated in homokaryotic (haploid) fruiting bodies. Knowledge of the mode of inheritance of the sporeless character is essential for breeding work with this character. However, this information is difficult to obtain by the usual technique of making matings, collecting spores from fruiting bodies, and analyzing these for the character being investigated, because the sporeless mutant does not produce spores, thus necessitating the use of other techniques.

In one technique the dikaryotic mycelium of the sporeless mutant is dedikaryotized to obtain the component monokaryotic mycelia for subsequent matings and genetic analysis.[34] Another technique makes use of di-mon matings to extract the component nuclei from the dikaryon for purposes of analysis. It is the di-mon mating technique that is presented here. First described is the study that Ohira made of a sporulation-deficient mutant of *P. pulmonarius*.[36]

The sporulation-deficient mutant of *P. pulmonarius* appeared spontaneously among dikaryotic stocks that had been used in other studies. This dikaryon had been formed from a pairing of

compatible monosporous mycelia derived from basidiospores of a fruiting body that had been collected in the field. The homokaryons that formed the sporulation-deficient fruiting body had not been saved, but cultures of all four mating types (*A*1*B*1, *A*2*B*2, *A*1*B*2, *A*2*B*1) from the original fruiting body collected in the field were available. When compatible matings were made, the fruiting bodies that subsequently formed showed normal sporulation:

*A*1*B*1 × *A*2*B*2 → Dikaryon and fruiting body with normal sporulation
*A*1*B*2 × *A*2*B*1 → Dikaryon and fruiting body with normal sporulation

Next, di-mon matings were utilized for analysis for the sporulation-deficient factor as follows:

Di × Mon

Mutant Spore-Deficient Dikaryons	Monokaryons	Resulting Dikaryons
*A*1*A*2*B*1*B*2 (Composition of nuclei of dikaryon unknown)	*A*1*B*1	*A*1*B*1 growth too slow to obtain result
	*A*2*B*2	Sporulation deficient
	*A*4*B*3 (from wild type)	Sporulation normal
	*A*6*B*2 (from wild type)	Sporulation normal

Interpretation:
1. In the di-mon mating involving *A*2*B*2, the nucleus contributed by the dikaryon was *A*1*B*1.
2. In the di-mon mating involving *A*6*B*2, it was an *A*1*B*1 nucleus that combined with *A*6*B*2, as an *A*2*B*2 nucleus would not have formed a dikaryon with *A*6*B*2 (*A*2*B*2 × *A*6*B*2 would be *A*≠*B*=).

Conclusion:
The nuclear constitution of the mutant dikaryon was *A*1*B*1 + *A*2*B*2.

Experimental Protocol:
1. A mutant dikaryon was treated with cholic acid to induce dedikaryotization, and 11 neohaplonts were recovered. The neohaplonts were all of mating type *A*1*B*1.
   Result: The neohaplonts were all of mating type *A*1*B*1.
2. Each of the neohaplonts was mated with the *A*2*B*2 tester strain that was previously used in the di-mon mating.
   Result: Sporulation-deficient fruiting bodies were formed in every case.
3. Each of the neohaplonts was mated with a wild-type *A*3*B*3.
   Result: Normal fruiting bodies were formed in every case.

General Conclusion:
This sporulation-deficient mutation in *P. pulmonarius* is the result of a recessive gene of spontaneous occurrence.

C. Breeding for High-Temperature Strains

Although there are reports[23,49] of cultivars of *Lentinula edodes* that are stimulated to produce fruiting body primordia at low temperatures, which are relatively high (22 to 32°C), in practice the best cultivars seem to be those that require a lower range of temperatures (3 to 12°C) to trigger fruiting. We do know that there is a need and demand for high-temperature fruiting cultivars. *Lentinula* that

is grown in tropical or subtropical regions is generally grown in mountainous areas where the temperature is lower, or it fruits seasonally following a period of low temperature.

If mycelial running is not good at high temperatures, then there will not be good growth in high-temperature tropical regions even if the conditions for stimulation of fruiting are satisfied. Consequently, it is important to consider mycelial growth at high temperatures, as well as fruiting. As a generalization, it is safe to say that for any mushroom a good yield requires that there is first good mycelial growth. It must be recognized that the conditions that affect mycelial growth favorably may not so affect fruiting. Mycelial growth and fruiting are different stages in the life cycle of the mushroom, and the optimal condition for one is not necessarily (or even likely) the optimal condition for the other. This means that both mycelial growth and fruiting must be studied and techniques devised for selection of high-temperature dikaryotic stocks for both phases.

Because it is essential for fruiting and is easier to approach experimentally, mycelial growth should be examined first. Obtaining the dry weight of the mycelium after a period of growth is the preferred method for measuring growth, but this is not suitable when many stocks and strains must be screened. Although not without drawbacks, linear growth rate measurements can be used. Such measurements are useful in screening procedures where only one variable is being altered (temperature in this case). It is true that linear growth rate measurements may not be indicative of total amount of growth as the hyphal density is not measured, and it is known that in some studies a very sparse mycelium may have the same growth rate as a dense mycelium with much three-dimensional growth. Along with the growth rate measurements, a qualitative estimate of mycelial density should be made. The medium can influence growth rate, so the same medium must be used.

As light inhibits the vegetative growth of *L. edodes* and some other edible fungi, incubation will be in the dark in temperature-controlled incubators.

The following sequence of studies should be undertaken:

1. **Growth rate determination of dikaryotic stocks** at 20, 25, 30, 35, and 37°C. This is to determine the maximum temperature at which growth occurs, and, in cases where growth does not occur, to determine if the cells are viable by putting the plates in which no growth occurred at a temperature at which good growth occurs. (Mycelial death has been reported after 40 minutes at 45°C.[49])
2. **Growth rate determination of monokaryotic components of the dikaryons**, or single-spore cultures (which can be mated to give a dikaryon). This is to compare dikaryotic and monokaryotic growth. When the monokaryotic cultures show significant growth rate differences, it is important to see if one or the other of the monokaryons influences the growth rate of the dikaryon. A number of studies have been made with other fungi to determine quantitatively the combining ability of various monokaryons in dikaryotic growth rate. Simchen and Jinks[46] made such a study in *Schizophyllum commune,* and Simchen[44,45] extended this to fruiting studies in *Schizophyllum,* while Williams et al.[53] examined variation within the extreme isolates for growth rates. That is, it has been possible to examine "the genetic contribution of different monokaryons to the growth rate and yield of dikaryons."[15]
3. **Mutagenic treatment of mycelium** by ultraviolet light, followed by selection of high-temperature stock or strain by placing a mycelial macerate on a plate of medium at a temperature above which the stock or strain had grown.

The combining ability of eight monokaryons in relation to dikaryotic growth rate and sporophore production in *Pleurotus* was studied.[52] From this study of *Pleurotus*, it could be concluded that there is a high correlation between monokaryotic and dikaryotic growth rates with dikaryons growing faster than their component monokaryons, a lack of correlation between growth rates of dikaryotic mycelia and yield, and a correlation in time to the initiation of primordia and eventual

yield. This means that screening for high-yielding stocks can be made without carrying out an experiment to assay the complete yield from each stock.

In another study, correlation was found between the number of primordia produced by monokaryotic strains of *Pleurotus* exposed to the fruiting conditions required by dikaryons and the fruiting potential of dikaryotic mycelia.[13] That is, this technique selects not for yield but for good fruiting strains and permits the elimination from further studies of poor fruiting strains.

There is also a good correlation between time to produce a certain yield (250 g) of mushrooms and the eventual yield. This permits a screening for yield potential without carrying the experiment to the end of fruiting.

D. Conservation of Germplasm

There has been much concern in recent years about decreases in natural resources and the steady diminishment of untouched natural land. With the encroachment on this ever-decreasing amount of untouched natural land by human activities, there is a concomitant loss of populations of species that inhabit that land and thus a loss of germplasm of various species, including edible fungi. For many important agricultural species, preservation of the germplasm in wild strains for future use in breeding programs is being preserved by the establishment of gene banks, and we suggest that it is important for this to be done with the edible fungi as well. Previously, we have suggested[33] a procedure for collecting and conserving the germplasm of *Lentinula edodes*, a tetrapolar basidiomycete. A brief description and rationale for this procedure is given below.

1. **Collection:** Specimens of the fruiting bodies for subsequent tissue and spore isolations should be collected from diverse geographical areas and ecological situations. The aid of colleagues should be solicited, so that specimens can be obtained from widely separated geographical areas. The various collections of fruiting bodies, spore prints, or tissue cultures should be sent to a central laboratory where the culture will be prepared for analysis.
2. **Analysis:** Matings of monosporous cultures from each collection will be made to determine the number of distinct mating types at the *A* and *B* loci of the tetrapolar species. This determination can be made by application of the formula:

$$N = \frac{n(n-1)/2}{r}$$

where N is the total number of distinct members of the series, n is the number of individuals in the sample, and r is the number of identical factors in the sample.[41] Unless there is bias toward the isolation of identical mating types by sample collections from the same mycelium (e.g., from the same part of a log), the assumption can be made that the percentage of mating types isolated will give an indication of the amount of germplasm of the species conserved in the collection. The mating type genes are appropriate for this kind of analysis, because there is no indication in any previous study of a relationship between mating type and climate or geographical location; that is, the mating type alleles are distributed randomly.
3. **Suggestions:** The cultures should be maintained as dikaryotic stocks, because dikaryotic stocks have twice the genetic material of a monokaryotic strain and recessive lethal mutations will not cause the loss of the culture. The stocks collected from nature should be maintained by techniques involving as little growth as possible, e.g., in liquid nitrogen, to minimize propagation of spontaneously occurring mutations that might adversely affect the usefulness of the culture.

V. POTENTIAL USE OF MUSHROOM MYCELIUM

With one third of the world's population of 6 billion people existing on protein-deficient diets and with the prospect that by the year 2050 the population will be 9 billion, the demands on food supplies, already inadequate in many parts of the world, will be tremendously increased. The search for solutions to a problem of this dimension should involve many approaches as this age-old matter of inadequate diet is not a simple problem, and different approaches may provide partial solutions, which can contribute to the alleviation of this tragic situation.

This book is concerned with the production of edible mushrooms, which, as we have repeatedly pointed out, are relatively high in protein and may be grown on the organic wastes of industry, agriculture, and households. These wastes are abundant and are frequently pollutants that are detrimental to the environment. Some of these waste products are less suitable than others for mushroom production, but some waste products of the food industry have the potential for being acted on by the fermentative activities of the mycelium of fungi to produce a good food for human consumption.

