CHAPTER 1

Overview of Mushroom Cultivation and Utilization as Functional Foods

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CONTENTS

1.1 INTRODUCTION

In 1957, R. Gordon Wasson, a world known amateur mycologist, proposed the division of people into two classes for which he coined the following terms:

Mycophiles—Those who love and know their mushrooms intimately.
Mycophobes—Those who fear, dislike, and do not know their mushrooms.

I think all readers of this book belong to the former and not the latter.

Knowledge of numerous new mushroom species has accumulated through time. The number of recognized mushroom species has been reported to be 14,000, which is about 10% of the total estimated mushroom species on the earth (Hawksworth, 2001). China is estimated to have about 1500–2000 edible mushroom species with 981 species identified. By 2002, 92 species have been domesticated while 60 of these have been commercially cultivated (Mau et al.,

Mushrooms as Functional Foods, Edited by Peter C. K. Cheung

2004). However, mushrooms have nearly always been around, with a very long and interesting history. Mushrooms have been found in fossilized wood that is estimated to be 300 million years old, and almost certainly prehistoric man used mushrooms collected in the wild as food. Recently, the importance of the role of mushrooms in history was evidenced by the fact that the desert truffle, *Terfezia arnenari*, was described in the Bible as “bread from heaven” and also “manna of the Israelites” (Pegler, 2002).

It may be interesting to have a charming mushroom poem as a beginning for this chapter: “Without leaves, without buds, without flowers, yet, they form fruit; as a food, as a tonic, as a medicine, the entire creation is precious” (Chang and Miles, 1989, p. 345). The first part describes the morphological and physiological characteristics of mushrooms while the second states the nutritional and medicinal properties of mushrooms.

Our attitudes to the phenomenon of nature are seldom based on simple observation. There are, however, examples throughout history where certain living things have inspired fear and loathing simply because they are regarded as ugly species with peculiar behavior and supposedly evil. For example, in some communities, bats, snakes, spiders, toads, and owls have all been associated with the devil or regarded as harbingers of illness and even of death. This is one of the reasons why some refer to the poisonous mushroom as a “toadstool.” Actually, the name has no scientific basis at all and should not be used in any situations, although it is possible to find a toad sitting beside or even on top of a mushroom. Mushrooms attract toads, not due to the mushroom itself, but because of the various insects which are harbored in them. Insects certainly are interested in mushrooms as a source of food (Chang, 2005).

It cannot be denied that some mushrooms, even though they represent less than about 1% of the world’s known mushrooms, are dangerous if eaten. Some are deadly poisonous. But perhaps a more likely explanation for the widespread abhorrence of wild mushrooms in communities is that they are by nature a rather strange and mysterious group of organisms, quite unlike the green plants. In some ancient communities, the seemingly miraculous manner of its growth without seed, without leaf, and without bud, its fruiting body’s sudden appearance after rain, especially after lightning and thunderstorms, its equally rapid disappearance, and its curious umbrellalike shape gave rise to a wealth of illusions and mythologies.

Fungi are found just about everywhere. Mushrooms, a special group of macrofungi, are rather more selective than other fungi in that the size of the fruiting body requires the availability of more nutrients than are required for the production of asexual spores by microfungi. Nevertheless, in damp places, such as tree-fern gullies and areas of rain forest, plentiful moisture leads to mushroom formation and mushrooms can be collected during most of the year. There may be a particular flora of mushroom species associated with the seasons of autumn, summer, and spring. Relatively few mushrooms are produced during the cold winter months, although there are perennial fruiting bodies that persist during the winter. But in drier regions mushrooms occur only after seasonal rains. Formation of mushroom fruiting bodies depends very much on the pattern of rain and, in some years, there may be virtually a complete lack of fruiting.