An example of a widely used food that is based upon the fermentative activities of fungal mycelium is tempe. **Tempe** is not prepared from waste materials but from soybeans, which are soaked, dehulled, partially cooked, and inoculated with molds of the genus *Rhizopus*. In 1 to 3 days, with incubation at 30 to 37°C, a cake that is 40% protein on a dry weight basis is formed by the growth of the mold mycelium throughout the soybeans.[47] An average of 100 g of tempe is estimated to be consumed daily by 10,000,000 people in Indonesia. Soybeans have also been used to make meat substitutes for several decades, and mold mycelium has been grown on a variety of carbohydrate sources and then used to give a fibrous texture to meat substitutes. Thus, both soybeans and fungal mycelium have a previous history of being combined for food.

A few years ago, we performed some preliminary studies on the formation of an acceptable food product (which we have tentatively named "mycomeat") by growing the mycelium of edible fungi on waste soybean residues from tofu production. The product formed had good taste and texture, with flavor determined by the edible mushroom used. Mixed cultures displayed potential as a means of combining desired characteristics of different edible fungi, e.g., texture and flavor.

The most successful use of fungi to produce protein for human consumption is the mycelium of the imperfect fungus *Fusarium graminearum,* which is produced from chemostat cultures in a medium having glucose as the carbon source, ammonium for nitrogen, plus other minerals. This product has been named "**Quorn**" and is available in Great Britain in the form of meatlike chunks and in precooked, packaged meals. The protein content of Quorn is similar to that of meat, but Quorn has the advantages of lacking cholesterol (recall that the sterol of fungi is ergosterol and not cholesterol as in animal cells), is low in fat, and is high in dietary fiber. The development of Quorn was a very significant advance in fungal biotechnology and marked the first example of a single-cell protein available for human consumption.

Mycelium grown in liquid culture has been used in the making of soups and teas. This is especially practiced with edible fungi that are thought to have medicinal or tonic values. In China, the mycelium of the golden ear mushroom, *Tremella aurantia* Schw. ex Fr., that is harvested from liquid culture is added to walnut cakes, biscuits, and bread. It is also put into drinks for older people and children. When the mycelium is added to flour, which may be used for making noodles, a concentration of mycelium of 1 to 2% is best for health benefits.

In studying the medical effects of both the mycelium and the fruiting bodies of *T. aurantia*, mice were used for testing, and it was found that the mycelium had a beneficial effect in lowering cholesterol and in treatment of coronary disease. Assays of the mycelium of the golden ear mushroom revealed that the values of protein, amino acids, crude fat, and crude fiber are all higher than in the fruiting bodies. The contents of vitamins, calcium, iron, copper, and other trace elements are similar to those in fruiting bodies.

The use of mycelia of edible fungi in the preparation of foods or supplements to foods for humans, by means of utilization of certain kinds of agricultural or food processing wastes, merits greatly increased consideration and scientific research.

VI. SOME OBSERVATIONS AND CONSIDERATIONS

A. Mushroom Cultivation

From accounts in the previous chapters, it is evident that the cultivation of mushrooms is not only an old agricultural practice, but it is also a modern technological enterprise. Even at the present time, mushroom cultivation can be a primitive rural farming activity or it can be a highly developed technological industry. Some consider that mushrooms are quite easy to grow, but others are aware that the reality is quite different. The whole process of mushroom cultivation is a complex business, and success in mushroom farming requires both basic knowledge and practical experience to conduct the series of steps or phases of this complex business. As mentioned in Chapter 1, mushroom science developed from the disciplines of microbiology, fermentation, and environmental engineering. In addition, genetics and physiology have also played important roles in the development of the industry and will certainly continue to do so.

1. Microbiology

The quality of the spawn used is a key step in obtaining a good yield of mushrooms. Spawn quality is principally dependent on obtaining and maintaining good cultures of the mushrooms. These cultures are obtained from spores or tissues of mushrooms and must be free from contaminating microorganisms. That is, they must be pure cultures. Pure culture mushroom spawn was first achieved in 1886 in Great Britain, in 1894 in France, and in 1902 in the United States.[16]

2. Fermentation

Composting utilizes the principles of fermentation and is achieved by manipulating the natural succession of microorganisms present in the raw materials. As pointed out earlier, the purpose of composting is to prepare a substrate in which the growth of mushroom mycelium is promoted and the competitor microorganisms are either eliminated or retarded in growth.

Around 1915, growers in the United States introduced a second stage or phase to composting, which they called "sweating out,"[19] This stage later became known as the pasteurization, or Phase II, of the composting process.

3. Environment

Mushroom development can be divided into two stages: the vegetative stage and the reproductive stage. The transition from the vegetative stage to the reproductive stage is principally controlled or affected by environmental factors, e.g., light, carbon dioxide concentration, temperature, and humidity.

4. Genetics

In the past, strain improvement in edible mushrooms was restricted mainly to selection of better strains from spontaneous or induced mutations. Mutagenesis and selection alone, without the ability to recombine genetic materials, have only short-term value in breeding. Today, breeding of edible mushrooms has expanded to the use of the techniques of genetic recombination based on a better knowledge of the manipulation of these fungi under controlled conditions, of their life cycles, and of their sexual patterns. By means of recombination, favorable characteristics found either in wild

strains or obtained following mutagenic treatment can be brought together in one organism. The basic knowledge of genetics and breeding of edible mushrooms has been reported.[13,15,21,32,40]

Through genetic studies, and a breeding program, it is possible to achieve a desirable strain, such as a strain that grows and fruits at a high or low temperature, or an early fruiting strain, or a strain of some desired appearance, or a strain that produces mushrooms with a nice aroma.

5. Nutritional Requirements and Enzyme Activities

The nutrients required for both mycelial growth and fruiting body development are closely related to enzyme activities. It has been reported that the carbon compounds of the mycelium that are depleted by the developing fruiting bodies are replaced through the activity of extracellular cellulase.[55]

B. Criteria for Study of Major Phases of Cultivation

A logical, systematic approach involving observation and experimental data and their scientific evaluation must be made for the various phases of mushroom cultivation, namely, establishment of a fruiting culture, spawn preparation, compost preparation, and management for mycelial running and fructification.

Further growth and development of mushroom science will come about through the establishment of hypotheses, based on observation and experimental data, which can be subjected to testing.

Any applied science must have a firm foundation in the basic sciences, and basic research has contributed to the mushroom industry and to mushroom science. For example, microbiological studies have led to the establishment and preservation of pure cultures and the development of spawn for the mushroom industry. Genetic improvement of fruiting cultures by breeding techniques and the preservation of such cultures have resulted mainly from basic investigations. In the area of physiology, basic studies provide a measure of understanding for such things as the degeneration of spawn, the necessity for proper aeration in the mushroom houses because of inhibition of fruiting and malformation of fruiting bodies in the presence of raised concentrations of carbon dioxide, and the role of light in inhibiting vegetative growth in some species and in triggering fruiting body formation in certain species. Morphogenetic studies have provided useful information on the development of fruiting bodies — one application of which has been directed toward the development of sporeless strains of *Pleurotus*.

We can anticipate that future research will lead to an increase in the number of species that can be grown commercially, an increase in the use of a greater variety of substrate materials, a decreased cropping period for a number of species, improved methods of protection against pests, and better techniques for preservation.

C. Problems in Cultivation in Developing Countries

Cultivation of *Agaricus* in developed countries has become a high-technology industry, and in fundamental research *Agaricus* has become the leader in mushroom science. Millions of dollars have been spent in Western countries to develop the industry. The mushroom farms are usually supplied with advanced equipment. The support from industry and governments for *Agaricus* mushroom research is most impressive in some developed countries. Such institutions as the Mushroom Research Center at the Pennsylvania State University in the United States; the Glasshouse Crops Research Institute in Littlehampton, England (now the Horticulture Research International, Wellesbourne, Warwick, England); the Mushroom Science Unit at Aston University, Birmingham, England; and the Mushroom Experimental Station and Mushroom Growers Training Center, Horst, the Netherlands, are the result of this type of support.

By contrast, the cultivation of mushrooms, particularly tropical mushrooms, in tropical and subtropical countries is still primitive. Several reasons, which may be directly or indirectly related to the slow development of mushroom cultivation in tropical regions, are presented.

1. Social Concept

Mushrooms are usually eaten for their gastronomic properties, providing a flavoring and garnish for other foods. They are cultivated with special techniques and are usually consumed by the more affluent people, as the price of mushrooms is usually much higher than that of most common vegetables. Because mushrooms are rich in protein and contain several vitamins and mineral salts, they should be considered high-protein vegetables to enrich human diets, and they should be produced in quantities sufficient to lower the price so that they can be available to all.

2. Lack of Support from Government and Industry

There is a lack of governmental and industrial support for research, even though research on tropical mushrooms is relatively inexpensive, requiring neither huge research establishments nor massive, highly complex equipment.

3. Lack of Interest of Academia

There is a lack of interest of academic scientists in the fundamental biological studies of edible mushrooms. This is responsible for delaying the massive production of edible mushrooms in the tropical regions, and fundamental knowledge of the biological nature of mushrooms cultivated in tropical regions is still meager. Without such basic knowledge, the development of a mushroom industry is difficult, because cultivation of mushrooms requires very strong regional and local adaptation. The greatest needs are for creation of national and regional laboratories or centers of fundamental biological and applied technical research on edible mushrooms in tropical or subtropical countries, and for the training of personnel to work in these laboratories or centers.

4. Additional Concerns

The three problems mentioned above are the most basic and important problems. There are, to be sure, several other problems, such as a shortage of technical expertise, a lack of regional conferences and cooperative activities, a lack of regulated marketing practices, etc.

In practice, because of the hot and humid climate, contamination occurs more easily; it is difficult to prolong the shelf-life of mushrooms; and the manufacture and preservation of pure culture mushroom spawn are also problems for mushroom growers who lack a basic knowledge of microbiology. On the whole, the yield of mushrooms produced in these regions is generally low and unpredictable.

The techniques used for cultivation of mushrooms are, as mentioned, mainly based on the principles of microbiology, fermentation, and environmental engineering; and continuous production of successful crops requires both practical experience and scientific knowledge.

Last, but not least, is the problem of keeping the techniques secret. Mushroom growers in general do not trust their competitors and, unfortunately, some mushroom researchers also adopt the same attitude toward their colleagues. They should share their experience and knowledge in order to enlarge and promote mushroom cultivation. By sharing their results, they will share the benefits.

D. Prospects for Mushroom Cultivation in Developing Countries

The prospects for cultivation of edible mushrooms in tropical regions are basically very good. Let us consider the following points:

1. **Mushrooms can convert waste materials into human food.** Tropical regions generally have a wet and warm climate and an abundant supply of agricultural wastes. These materials are resistant to degradation because they contain mainly cellulose, hemicellulose, and lignin. The mycelium of mushrooms excretes extensive enzyme complexes,

which can directly attack and degrade these components. Therefore, mushrooms can use these wastes as nutrients for their growth, and the mushrooms produced become food for human consumption.

2. **Mushrooms are relatively fast-growing organisms**. Some tropical mushrooms can be harvested and consumed within 10 days after spawning. By using different varieties, mushrooms can be produced all year. They can be cultivated by using primitive farming techniques in rural areas or by using high technology in populated regions.
3. **Mushroom cultivation is labor intensive**; however, labor is plentiful in many tropical regions.
4. While land availability is usually a limiting factor in most types of primary production, **mushroom culture can be concentrated within a relatively small space**.
5. **Mushrooms have been accepted as human food** from time immemorial and can immediately supply additional protein to the human diet. Other sophisticated and unconventional sources of food protein, such as yeast, algal cultures, and single-cell proteins, have relatively more complicated requirements and need to be processed before they can be consumed.
6. **Mushrooms should be used as a type of vegetable**. After improving the culture and cultivation techniques, it should be possible to grow them as widely and cheaply as other common vegetables, which would be greatly beneficial to the public.
7. In view of their pleasing flavor, adequate protein, and health values, mushrooms unquestionably represent **one of the world's greatest relatively untapped sources of nutritious and palatable food for the future**.

In spite of the many problems that exist in the cultivation of mushrooms in developing countries in tropical regions, there is definitely a possibility of using mushrooms in a more important role as a source of protein to enrich human diets in these regions where the shortage of protein is most marked. There is no reason both rural and urban areas cannot share in this new possibility and prospect.

To illustrate what can be accomplished, an examination of mushroom production in China is very informative.[28] In 1986, the total production of edible mushrooms in China was 540,000 MT, having a value of U.S. $429 million. The mushrooms exported earned U.S. $160 million. Compared to 1980, the 1986 figures represent an increase of 289% in production, an increase of 300% in value, and an increase of 90% in export value. In 2000, the total production of cultivated edible mushrooms in China was 6.63 million MT,[62] with 479,531 MT exported earning U.S. $602 million. The home consumption accounts for 92.8% with only 7.2% of total production exported.

The continuing challenge remains today to introduce new technology, not necessarily involving expensive equipment, to maximize mushroom production and mushroom products per unit area with minimum costs to provide a cheap source of food protein, mushroom dietary supplements, and nutriceuticals for people in the developing countries of the world. Furthermore, the spent composts or substrates can be utilized for organic fertilizers or soil conditioners. Based on an analysis by the China Agricultural University,[62] the content of organic matter remaining after the production of edible mushrooms is more than 10%. Other indices reached or surpassed those of human waste, pig manure, and cow manure. Mushroom cultivation provides (1) edible mushroom production for food protein, (2) medicinal mushroom products for nutriceuticals, (3) diminution of unused lignocellulosic wastes from activities of agriculture, forestry, and food processing and minimization of environmental pollution arising from burning, and (4) production of organic fertilizer and the promotion of organic agriculture. This list is also indicative of their ranking in terms of their importance in relation to production volumes and market values worldwide.

REFERENCES

1. Anne, J. and Peberdy, J.F., Protoplast fusion and interspecies hybridization in *Pencillium*, in *Fungal Protoplasts*, Peberdy, J.F. and Ferenczy, L., Eds., Marcel Dekker, New York, 259–277, 1985.
2. Arst, H.N., A new cloning strategy for filamentous fungi, *Microbiol. Sci*., 2(6), 167, 1985.
3. Bajon, A.M., Yuen, T.L.S.T.H., Fong, J.C.L.S., and Olah G.M., Preliminary screening of some fungal strains for protein upgrading of sugar-beet pulp, *Biotechnol. Lett*., 7(3), 203–206, 1985.
4. Chang, S.T., Cultivation of *Volvariella* mushrooms in Southeast Asia, *Mushroom Newsl. Trop*., 1(1), 5–10, 1980.
5. Chang, S.T., Mushroom production in southeast Asia, *Mushroom Newsl. Trop*., 1(2), 18–22, 1980.
6. Chang, S.T. and Hayes, W.A., *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, 1978.
7. Chang, S.T. and Miles, P.G., A new look at cultivated mushrooms, *BioScience*, 34, 358–362, 1984.
8. Chang, S.T. and Yau, C.K., Production of mushroom food and crop fertilizer from organic waste, in *Global Impacts of Applied Microbiology VI*, Emejuaiwe, S.O., Ogumbi, O., and Sanni, S.O., Eds., Academic Press, New York, 647–652, 1981.
9. Chang, S.T., Li, G.S.F., and Peberdy, J.F., Isolation of protoplasts from edible fungi, *MIRCEN J. Appl. Microbiol. Biotechnol*., 1, 185–194, 1985.
10. Chang, S.T., Yu, M.L., and Lau, W.C., A sporeless strain in *Pleurotus florida, Mushroom Newsl. Trop*., 5(3), 19–21, 1985.
11. Croft, J.H., Protoplast fusion and incompatibility in *Aspergillus*, in *Fungal Protoplasts*, Peberdy, J.F. and Ferenczy, L., Eds., Marcel Dekker, New York, 225–240, 1985.
12. Duff, S.J.B., Cooper, D.G., and Fuller, M., Cellulase and β-glucosidase production by mixed culture of *Trichoderma reesei* Rut C 30 and *Aspergillus phoenicis, Biotechnol. Lett*., 7(3), 185–190, 1985.
13. Eger, G., Rapid method for breeding *Pleurotus ostreatus, Mushroom Sci*., 9(1), 567–573, 1976.
14. Eger, G., Eden, G., and Wissig, E., *Pleurotus ostreatus* — breeding potential of a new cultivated mushroom, *Theor. Appl. Genet*., 47, 155–163, 1976.
15. Elliott, T.J., Genetics and breeding of cultivated mushrooms, in *Tropical Mushroom — Biological Nature and Cultivation Methods*, Chang, S.T. and Quimio, T.H., Eds., Chinese University Press, Hong Kong, 11–30, 1982.
16. Elliott, T.J., Spawn-making and spawns, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, 131–139, 1985.
17. Ferenczy, L., Microbial protoplast fusion, in *Genetics as a Tool in Microbiology*, Glover, S.W. and Hopwood, D.A., Eds., Cambridge University Press, New York, 1–34, 1981.
18. Fermor, T.R. and Wood, D.A., The role of microbial biomass in mushroom nutrition, *Mushroom Inf.*, 1(4), 10, 1984.
19. Fermor, T.R., Randle, P.E., and Smith, J.F., Compost as a substrate and its preparation, in *The Biology and Technology of the Cultivated Mushroom*, Flegg, P.B., Spencer, D.M., and Wood, D.A., Eds., John Wiley & Sons, New York, 81–109, 1985.
20. Franklin, F.C.H., The impact of biotechnology on agriculture, *Biotechnol. News*, 5, 7, 1985.
21. Fritsche, G., Breeding work, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 239–250, 1978.
22. Hong, S.W. and Yoon, Y., Formation and regeneration of protoplasts in *Lentinus edodes, Mushroom Newsl. Trop*., 5(4), 4–9, 1985.
23. Ito, T., Cultivation of *Lentinus edodes*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 461–473, 1978.
24. Kahlon, S.S. and Parveen, N., Protein enrichment of wheat straw with non-toxic fungus *Pleurotus ostreatus, J. Res. Punjab Agric. Univ.,* 20, 327–231, 1983.
25. Kamra, D.N. and Zadrazil, F., Influence of oxygen and carbon dioxide on degradation in solid state fermentation of wheat straw with *Stropharia rugosoannulata, Biotechnol. Lett*., 7(5), 335–340, 1985.
26. Kevei, F. and Peberdy, J.F., Interspecies hybridization after protoplast fusion in *Fungal Protoplasts*, Peberdy, J.F. and Ferenczy, L., Eds., Marcel Dekker, New York, 241–257, 1985.
27. Lau, W.C., Dhillon, K.S., and Chang, S.T., Isolation and reversion of protoplasts of *Pleurotus sajor-caju, MIRCEN J. Appl. Microbiol. Biotechnol*., 345–353, 1985.
28. Liu, Y.D., National conference in Shanghai on production of edible fungi, *Edible Fungi*, 4, 1, 1987.