There has been a recent upsurge of interest in mushrooms not only as a health vegetable (food) which is rich in protein but also as a source of biologically active compounds of medicinal value. Uses include complementary medicine/dietary supplements for anticancer, antiviral, immunopotentiating, hypocholesterolemic, and hepatoprotective agents. This new class of compounds, termed *mushroom nutriceuticals* (Chang and Buswell, 1996), are extractable from either the mushroom mycelium or fruiting body and represent an important component of the expanding mushroom biotechnology industry. It has been shown that constant intake of either mushrooms or mushroom nutriceuticals (dietary supplements) can make people fitter and healthier. In addition, mushroom cultivation can also help to convert agricultural and forest wastes into useful matter and reduce pollution in the environment. Therefore, mushroom cultivation can make three important contributions: production of health food, manufacture of nutriceuticals, and reduction of environmental pollution.

1.2 WHAT ARE MUSHROOMS?

1.2.1 Definition of a Mushroom

Mushrooms along with other fungi are something special in the living world, being neither plant nor animal. They have been placed in a kingdom of their own, called Myceteae (Miles and Chang, 1997). But what are mushrooms? The word *mushroom* may mean different things to different people and countries. It was reported (Chang and Miles, 1992) that specialized studies and the economic value of mushrooms and their products had reached a point where a clear definition of the term mushroom was warranted. In a more broad sense "mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang and Miles, 1992). Thus, mushrooms need not be Basidiomycetes, or aerial, or fleshy, or edible. Mushrooms can be Ascomycetes, grow underground, have a nonfleshy texture, and need not be edible. This definition is not a perfect one but can be accepted as a workable term to estimate the number of mushrooms on the earth (Hawksworth, 2001). The most common type of mushrooms is umbrella shaped with a pileus (cap) and a stipe (stem), that is, *Lentinula edodes*. Other species additionally have a volva (cup), that is, *Volvariella volvacea*, or an annulus (ring), that is, *Agaricus campestris*, or both, that is, *Amanita muscaria*. Furthermore, some mushrooms are in the form of pliable cups; 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 very much 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 soil, compost, wood log, or other lignocellulosic material on which the fungus may be growing. After a period of growth and under favorable conditions, the established (matured) mycelium could produce the fruit structure which we call the mushroom. Accordingly mushrooms can be grouped into four categories: (1) those which are fleshy and edible fall into the edible mushroom category (e.g., *Agaricus bisporus*); (2) mushrooms which are considered to have medicinal applications are referred to as medicinal mushrooms (e.g., *Ganoderma lucidum*); (3) those which are proven to be or suspected of being poisonous are named poisonous mushrooms (e.g., *Amanita phalloides*); and (4) a miscellaneous category, which includes a large number of mushrooms whose properties remain less well defined, may tentatively be grouped together as "other mushrooms." Certainly, this approach of classifying mushrooms is not absolute and not mutually exclusive. Many kinds of mushrooms are not only edible but also possess tonic and medicinal qualities.

1.2.2 Ecological Classification of Mushrooms

Mushrooms can be ecologically classified into three categories: saprophytes, parasites, and mycorrhiza.

There are only a few parasitic mushrooms. Most of the cultivated gourmet mushrooms are saprophytic fungi. Some are mycorrhizal mushrooms, for example, Perigold black truffle (*Tuber melanosporum*) and matsutake mushroom (*Tricholoma matsutake*). It is difficult to bring these pricey wild gourmet species into cultivation because they are mycorrhiza. These mushroom species have a symbiotic relationship with some vegetation, particularly trees, that is, there is a relationship of mutual need. Therefore, the substratum (host) should be carefully recorded, as this can be an important feature in identification and in classification, for example, whether the mushroom is growing on dung, wood, bark, living trees, litter, or soil. If the mushroom is growing on a living plant or on dead parts recognizable as belonging to a nearby plant, flowers, fruits, or other parts of the plant, these should be collected for identification of the host or substrate if its name is not known.

Saprophytes obtain nutrients from dead organic materials; parasites derive food substances from living plants and animals, causing harm to the hosts; and mycorrhiza live in a close physiological association with host plants and animals, thereby forming a special partnership where each partner enjoys some vital benefits from the other.