29. Lohr, Y.I., O'Brien, R.G., and Coffey, D.L., Spent mushroom compost in soilless media and its effects on the yield and quality of transplants, *J. Am. Soc. Hortic. Sci*., 109, 693–697, 1984.
30. Lou, L.H., Cultivation of edible mushrooms in the tropical and subtropical regions of the Peoples' Republic of China, *Mushroom Newsl. Trop*., 1(2), 14–18, 1980.
31. Macris, B.J., Paspaliari, M., and Kekos, D., Production and cross-synergistic action of cellulolytic enzymes from certain fungal mutants grown on cotton and straw, *Biotechnol. Lett*., 7(5), 369–372, 1985.
32. Miles, P.G. and Chang, S.T., Application of biotechnology in strain selection and development of edible mushrooms, *ASEAN Food J*., 2(1), 3–10, 1986.
33. Miles, P.G. and Chang, S.T., The collection and conservation of genes of *Lentinus, Proc. Int. Symp. Scientific and Technical Aspects of Cultivating Edible Fungi*, Elsevier Science, Amsterdam, 227–234, 1987.
34. Miles, P.G. and Raper, J.R., Recovery of the component strains from dikaryotic mycelia, *Mycologia*, 48, 484–494, 1956.
35. Mukherjee, M. and Sengupta, S., Mutagenesis of protoplasts and regeneration of mycelium in the mushroom *Volvariella volvacea, Appl. Environ. Microbiol*., 52, 1412–1414, 1986.
36. Ohira, I., Sporulation-deficient mutant in *Pleurotus pulmonarius*, Fr., *Trans. Mycol. Soc. Jpn.*, 20, 107–114, 1979.
37. Pack, M.Y., Korea Advanced Institute of Science, personal communication, 1986.
38. Peberdy, J.F., Protoplast fusion — a tool for genetic manipulation and breeding in industrial microorganisms, *Enzyme Microbiol. Technol*., 2, 23–29, 1980.
39. Peberdy, J.F. and Ferenczy, L., Eds., *Fungal Protoplasts*, Marcel Dekker, New York, 1985.
40. Raper, C.A., Sexuality and breeding, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 83–117, 1978.
41. Raper, J.R., Krougelb, G.S., and Baxter, M.G., The number and distribution of incompatibility factors in *Schizophyllum commune, Am. Nat*., 92, 221–232, 1958.
42. Santiago, C.M., Jr., Production of *Volvariella* protoplasts by use of *Trichoderma* enzyme, *Mushroom Newl. Trop*., 3(2), 3–6, 1982.
43. Schneiderman, H.A., Partnership between university and industry in biotechnology, *ASM News*, 51(6), 264–267, 1985.
44. Simchen, G., Fruiting and growth rate among dikaryotic progeny of single wild isolates of *Schizophyllum commune, Genetics*, 53, 1151–1165, 1966.
45. Simchen, G., Monokaryotic variation and haploid selection in *Schizophyllum commune, Heredity*, 21, 241–263, 1966.
46. Simchen, G. and Jinks, J.L., The determination of dikaryotic growth rate in the basidiomycete *Schizophyllum commune*, a biometrical analysis, *Heredity*, 19, 629–649, 1964.
47. Steinkraus, K.H., *Handbook of Indigenous Fermented Foods*, Marcel Dekker, New York, 1983.
48. Togomasu, T., Matsumoto, T., and Mori, K., Interspecific protoplast fusion between *Pleurotus ostreatus* and *Pleurotus salmoneo-stramineus, Agric. Biol. Chem*., 50, 223–225, 1986.
49. Tokimoto, K. and Komatsu, M., Biological nature of *Lentinus edodes*, in *The Biology and Cultivation of Edible Mushrooms*, Chang, S.T. and Hayes, W.A., Eds., Academic Press, New York, 445–449, 1978.
50. Vedder, P.J.C., *Modern Mushroom Growing*, Educaboek, Culemborg, the Netherlands, 1978.
51. Wang, S.H.L., Lohr, V.I., and Coffey, D.L., Spent mushroom compost as a soil amendment for vegetables, *J. Am. Soc. Hortic. Sci*., 109, 698–702, 1984.
52. Wang, S.S. and Anderson, N.A., A genetic analysis of sporocarp production in *Pleurotus sapidus, Mycologia*, 64, 521–528, 1972.
53. Williams, S., Verma, M.M., Jinks, J.L., and Brasier, C.M., Variation in natural population of *Schizophyllum commune*. II. Variation within the extreme isolates for growth rate, *Heredity*, 37, 365–375, 1976.
54. Wood, D.A., Microbial processes in mushroom cultivation: a large scale solid substrate fermentation, *J. Chem. Tech. Biotechnol*., 34B, 232–240, 1984.
55. Wood D.A., Production and roles of extracellular enzymes during morphogenesis of basidiomycete fungi, in *Developmental Biology of Agarics*, Moore, D.M., Ed., Symposium Series, British Mycological Society, Cambridge University Press, Cambridge, U.K., 375–388, 1985.

56. Wood, D.A. and Fermor, T.R., Nutrition of *Agaricus bisporus* in compost, *Mushroom J*., 114, 194–197, 1982.
57. Yamada, O., Magae, Y., Kashiwage, Y., Kakimoto, Y., and Sasaki, T., Preparation and regeneration of mycelial protoplasts of *Collybia velutipes* and *Pleurotus ostreatus, Eur. J. Appl. Microbiol. Biotechnol*., 17, 298–300, 1983.
58. Yoo, Y.B., Byun, M.O., Go, S.J., You, C.H., Park, Y.H., and Peberdy, J.F., Characteristics of fusion products between *Pleurotus ostreatus* and *Pleurotus florida* following interspecific protoplast fusion, *Korean J. Mycol*., 12, 164–169, 1984.
59. Yu, M.L. and Chang, S.T., Effects of osmotic stabilizers on the activities of mycolytic enzymes used in fungal protoplast liberation, *MIRCEN J. Appl. Microbiol. Biotechnol*., 3, 161–167, 1987.
60. Zadrazil, F. and Brunnert, H., Solid state fermentation of lignocellulose containing plant residues with *Sporotrichum pulverulentum* nov. sp. and *Dichomitus squalens* (Karts.) Reid, *Eur. J. Appl. Microbiol. Biotechnol*., 16, 45–51, 1982.
61. Zadrazil, F., Grinbergs, J., and Gonzalez, A., "Palo podrido": Decomposed wood used as feed, *Eur. J. Appl. Micobiol. Biotechnol*., 15, 167–171, 1982.
62. Zheng, S., Liu, Q., Wang, H., and Ng, T.B., Can edible mushrooms promote sustainability in Beijing? *Mycol. Res*., 106, 754–756, 2002.

Glossary

A

***a priori*:** Reasoning from definitions formed or principles assumed; without examination or analysis.

Actin: One of the protein components into which actomyosin (the essential contractile substance of muscle) can be split. Actin can exist in a fibrous form (F-actin) or a globular form (G-actin).

Actinomycetes: A class of prokaryotes with fungal characteristics including hyphae and the formation of conidia.

Aerophilic: An organism requiring air for growth and development.

Afforestation: The act or result of turning ground into forest or woodland.

Agar: A hydrocolloid derived from some red seaweeds (e.g., *Gracilaria, Gelidium,* etc.), commonly used for solidifying culture media for supporting the growth of mushroom cultures and various microbial organisms.

Agaric: A gill-bearing mushroom. A member of the family Agaricaceae.

AIDS: Acquired Immune Deficiency Syndrome. The syndrome of opportunistic infections that occurs as the final stage of infection by the human immunodeficiency virus (HIV).

Aleuroconidium: A thick-walled, terminal conidium thought to be resistant to harsh conditions.

Allozymes: Enzymes that differ in their electrophoretic motility because of allelic differences in a single gene.

Ampoule: A small hermetically sealed glass vial for holding one dose of a sterile solution.

Anaerobic: Not requiring free, molecular oxygen for respiration.

Anastomosis: The fusion between branches of the same or different hyphae to form a network.

Angiocarpic: Development of primordium, which proceeds inward and is enclosed.

Annulus: A ringlike partial veil around the stipe after expansion of the pileus.

Anorexia: Loss of appetite. Occurs in cases of depression, malaise, the commencement of fevers and illnesses, and in a number of disorders (e.g., alcoholic excesses and drug addiction).

Anti-HIV: Agents effective against the human immunodeficiency virus (HIV).

Antihypertension: The prevention or control of high blood pressure.

Antinociceptive: Acting against pain.

Antioxidant: An agent that prevents or inhibits oxidation. An antioxidant can prevent rancidity of oils or fats and the deterioration of other materials through oxidative processes.

Antiplatelet: A substance that manifests a lytic or an agglutinative action on the blood platelets, thereby inhibiting or destroying the effects of the platelets.

Apoplexy: Sudden loss of consciousness followed by paralysis due to cerebral hemorrhage or blocking of an artery of the brain by an embolus or thrombus that occludes an artery. Copious effusion of blood into an organ.

Apoptosis: The disintegration of cells into membrane-bound particles that are then phagocytosed by other cells. This process may be important in limiting the size of tumors.

Arbitrarily primed polymerase chain reaction (AP-PCR): Method makes use of markers based upon DNA probes and gives direct assessment of the genetic diversity of different strains.

Arthrosclerosis: Stiffening or hardening of the joints.

Ascomycetes: A major class of fungi in which fusion of compatible nuclei (karyogamy) and a subsequent nucleus reductional division (meiosis) take place in a sac-shaped structure (ascus), which contains the meiospores (called here ascospores).

Ascus (pl. asci)**:** A saclike cell typical of the class Ascomycetes, usually containing eight meiospores.

Atherosclerosis: The most common form of arteriosclerosis (narrowing of the lumen of an artery).

Autoclave: A strong vessel used for sterilization by generating sterilizing temperatures under steam pressure to prevent liquids from boiling over. Commonly for 20 minutes at a temperature of 121°C at 15 lb/in^2 is used. Autoclaves are commonly used for preparing culture media, for preparing mushroom spawn substrates, and for routine sterilization of other materials.

Autolysis: Self-dissolution or self-digestion that occurs in the tissues or cells by enzymes in the cells themselves, such as occurs after death or in some pathological conditions.

Autoradiography: The production of a picture by the rays from a radioactive substance in the material being photographed.

Auxotroph: A strain that will not grow on a chemically defined (minimal) medium that supports the growth of wild-type strains. The auxotroph has lost the ability to carry out some essential biochemical step.

B

Bagasse: The crushed juiceless remains of sugarcane as it comes from the mill.

Basidiocarp: Fruiting body of the Basidiomycetes.

Basidiomycetes: The class of fungi in which compatible nuclei fuse in a club-shaped structure called a basidium with the resulting diploid nucleus undergoing meiosis. The four haploid products of meiosis move through stalks (sterigmata) to the outside of the basidium where they enter developing spores (meiospores known as basidiospores). The gill, pore, tooth, and some cup mushrooms are Basidiomycetes.

Basidiospore: A fungal sexual spore (meiotic spore) produced following a sexual process and developed on the outside of a basidium.

Basidium (pl. basidia)**:** A microscopic club-shaped structure on the outside of which are attached several spores (usually four) and developing as the result of a sexual process.

Batch culture: A closed liquid culture.

Bioconversion: The process of conversion of biomass (organic matter — mostly from plants) into food, feed, biogas, or biofertilizer through the activity of living organisms, mostly by fungi.

Biological efficiency (BE): A practical estimate of the ability of mushrooms to convert substrate into fruiting bodies. Calculated by dividing the total fresh weight of mushrooms harvested from a crop (several flushes) by the total air-dry weight of substrate and expressing the fraction as percent. May be greater than 100%.

Biomass: The total organic mass in a specified area or volume.

Bioremediation: Refers to the process of remedying some harmful or potentially harmful situation by biological means. These harmful situations may involve polluted water, soils with harmful pesticides, materials that are contaminated with toxic or difficult-to-manage organic pollutants.

Biota: The plant and animal life of a region.

Biotechnology: Those processes that produce commercial quantities of useful substances through the use of microorganisms, plant cells, animal cells, or parts of cells such as enzymes.

Bipolar mating system: The mating competence is determined by incompatibility factors of a single series, the *A* factor. Therefore, only two mating types are produced in equal frequency by a single fruiting body, e.g., *Pholiota nameko*.

Blanching: Destructive of color; making white.

Bordeaux mixture: A fungicide composed of lime and copper sulfate, developed by Millardet. The first chemical fungicide.

Bran: The broken coat of the seed of wheat, rye, or other cereal grain separated from the kernel. Generally, relatively high in vitamins and commonly used for this purpose in substrates for growing fungi.

Brine: Water saturated or strongly impregnated with common salt.

Brown rot mushroom/fungi: Mushrooms utilizing only cellulose and hemicellulose; the lignin part remains unutilized or less utilized, resulting in the decomposed substrate having a brownish appearance.

Buller phenomenon: In a confrontation between homokaryotic mycelium and dikaryotic mycelium the homokaryotic mycelium becomes dikaryotized with the formation of clamp connections. Also known as a di-mon mating.

Button: A young fruiting body before it has opened.

C

Cap: Technically, the pileus, or upper portion of certain types of ascocarps and basidiocarps.

Carcinogenic: A cancer-causing agent.

Carcinostatic: An agent that has an arresting or inhibitory effect on the development or progression of a carcinoma.

Cardiovascular: Pertaining to the heart and blood vessels.

Caropophore: The fruiting body of higher fungi; the scientific term for the mushroom proper.

Cartilaginous: Relating to or consisting of cartilage (a specialized type of dense connective tissue characterized by its nonvascularity and firm consistency); firm and tough like cartilage.

Casing: The covering consisting of a thin layer of soil (or peat), which is placed on the compost in a mushroom bed to induce fruiting after the mycelium from the spawn has penetrated the bed.

Cellulose: A polysaccharide, made up of glucose units $(C_6H_{10}O_5)_n$, that constitutes the chief component of cell walls and woody parts of plants.

Cheilocystidia: Cystidia that are located along the edge of a lamella.

Chemotherapy: The treatment of disease by means of chemical substances or drugs. The application of chemical reagents that have a specific and toxic effect on the disease-causing cells or tissues.

Chlamydospore: A thick-walled asexual spore made from a hyphal cell that becomes segregated from the parent mycelium. Commonly imparts resistance to environmental extremes.

Cholesterol: A monohydric alcohol, $C_{27}H_{45}OH$. This sterol is widely distributed in animal tissues, especially in the membranes.

Cladding: Something that covers or overlays.

Clamp connection: An outgrowth of a cell of the secondary mycelium that at cell division serves as a connecting bridge to the penultimate cell. This bridge permits the passage of one of the products of the conjugate (simultaneous) division of the dikaryotic nuclei into the penultimate (next to the last) cell. This is important in maintaining the dikaryotic condition (two compatible nuclei in the same cell), which is characteristic of most Basidiomycetes.

Clastogenicity: Having the capability of breaking chromosomes.

Clone: Arising from a single cell or plants propagated from one seedling or stock.

Coenocyte: A mass of protoplasm in which there are no cell membranes between the nuclei. A multinucleated mass of protoplasm.

Cofactor: A compound essential to the workings of an enzyme; e.g., a coenzyme.

Compost: A mixture usually consisting largely of decaying organic matter. Different types of mushrooms require different types of compost/substrate for the selective growth of edible mushrooms. *Agaricus bisporus* grows on fermented compost, which is traditionally developed from wheat straw mixed with horse manure, and it requires higher nitrogen content. The other cultivated mushrooms are grown on almost raw or less composted lignocellulosic materials, which are usually referred to as substrates.

Composting: A process of microbial fermentation. Through composting, a mixture of rich organic materials is converted into a stable medium, which is selective for the growth of a particular mushroom but is not suitable, or is less favorable, for the growth of competing microorganisms. The basis of this selectivity, however, cannot be attributed to one factor or one aspect of the entire system. The physical, chemical, and biological aspects of composting are fundamentally interrelated, but can be artificially separated for the convenience of investigation and discussion.

Contaminant: Any organism other than the one desired to be cultivated.

Corn steep liquor: A sticky goo left over when starch is extracted from corn. Used as a substrate for growth and antibiotic production by *Penicillium notatum.*

Cryoprotectant: A drug that permits cells to survive freezing and thawing.

Cultivation technology: The technology for mushroom cultivation can be primitive as in rural farming in developing countries. It can also be highly industrialized. The latter requires advanced knowledge of mushroom biology and technology, and the use of sophisticated equipment.

Culture: The growing of mushroom mycelium or tissues in prepared nutrient medium and maintained under sterile conditions.

Culture collection: A reference collection and preservation of different species and strains of mushrooms or cultured cells.

Cup: Technically, the volva, which is located at the base of the stipe of certain mushrooms. The cup is the remnant of the universal veil.

Cyclic AMP: Adenosine 3′,5′-cyclic monophosphate. A cyclic nucleotide participating in the activities of many hormones; must complex with a catabolite gene activator protein (CAP) before transcription of certain genes can take place. Has suggested role of a fruiting inducing substance (FIS) in certain fungi.

Cystidium (pl. cystidia)**:** A sterile, generally light-colored conical or cylindrical end of the hypha in the hymenium of certain Basidiomycetes, differing morphologically from the basidium.

Cytocidal: Lethal to cells.

Cytokines: A group of extracellular factors that may be produced by a variety of cells including monocytes, lymphocytes, and other nonlymphoid cells. Cytokines include interleukins, some interferons, and tumor necrosis factor. Cytokines are important in controlling local and systemic inflammatory response.

Cytoplasm: The living matter of a cell exclusive of the cell wall, cell membrane, and the nucleus.

Cytotoxic: The state of being poisonous to the cell.

D

Dedikaryotization: The separation of a dikaryon into its homokaryotic components.

Dictyosomes: Clusters of vesicles located near the apex of the hypha, probably derived from the Golgi apparatus and originating from the endoplasmic reticulum. These secretory vesicles are thought to play a role in the formation of new wall components.

Dietary supplements: Ingredients extracted from foods, herbs, mushrooms, and plants that are taken without further modification from food for their presumed health-enhancing benefits.

Dikaryon: Two closely associated, sexually compatible nuclei.

Di-mon mating: *See* Buller phenomenon.

Dimer: A compound formed between two identical molecules and thus having the same percentage composition but twice the molecular weight of one of the original molecules. The consequence of dimer formation of pyrimidine nucleotides such as thymine or cytosine is an inhibition of normal DNA synthesis.

Dimitric: Fruiting bodies made up of both generative and skeletal hyphae.

Dolipore septum: A septum with a central pore surrounded by a barrel-shaped swelling of the septal wall. The swellings are covered on both sides by a perforated membrane (called the septal pore cap or the parenthesome). This structure is characteristic of many Basidiomycetes, but is not found in rusts and smuts.

Double blind study: A method of scientific investigation in which neither the subject nor the investigator working with the subject or analyzing data knows what treatment, if any, the subject is receiving.

Dry weight: The weight of the biomass of fungal mycelium after the removal of water by drying by heat at around 85°C for approximately 20 hours. A common means to measure growth.

Dysentery: A term applied to a number of intestinal disorders, especially of the colon, characterized by inflammation of the mucous membrane, frequent watery stools, and often with blood.

E

Egg: The button stage of a mushroom with a universal veil (e.g., *Volvariella volvacea*).

Electrophoresis: The movement of charged colloidal particles through the medium in which they are dispersed as a result of electrical potential. Electrophoretic methods are used in the analysis of protein mixtures, as protein particles move with different velocities depending on the number of charges carried by the particle.

Endocrinology: The study of the endocrine gland (a gland that secretes directly into the bloodstream) or ductless glands and the functions of the internal secretions of the body.

Endoplasmic reticulum (ER): A network of lamellae or tubules that run through the nucleus and cytoplasm of the cell. Observed by electron microscopy and essential to the metabolic function of cells.

Entomology: The branch of biology that deals with insects.

Enzyme: A protein produced by living cells that acts as a catalyst in that enzymes produce chemical changes in other substances without being changed themselves. Enzymes are reaction specific and are capable of accelerating the speed of chemical reactions.

Epibasidium: That part of a phragmobasidium or teliobasidium in which meiosis occurs.

Epigeous: Fruiting on or above the ground.

Ergosterol: An unsaturated hydrocarbon of the sterol group, $C_{28}H_{43}OH$. Present in the membranes of fungi, unlike the membranes of other eukaryotic organisms, which have cholesterol.

Erythrocyte: A mature red blood cell or corpuscle.

Ethanol: Ethyl alcohol, C_2H_5OH. Ethanol is produced by the yeast *Saccharomyces cerevisiae* and several filamentous fungi.

Etiology: The study of the causes or origins of diseases.

Eukaryotic: Organisms having membrane-bounded nuclei, plastids, Golgi apparatus, and mitochondria.

F

False clamp connections: Differ from true clamp connections in that in the case of the false clamp connection the hook cell does not fuse with the subterminal cell of the hypha.

Fermentation: The oxidative breakdown of complex substances caused by the influence of enzymes or ferments; e.g., lactic acid bacteria contain the enzymes that are responsible for the souring of milk.

Fibrosarcoma: A spindle-celled sarcoma (cancer arising from connective tissue such as muscle or bone and which may affect bones, bladder, kidneys, liver, lungs, parotids, and spleen) containing much connective tissue.

Fibrosis: Abnormal formation of fibrous tissue.

Flush: The term used for the appearance of mushrooms at intervals (rhythmic cycle) for harvesting.

Foot-candle: A measurement of the intensity of light, equivalent to 10.7639 lux. A foot-candle is the amount of light from 1 lumen at a distance of 1 ft over a surface area of 1 ft^2.

Fructification: Fruiting body.

Fruiting: The process of mushroom formation and development.

Fruiting body: The sexual spore-bearing structure in fungi, e.g., the basidiocarp is the sexual fruiting body of Basidiomycetes, ascocarp is the sexual fruiting body of Ascomycetes, and mushroom is the sexual fruiting body of macrofungi.

Fruiting culture: A pure culture of mushrooms with the genetic capacity to form fruiting bodies under suitable growth conditions; e.g., a single-spore isolate from a heterothallic mushroom species is a pure culture and, after mating with a compatible partner single-spore isolate, a fruiting culture can be established. A pure tissue culture from fruiting bodies is a fruiting culture.

Functional food: "Foods for specified health use." Many of the early products promoted the growth of intestinal bacteria, such as Yakult, developed in the 1930s when water supplies were unreliable.

Fungal immunomodulatory protein (Fip): A distinct family of fungal proteins defined on the basis of similarities in their amino acid sequences and immunological effects.

G

Gastric: Pertaining to the stomach.

Genotype: The total genetic heritage or constitution of an organism, from which individual phenotypes are expressed.

Geographical races: Denotes stocks of a fungal species collected from widely separated locations.

Geotropism: Any movement or growth of an organism in response to a one-sided stimulus of gravity.