However, some mushrooms do not fall neatly within these man-made categories and can share two of these categories (Figure 1.1). For example, some *Ganoderma* spp., including *G. lucidum*, are common saprophytes but can be pathogenic too; also *T. matsutake*, while initially appearing to be mycorrhizal on young roots, soon becomes pathogenic and finally exhibits some saprophytic ability.

1.2.3 Identification of Mushrooms

Successful identification of wild mushrooms requires a basic knowledge of the structure of fungi and of the way in which they live. To identify a given mushroom,

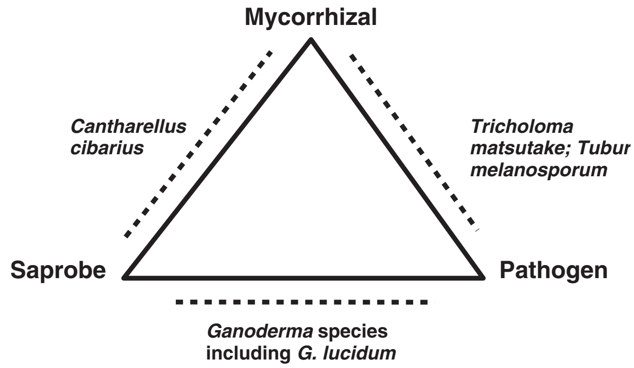

Figure 1.1 Modified triangular model for ecological classification of mushrooms (Hall et al., 2003b).

it is necessary to examine the fruiting bodies with the utmost care. A fresh fruiting body is much easier to identify than a pickled (preserved in formalin) or a dried one. A good reference material, usually a book with color, pictures of the different mushrooms known, is a basic requirement. A key is usually provided to simplify identification in most reference texts (Arora, 1986; Carlucccio, 2003; Chang and Mao, 1995; Fuhrer, 2005; Shepherd and Totterdell, 1988; van der Westhuizen and Eicker, 1994).

In using the reference, it is essential that one knows some specific characteristics of the mushroom being identified. These characteristics are (1) size, color, and consistency of the cap and the stalk; (2) mode of attachment of the gills to the stalk; (3) spore color in mass; and (4) chemical tests or reactions.

Although the color of the gills is a good indication of the spore color, there are instances when the experienced mycologist will have to resort to what is called "spore print" examination to determine the real color of the spores. For specimens with a distinct cap and stem, the cap is removed and placed fertile-side down, preferably on a microscope slide, but in the absence of such, on white paper, black paper, or cellophane. Then it is covered with a bowl or similar object to prevent air currents. A thin spore print is often visible after as little as a half hour, but a useful deposit usually requires longer time (up to 2 hours or more). The print is necessary to determine overall spore color. It is also a source of mature spores for microscopic examination and measurements.

The mode of gill attachment to the stem indicates the genus of the mushroom and should be carefully noted. To determine the mode of attachment, the mushroom is cut longitudinally through the cap, exposing the point of attachment of the gills to the stem.

The environment in which the mushroom was picked should also be noted. It is important to know whether the mushroom grows directly on the ground, on decaying wood, on a living tree trunk, or on compost. One should not overlook the species of those on which the mushrooms are found growing or the type of grasses or moss present in the area where the mushrooms are collected.

There is no single reference work in which all mushrooms are illustrated or described. In most cases, mushroom species in publications are grouped by region or locality, for example, North American mushrooms, mushrooms of the Western Hemisphere, and mushrooms of South Africa. While certain mushrooms are easy to identify, many are not. In fact, there is a great number of look-alikes. To avoid any unpleasant experiences, especially when identifying mushrooms for the purpose of determining edibility, experts should always be consulted (Quimio et al., 1990).

Collectors should always remember when using keys that the mushroom they have in hand might not be included in the book they are consulting (or in any other book, for that matter). Once they have obtained a name with a key, they must read the detailed description provided for the mushroom and compare it with the one they are trying to identify. If the description does not fit the specimen, then they must go back to the key and try again, following a different route. If they exhaust all of the possible routes and still cannot find a description that fits, they should assume that the fungus in hand is not in the books being consulted. Using the information gained, they may then consult other appropriate references that may be available or they may seek the assistance of specialists working with the group in question. They should never attempt to force a specimen into a category where it does not fit.