Germ tube: The first, still short and undivided, appearance of a hypha that gives rise to the primary mycelium. It is produced by the germinating spore and has the shape of a tubular process.

Gills (or lamellae): Hymenium covered spore-producing blades on the underside of an agaric mushroom cap, radiating from center to margin.

Gleba: The inner, fertile portion of the basidiocarp of a gasteromycete. The sporogenous tissue forming the central mass of the sporophore in some Basidiomycetes such as puffballs and stinkhorns.

Glycogen: A glucosan of high molecular weight formed from glucose, fructose, galactose, lactic and pyruvic acids, pyruvic aldehyde, or dihydroxyacetone. Known as animal starch. It is converted when needed by the tissues into glucose. It also serves as an intracellular storage polysaccharide of fungi.

Gymnocarpic: Development of primordium, which proceeds outward and is unenclosed.

H

Hallucinogen: A drug or chemical substance whose most prominent pharmacological action is on the central nervous system, producing optical or auditory hallucinations, perceptual disturbances, and disturbances of the thought process.

Hardwood: Used here in a broad sense to denote any tree that is not a conifer. Note that not all hardwoods are in fact harder than all softwoods (wood from coniferous trees).

Hectare: A metric unit of area equal to 2.47 acres or 10,000 square meters.

Hematology: The medical specialty that pertains to the structure, physiology, pathology, symptomatology, and therapeutics related to the blood and blood-forming tissues.

Hemicellulose: A gummy substance intermediate in chemical composition between the sugars and cellulose. Hemicelluloses occur abundantly in plant walls, interspersed with cellulose and lignin. Hemicelluloses are second only to cellulose as the most abundant compound in the plant cell wall.

Hemicompatible di-mon reaction: In this reaction the monokaryon is compatible with only one nucleus of the dikaryon.

Hemolysis: The alteration, dissolution, or destruction of red blood cells resulting in the liberation of hemoglobin (the red, respiratory protein of erythrocytes) into the medium in which the cells are suspended.

Hepatitis: Inflammation of the liver clinically characterized by fever, jaundice, and an enlarged liver.

Hepatocarcinogenesis: Causing cancer of the liver.

Hepatocarcinogenic: A substance causing liver cancer.

Hepatocyte: A parenchymal (parts of organ concerned with function in contradistinction to framework) liver cell.

Hepatopathy: Any disease of the liver.

Hepatoprotective: Agent effective against liver ailments.

Heteroallelic: Different alleles at the subunits of a gene, such as the mating type factors of *Schizophyllum commune*.

Heterokaryon: A cell or mycelium containing more than one genetically distinct type of nucleus in a common cytoplasm.

Heterothallism, heterothallic: Condition of sexual reproduction of mushroom mycelium in which conjugation is possible only through the interaction of different mating types, which are morphologically similar strains; self-sterility.

Heterotrophic: Term that refers to organisms that must obtain their food from organic matter; unable to use inorganic matter to form proteins and carbohydrates.

Heterotrophic nutrition: Nutrition based on organic compounds, in contrast to autotrophic nutrition in which organisms are capable of synthesizing protoplasm from entirely inorganic substances.

Heterotypic division: The first meiotic division, which is reductional in chromosome number.

Heterozygous: With alternative forms of a gene (alleles) at a given locus, one allelic form on each of the two homologues.

Hilum: The scar on a spore at the point of attachment to the sterigma.

Homogenize: To make more uniform throughout in texture and mixture by breaking down and blending the particles.

Homokaryon: A cell or mycelium containing only one genetic type of nucleus.

Homothallism, homothallic: A condition in which the thallus (vegetative phase) is self-fertilizing (i.e., does not need copulation with another different mating haploid mycelium; self-fertile).

Homotypic division: The second division in meiosis, similar to typical mitosis.

Homozygous: With the same allele present at a given locus on both homologous chromosomes.

Human immunodeficiency virus (HIV): The causative agent of acquired immune deficiency syndrome (AIDS).

Humus: The dark organic portion of soil consisting largely of partially decaying plant matter.

Husk: The outer covering of a kernel or seed.

Hydrolysis: A chemical reaction in which a compound reacts with the ions of water (H^+ and OH^-) to produce a weak acid, a weak base, or both. A chemical reaction of water in which the bond of the reactant other than water is split and hydrogen and hydroxyl are added with the formation usually of two or more new compounds.

Hygroscopic: Absorbing moisture readily.

Hymenium: The spore-bearing layer of a gill.

Hypercholesterolemia: Excessive amount of cholesterol in the blood.

Hyperlipidemia: An excessive quantity of fat in the blood.

Hypertension: A condition in which the patient has a higher blood pressure than that judged to be normal; high arterial blood pressure.

Hypha (pl. hyphae)**:** A tubular, filamentous structure, which grows at the tip and is the basic unit of any mushroom.

Hypobasidium: A special cell constituting the base of the basidium in various fungi of the orders Auriculariales and Tremellales in which haploid nuclei fuse and from which the epibasidium arises.

Hypocholesterolemic: Decreased, abnormally small amounts of blood cholesterol.

Hypogeous: Fruiting underground.

Hypoglycemia: Deficiency of sugar in the blood.

Hypoglycemic: Causing hypoglycemia (deficiency of sugar in the blood).

Hypotension: Low arterial blood pressure.

I

Illegitimate di-mon reaction: In this reaction the nucleus of the monokaryon is not compatible with either of the components of the dikaryon.

Immunomodulation: Term that refers to the effects of various chemical mediators, hormones, and drugs on the immune system.

Immunopotentiation: The augmentation with a second drug that gives a response greater than the predictable additive effects on the immune system.

In vitro: In a test tube. A test done in the laboratory.

In vivo**:** In the living body. A test performed on a living organism.

Incompatibility factor: Specific genetic factor determines the pattern of mating and does not involve morphological differentiation.

Indigenous: Native; born growing or produced naturally in a country or region.

Indusium: A skirtlike structure hanging from the receptacle of the expanded fruiting body of *Dictyophora*.

Inoculum: The material (e.g., bacteria, viruses, spores, etc.) used in making an inoculation (i.e., introducing the inoculum into an organism or substrate material that will support its growth).

Insulin: A polypeptide hormone essential for the proper metabolism of blood glucose and for maintenance of the proper blood sugar level.

Interferon: A group of proteins released by white blood cells and fibroblasts following invasion by a virus. Interferons inhibit production of the virus within infected cells, prevent the spread of the virus to other cells, enhance the activity of macrophages, natural killer cells, and cytotoxic T cells. They may inhibit the growth of certain tumor cells.

Intraperitoneal: Within the serous cavity (e.g., the peritoneum) lining the abdominal cavity.

Intravenous: Within or into a vein.

Intussusception: The deposition of new particles of formative material among those already embodied in a tissue or structure. Intussusception is distinguished from accretion and apposition.

Inverse trama: Trama in which the hyphae run from the edge upward, curving out in the outer zone and directing themselves toward the hymenium.

Irradiation: Exposure to rays (e.g., ultraviolet light, x rays, or alpha particles).

Isozymes: Two or more enzymes capable of catalyzing the same reaction but differing slightly in their structures and thus in their efficiencies under various environmental conditions.

K

Karyogamy: The fusion of two compatible nuclei after cell fusion.

Karyolymph: Nucleoplasm, nuclear sap.

L

Lamellae: The gills of a mushroom.

Lanosterol: A crystalline tetracyclic alcohol $C_{30}H_{49}OH$ that occurs in wool grease and yeast and may be regarded as a triterpenoid sterol.

Lectin: A sugar-binding (glyco) protein that agglutinates cells; plant proteins that stimulate lymphocytes to proliferate.

Legitimate di-mon reaction: In this reaction the mating type of the monokaryon is compatible with both nuclei of the dikaryon.

Leucocyte: White blood corpuscle. Small, colorless cells in the blood, lymph, and tissues, which move like amoebae and destroy organisms that cause disease. Leucocytes are the primary effector cells against infection and tissue damage. They are formed from stem cells in the bone marrow.

Life cycle (life history): The stage or series of stages between one spore form and the development of the same kind of spore again. It includes the order and relative duration of successive developmental phases as they involve nuclear and morphological events.

Light unit: A foot-candle. The amount of light measured one foot from a standard candle.

Lignicolous mushrooms: Mushrooms that grow on wood.

Lignin: A polysaccharide present in plants that combines with cellulose to form the cell walls. A polymer of coniferyl alcohol; present in vegetable fiber and wood cells to the extent of 25 to 30%.

Lipid: Any one of a group of fats or fatlike substances characterized by their insolubility in water and solubility in fat solvents such as alcohol, ether, and chloroform.

Lomasome: Polyvesicular body attached to the cytoplasmic membrane against the cell wall.

Lumen: A unit of light. The amount of light emitted in a unit solid angle by a uniform point source of one international candle. The cavity of a tubular organ.

Lux: A unit of light intensity equivalent to one lumen per square meter.

Lymphocyte: Colorless corpuscles present in the blood and lymphatic tissue. They are derived from stem cells (the origin of all blood cells) and constitute the main means of providing the body with immune capability.

Lymphokines: Substances released by sensitized lymphocytes when they contact specific antigens. They help to produce cellular immunity by stimulating macrophages and monocytes.

Lyophilization: The process of rapidly freezing a substance at an extremely low temperature and then dehydrating in a high vacuum.

M

Macerate: To cause solid matter to become soft or separated into constituent elements by steeping in fluid and by pounding.

Macrophage: A monocyte (i.e., a mononuclear phagocytic white blood cell derived from the mycloid stem cells). Monocytes are short-lived, circulate in the bloodstream from which they move into tissues, and then mature into macrophages. Macrophages have the ability to recognize and ingest all foreign antigens through receptors on the surface of their cell membranes. These antigens are then destroyed by lysosomes. Macrophages have a lifespan of several months.

Malathion: An organic phosphate ($C_{10}H_{19}O_6S_2P$) used as an insecticide.

Mating systems (patterns of sexuality): Mushrooms contain both self-fertile and self-sterile species. Self-fertilization (homothallism or homomixis) is probably the most common mode of sexual reproduction in the fungi as a whole, but in the Basidiomycetes, self-fertile species are in a distinct minority of only about 10%; self-sterile species (heterothallism or dimixis) are about 90%, of which 25% are bipolar and 65% are tetrapolar.

Mating type genes: Genes that impart genetic control of the fusion of individuals during sexual reproduction.

Meiosis: Two successive nuclear divisions in which the chromosome number is halved and genetic segregation and recombination occur.

Melanin: Dark brown or black pigments in animals or plants. Tyrosinase, a phenol oxidase, is involved in the polymerization reaction that makes melanin.

Mesophiles: Microorganisms have minima growth above 0°C, maxima below 50°C, and optima between 5 and 40°C.

Mites: Any of a large number of arachnids, many of which live as parasites on fungi.

Mitochondrion: A self-reproducing organelle that occurs in the cytoplasm of all cells except bacteria and blue-green algae. It is surrounded by a double-limiting membrane. The mitochondrion is the site of energy release in cellular respiration.

Mitogenic: Causing mitosis.

Mitosis: A nuclear division involving chromosomes that are replicated and distributed equally between the nuclear progeny.

Mixed culture: A culture that contains two or more species as opposed to a pure culture in which there is only one species.

Monokaryon: A cell or hypha in which there are one or more haploid nuclei, all of a single genetic type.

Monomitric: Fruiting bodies made up of only generative hyphae.

Morphogenesis: Literally, "the origin of form"; concerned with the process of development in which organisms acquire their characteristic form by changes in their component cells and tissues, and this determination of form is under genetic control.

Morphology: The science of structure and form without regard to function, but considering both ontogenetic development and phylogenetic relationships.

Multiple alleles: Three or more forms of a gene, any one of which can occur at a given locus on a specific chromosome.

Muscarine: A highly poisonous alkaloid, $C_8H_{19}O_3N$, found in certain mushrooms including *Amanita muscaria*. Its action is similar to acetylcholine in that it causes cardiac inhibition and stimulation of the gastrointestinal tract.

Mushroom: A macrofungus with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand. It can be a basidiomycete or an ascomycete.

Mushroom biology: The branch of mycology that is concerned with the scientific study of mushrooms; it consists of two main components: mushroom science and mushroom biotechnology.

Mushroom biotechnology: Term concerned with mushroom products (mushroom derivatives), encompassing microbiology, fermentation technology, processing, marketing, and management.

Mushroom cultivation/farming: A complicated operation involving a number of different operations, including the selection of an acceptable fruiting culture of the mushroom species, preparation of spawn and compost/substrate, inoculation of compost, crop care, harvesting, preservation of the harvested mushrooms, and marketing.

Mushroom nutriceuticals: A refined or partially refined extract from either the mycelium or the fruiting body of the mushroom, which is consumed in the form of capsules or tablets as a dietary supplement (not a food) and which has potential therapeutic applications.

Mushroom science: Science concerned with mushroom production (mushrooms themselves), encompassing microbiology, composting technology, and environmental engineering, as well as marketing and management.

Mutagen: Any agent that can cause an increase in the rate of mutation in an organism.

Mycelium (pl. mycelia)**:** A mass or network of threadlike hyphae constituting the somatic thallus of a fungus.

Mycoparasite: An organism that lives inside a fungus from which it derives sustenance or protection.

Mycophagous: Feeding on fungal matter.

Mycophagy: The eating of fungi (e.g., mushrooms).

Mycophiles: People who love and have knowledge of mushrooms.

Mycophobes: People who fear mushrooms.

Mycorrhiza: An association of roots or rhizomes with a fungus.

Mycorrhizal fungi: Fungi that form a mutually beneficial symbiotic relationship with the rootlets of plants.

N

Neohaplont: The homokaryotic components obtained following dedikaryotization.

Nephritis: An acute or chronic disease of the kidneys characterized by inflammation, degeneration, fibrosis, etc.

Nephron: The structural and functional unit of the kidney. Role is formation of urine by filtration in the renal corpuscles.

Neurasthenia: A condition of nervous exhaustion, unexplained chronic fatigue, and lassitude. A functional neurosis marked by intense nervous irritability and weakness.

Niacin: Nicotinic acid, $C_6H_5O_2N$. As the component coenzyme of two important enzymes, it is important in glycolysis, tissue respiration, and fat synthesis. Soluble in hot water and alcohol.

Nongreen revolution: Bioconversion of the huge lignocellulosic biomass waste materials by mushrooms by a multidisciplinary technology. This benefits human welfare threefold by increasing food supplies, enhancing health care, and reducing environmental pollution.

Nucleic acid: High-molecular-weight substances found in cells of all living things. Nucleic acids have a complex chemical structure consisting of sugars (pentoses), phosphoric acid, and nitrogen bases (purines and pyrimidines).

Nutraceuticals: (1) Defined according to DeFelice (*The Bulletin,* July 9, 1979) as foods, or parts of food, that provide medical or healthy benefits, including the prevention and treatment of disease; (2) defined according to Zeisel (*Science,* 285, 1853–1855, 1999) as diet supplements that deliver a concentrated form of a presumed bioactive agent from a food (i.e., at dosage levels exceeding those that could be obtained from a normal food), presented in a nonfood matrix, and used to enhance health.

Nutriceuticals: A refined or partially defined extract (e.g., from mushrooms or other foods) that is consumed in the form of capsules or tablets as a dietary supplement and that has potential therapeutic applications.

O

Obligate parasite: Organism limited to the condition of living on or within another organism from which it derives sustenance or protection. It is incapable of living as a saprophyte.

Osmotrophic nutrition: Also called absorptive nutrition. A form of nutrition characteristic of fungi in which organic materials in solution are taken into fungal cells.

Osteoporosis: A general term for describing any disease process that results in reduction in the mass of bone per unit volume.

P

Palatability: The quality of being agreeable to the taste.

Paraphysis: A narrow sterile accessory hymenial structure located among reproductive cells or organs.

Parasite: An organism that derives its nourishment from another living organism.

Pasteurization: A process of partial sterilization, usually involving heat treatment or irradiation to kill most of the mesophilic microorganisms present. When pasteurizing a substrate the harmful competitor (usually mesophilic) organisms are at a disadvantage, thus allowing the beneficial (thermophilic) organisms in the substrate to flourish.

PDA (potato dextrose agar): The simplest and the most popular medium for growing mycelia of most cultivated mushrooms. It can be purchased commercially as a ready-mixed powder, which can be used directly to make the medium in the laboratory; alternatively, it can be prepared in the laboratory with the following ingredients: potato, diced, 200 g; dextrose, 20 g; powdered agar or agar bars, 20 g; water, 1 liter.

Perithecium: An ascocarp with a terminal ostiole (a tubular passage terminating in a pore, which extends through the neck of a perithecium).

Perlite: A glassy volcanic rock with a pearly luster.

pH value: A logarithmic index for the hydrogen ion concentration in an aqueous solution, used as a measure of acidity or alkalinity (measured at 25°C). Most mushrooms grow best at approximately pH 7, but tolerate a wide range, from pH 4 to 10.

Phagocytosis: The action or process of leukocytes attacking bacterial cells.

Pharmaceuticals: A defined chemical preparation that possesses medicinal properties and is used therapeutically for the treatment of specific medical conditions.

Pharmaceutics: The science of dispensing medicines; pharmacy.

Phase I: The initial steps in composting. Phase I includes adding water and other ingredients of the substrate, and turning (mixing). The process requires about 10 days to 2 weeks depending on the materials used and weather conditions.

Phase II: The second, finishing process, in composting. Compost that has completed Phase I is taken inside the mushroom house and allowed to compost further; then steam is added to raise the air temperature. Finally, it is cooled slowly for spawning.

Phase III: The compost is spawn run in a bulk tunnel, and is ready for casing when delivered to the grower. In its simplest form, Phase II compost is taken from a bulk tunnel and spawn run for 2 weeks before emptying and dispatching to the growing unit.

Phenotype: The observable physical characteristics of a living organism. The phenotype results from the interaction between genotype and the environment.

Phototropism: Any movement or growth of an organism in response to a one-sided stimulus of light.

Phylogenesis or phylogeny: The evolutionary development of any plant or animal species. The ancestral history of the individual as opposed to ontogenesis (ontogeny), or the development of the individual.

Physiology: The branch of biology dealing with the functions and vital processes of living organisms and their components.

Phytochemical: Plant-derived chemicals, such as isoflavones and phenolic acids found in soy, which may have anticarcinogenic properties. Other examples are lycopene, a chemical found in tomatoes that may prevent prostate cancer, and Omega-3, a fatty acid found in fish oil, said to reduce the risk of heart disease.

Pileus: The caplike part of the fruiting body, bearing the spore layer on its under surface.

Pin (pl. pins)**:** The mushroom primordia. The first knots of mycelium that can be distinguished and will form mushrooms.

Pinhead: A dotlike visible indication of mushroom primordium formation in a mycelium, which ultimately develops into a mushroom.

Pipettor: A device with a graduated glass tube or cylinder, which delivers a desired volume of liquid.

Plasmogamy: The fusion of protoplasts of two haploid cells without the fusion of their nuclei, as in certain fungi; cell fusion.

Pleomorphic: The occurrence of more than one distinct form in the life cycle.

Pleurocystidium: A cystidium in the hymenium at the side (surface) of a lamella.

Polygenic: Inheritance involving alleles at many genetic loci.

Polymer: A substance formed by a combination of two or more molecules (up to millions) of the same substance. The substance is usually of high molecular weight and is made up of a chain of repeated units that may usually be liberated by hydrolysis. Starch and cellulose are examples.

Polymerase: Any enzyme catalyzing a polymerization (i.e., a reaction in which a high-molecular-weight product is produced by successive addition or condensations of a simple compound). For example, the polymerization of nucleotides to polynucleotides.

Polymerase chain reaction (PCR): Process that duplicates specific pieces of DNA. It is an *in vitro* enzymatic amplification of a specific DNA sequence that was present in a minute amount.

Polypore: Any of a large group of mostly tough, wood-inhabiting mushrooms, which bear their spores in pores.

Polypropylene: A polymer of propylene used for the manufacture of plastic bags for cultivation of mushrooms since this polymer can withstand the temperature required in autoclaving for sterilization (121°C).

Polysaccharide: A carbohydrate containing a large number of sugar groups $(C_6H_{12}O_6)_n$ and which upon hydrolysis yields more than two molecules of simple sugars.

Polystyrene: A rigid transparent thermoplastic characterized by good physical and electrical insulating properties.

Polythene: Polyethylene used as a plastic.

Pores: The mouths of the tubes in boletes and polypores.

Primary homothallism: A homokaryotic mycelium, established from a single meiotic nucleus, that has the potential to progress through heterokaryosis to the completion of the sexual cycle, e.g., *Volvariella volvacea.*

Primary mycelium: The haploid mycelium derived from a basidiospore.

Primordium (pl. primordia)**:** The pinhead.

Protein: One of a class of complex nitrogenous compounds that occur naturally in plants and animals. They consist of a union of amino acids and contain carbon, nitrogen, oxygen, and frequently sulfur. They provide the amino acids that are essential for the growth and repair of tissues.

Prototroph: A strain that grows on a chemically defined (minimal) medium without supplementation.

Pseudoparenchyma: A fungal tissue consisting of more or less isodiametric cells and resembling the parenchyma (thin-walled living cells) of higher plants.

Psychotropic: Denoting drugs that affect psychic function, behavior, or experience.

Pulp: A moist, slightly cohering mass consisting of soft undissolved animal or vegetable matter. The soft succulent part of fruit or the soft pith of various stems.

Pure culture: A culture that contains a population of only one species.

Pyrethrins: Substances derived from chrysanthemums that are used as insecticides.

R

R-glucan: The alkali-resistant polysaccharide in the cell wall of *Schizophyllum commune*.

Radiotherapy: The treatment of disease by application of x rays, radium, ultraviolet, and other radiations.

Random amplified polymorphic DNA (RAPD): A modified polymerase chain reaction. The advantages of RAPD analysis are that it is simple and that it requires few materials, no Southern hybridization, and no radioactive materials for the amplification products to be visualized.

RAST (radioallergosorbent test): A blood test for allergy, which can measure minute quantities of immunoglobulin E in blood. It is useful for persons who are allergic to foreign substances, as the antibodies that develop can be detected by this test.

Renal: Relating to the kidney or kidneys.

Respiration: The processes by which an organism or cell takes in oxygen, utilizes it in tissues, and gives off products of oxidation, mainly carbon dioxide and water.

Restriction fragment length polymorphisms (RFLPs): Variations found within a species in the length of the DNA fragments generated from a particular DNA region by a specific restriction enzyme.

Reticulocyte: A red blood cell containing a network of granules or filaments representing an immature stage in development.

Rhizomorph: A thick strand of somatic hyphae in which the hyphae have lost their individuality, with the whole mass behaving as an organized unit. In *Dictycophora* rhizomorphs grow at the bottom of the volva and extend out into the soil.

Riboflavin: Vitamin B_2, $C_{17}H_{20}O_6N_4$. A constituent of certain flavoproteins that function as coenzymes in cellular oxidations. Water soluble.

Ribonucleic acid (RNA): A nucleic acid that controls protein synthesis in all living cells. It is present in the cytoplasm of all cells and is composed of long chains of phosphate and the sugar ribose along with the pyrimidine uracil rather than thymine as in DNA as one of its bases. One form of RNA (messenger RNA) is the carrier of genetic information from nuclear DNA to the cytoplasmic ribosomes.

Ribosome-inactivating proteins (RIP): Proteins possessing RNA *N*-glycosidase activity, which depurinate specific nucleotides and cause damage to eukaryotic ribosomes.

Ring: The annulus.

S

S-glucan: The glucan of *Schizophyllum commune* that is soluble in cold alkali.

Saponification: The chemical conversion of fats into soap. The hydrolysis or the splitting of fat by an alkali yielding glycerol and three molecules of alkali salt of the fatty acid.

Saprophagous: Feeding on decaying matter.

Saprophytic fungi: Fungi that live on dead or decaying organic matter for their nutrition.

Sarcoma: Cancer arising from connective tissue such as muscle or bone.

Schizophyllan: An extracellular polysaccharide of *Schizophyllum commune* which has $\beta(1\rightarrow3)$ linkages at every third unit along the chain, on the average. Schizophyllan is used in the treatment of cervical cancer.

Secondary homothallism: A fertile dicaryotic mycelium is established from a single basidiospore in which two meiotic nuclei of different mating types have entered, e.g., *Agaricus bisporus.*

Secondary mycelium: Dikaryotic mycelium resulting from plasmogamy of two compatible primary mycelia; mycelium developed from the tissue of a fruiting body of most Basidiomycetes.

Sector: Usually used to describe fans of morphologically differentiated regions of mycelium, due to mutation mostly.

Semicultivation: A technique of mushroom cultivation in which a plant and a fungus are grown together in order to obtain fruiting bodies. For example, a mycorrhizal association.

Septicemia: A systemic disease caused by the presence of microorganisms or their toxins in the circulating blood.

Septum (pl. septa)**:** The transverse divisional cross wall between two cells of a hypha.

Sexuality: Process consisting of three important stages: plasmogamy (fusion of cytoplasm), karyogamy (nuclear fusion), and meiosis (the reductional division in the number of chromosomes). Through the process of sexuality, genetic recombination and segregation subsequently occurs, providing for variation among the offspring.

Softwoods: Wood from coniferous trees.

Sonic oscillation: Vibration due to sound. Used to break up cells and rupture membranes.

Southern blot: A blot analysis technique used in molecular genetics to analyze a very small portion of genomic material, DNA.

Spatulate: Spoon shaped.

Spawn: The substrate in which mushroom mycelium has developed, and which will be used as a "seed" in propagation for mushroom production. The genetic and cultural characteristics of the mushroom species are carried in the spawn.

Spawning: The planting of mushroom spawn into the prepared substrate or compost.

Species: A biologically discrete group of closely related individuals, which are capable of interbreeding and giving rise to fertile progeny.

Spectrophotometer: An instrument for measuring the intensity of various wavelengths of light transmitted under standard conditions by a substance under study.

Splenocyte: A unicellular leukocyte or lymphocyte of the spleen.

Spore: A microscopic cell capable of developing into an adult organism but, unlike a seed, not containing a preformed embryo.

Spore culture: A culture derived by isolation of a single spore and its subsequent germination and development into a mycelium.

Sporulation: Production of spores. Sometimes used for the formation of fruiting bodies in the Basidiomycetes.

Stalk: Technically, the stipe.

Sterigma (pl. sterigmata)**:** The tiny spicule-like extension at the apex of a basidium, on which the basidiospore develops.

Sterilization: The rendering of a substrate into a state in which all living organisms have been made nonviable. Sterilization by heat (steam) is the most commonly employed method in mushroom cultivation.

Steroid: The term applied to any one of a large group of substances chemically related to sterols; for example, D vitamins, bile acids, certain hormones, saponins, and sterols.

Sterol: One of a class of solid cyclic alcohols; a subdivision of the steroids. Sterols are waxy materials derived from animal tissues (e.g., cholesterol) and plant tissues (e.g., ergosterol).

Stipe: A stalklike support, or the scientific term for the stem of a mushroom.

Stroma: A dense, cushionlike aggregation of mycelium formed on the surface of the substrate, which generally does not lead to forming fruiting bodies.

Stylet: A relatively rigid, slender, elongated organ or appendage on an animal.

Subhymenium: The middle layer of the gill, where the hyphae are densely woven.

Sublimation: The alteration of the state of a gas or solid without first changing it into a liquid.

Substratum (or substrate): Usually referring to the almost raw or less-composted lignocellulosic materials on which mushroom mycelium will grow; any materials used for growth of microorganisms.

Superoxide: A highly reactive form of oxygen that is produced when oxygen is reduced by a single electron.

Synergy: The combined or cooperative action of two agents producing an effect that is greater than the total effect of each agent operating by itself.

Synthetic compost: Any substrate that is composed of agricultural and/or industrial waste materials mixed with various organic supplements.

T

Tactile: Perceptible to touch.

Tantalum: A lustrous, platinum-gray, hard, ductile metallic element (atomic number 73, atomic weight 180.95) that has a very high melting point and is resistant to attack by most chemicals except hydrofluoric acid. Since it does not corrode, it is used surgically as plates, as a wire mesh, or as a wire suture.

Taxon (pl. taxa)**:** A taxonomic group or entity.

Taxonomy: The science of classification; the laws and principles covering the classification of organisms.

Termite: Pale-colored, soft-bodied social insects of the order Isoptera that live in colonies. Termites build or tunnel out large nests and feed on wood. They are very destructive to wooden structures and trees.

Tetrad: The four nuclei or four cells that are the immediate results of meiosis in a parent cell.

Tetrapolar mating system: Mating competence is determined by incompatibility factors of two series, *A* and *B*, which assort and segregate independently at meiosis. There are four rather than two mating type genes on two chromosomes produced in equal frequency by a single fruiting body, e.g., *Lentinula edodes.*

Thermophiles: Microorganisms that can make active growth at high temperature, have minima above 20°C, maxima at or greater than 50°C, and optima around 35°C or slightly higher.

Thiabendazole: An antihelminthic drug.

Tissue culture: A means of cloning living specimens while preserving their genetic constitution. Any part of a young mushroom may be used, but it is preferable to take tissue from the upper part of the stipe since this area is a region of rapid cell elongation.

Tracheitis: An inflammation of the trachea (windpipe).

Trama: The layer of hyphae in the central part of a gill.

Translocation: The movement of substances in plants.

Triglyceride: An ester of glycerol with three different fatty acids. A large proportion of the lipids in the blood are triglycerides, which are insoluble in water.

Trimitric: Fruiting bodies made up of generative, skeletal, and binding hyphae.

Triterpene: A molecule based on six isoprene units (isoprene is a five-carbon branched chain molecule). Triterpenes in *Ganoderma lucidum* have been found to have pharmacological activities.

Triterpenod: A triterpene or triterpene derivative, such as lanosterol or oleanolic acid.

Tritiated: Containing molecules of tritium (H_3), a radioactive isotope of hydrogen, in the molecule.

Tumor necrosis factor (TNF): A monokine produced in various parts of the body by activated phagocytes. TNF is important in stimulating leukocytosis (an increase in number of leukocytes in the blood), fever, and necrosis of some tumors.

U

Ultrastructure: Structures (fine structures) seen with the ultramicroscope or the electron microscope.

Umbo: The knob in the center of the pileus (cap) of certain mushrooms.

Universal veil: A protective layer of tissue covering all or most of the young fruiting body of certain mushrooms.

V

Vacuolate: Having vacuoles.

Vacuole: A clear space in cell protoplasm filled with fluid or air that is surrounded by a membrane called the tonoplast.

Veil: A tissue covering mushrooms as they develop.

Vermiculite: A micaceous mineral whose granules expand greatly at high temperatures to give a lightweight, highly water-absorbent material that is used in seed beds as a mulch.

Viscid: Adhering, glutinous, sticky.

Volva: The remnants of the universal veil at the base of the stalk in certain mushrooms, usually in a cuplike form or series of concentric rings or scales.

W

White rot fungi: Fungi that utilize cellulose, hemicellulose, and lignin components in substrate with the help of cellulases and ligninolytic enzymes, resulting in the decomposition of lignin (delignification); the substrate is rendered light in color, e.g., *Pleurotus sajor-caju.*

Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z