Some mushrooms are very palatable due to their exotic taste, but some mushrooms are very poisonous. Unfortunately, there are no general guidelines for distinguishing between the poisonous and edible species. The only means by which a nonspecialist can determine the edibility or toxicity of a given mushroom is to carry out an accurate identification of the specimen. Such identification may be obtained by consulting the relevant literature, preferably with illustrations, or experts in the subject. Identification of a mushroom at the generic level is inadequate since, within a given genus (e.g., *Lepiota*) some species are edible while other species are highly poisonous.

Several species of *Amanita* are extremely poisonous, but obvious symptoms do not appear until 8–12 hours after ingestion. The poisonous compound, amatoxin, is not destroyed by boiling or processing. Some less poisonous mushrooms produce only nausea or gastric upset within 30–60 minutes of ingestion (Hall et al., 2003a; Quimio et al., 1990). Mushrooms partially eaten by animals or insects are not necessarily fit for human consumption. When the mushroom is in doubt, throw it out. *If you are not absolutely sure whether a given mushroom is edible or otherwise, do not touch it. Leave the strange mushroom alone*.

1.3 CONCEPT OF MUSHROOM BIOLOGY AND APPLIED MUSHROOM BIOLOGY

1.3.1 Mushroom Biology

The biological science that is concerned with fungi is called mycology. Mushroom biology is the branch of mycology that deals with mushrooms 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, molecular biology, virology, fungal physiology, embryology, endocrinology, phycology, and entomology. These names indicate either a group of organisms (e.g., bacteria, algae, and insects) and/or an approach to the study (e.g., disease, development, and physiology).

Although several terms for this important branch of mycology that deals with mushroom have been used, and each of these has its merit, when we get down to the matter of definitions, it seems that there is a place for a new term—*mushroom biology* (Chang and Miles, 1992). Mushroom biology is a new discipline concerned with any aspect of the scientific study of mushrooms, such as taxonomy; physiology, and genetics.

1.3.2 Applied Mushroom Biology

Applied mushroom biology is concerned with all aspects of the application of mushroom biology. It consists of three main components: mushroom science, mushroom biotechnology (Chang, 1993), and mushroom bioremediation (Figure 1.2). *Mushroom science* deals with mushroom cultivation and production (mushrooms themselves) and encompasses the principles of mushroom biology/microbiology, bioconversion/composting technology, and environmental engineering (Figure 1.3); *Mushroom biotechnology* is concerned with mushroom products (mushroom derivatives) and encompasses the principles of mushroom biology/microbiology, fermentation technology, and bioprocess (Figure 1.4). Mushroom biotechnology, both as a technology and as the basis for new mushroom products, requires industrial development. It, like many bioscience

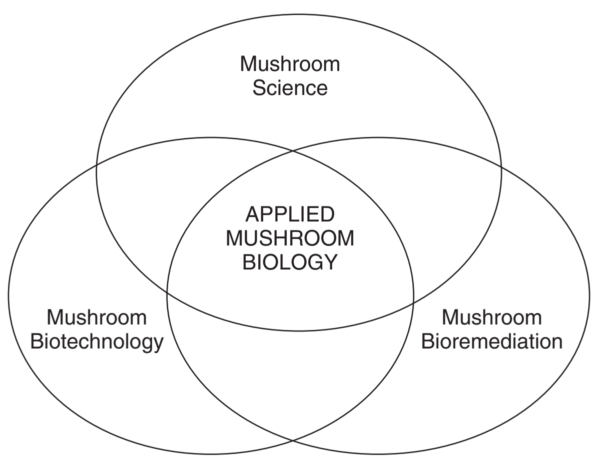

Figure 1.2 Applied mushroom biology consists of three components: mushroom science, mushroom biotechnology, and mushroom bioremediation.

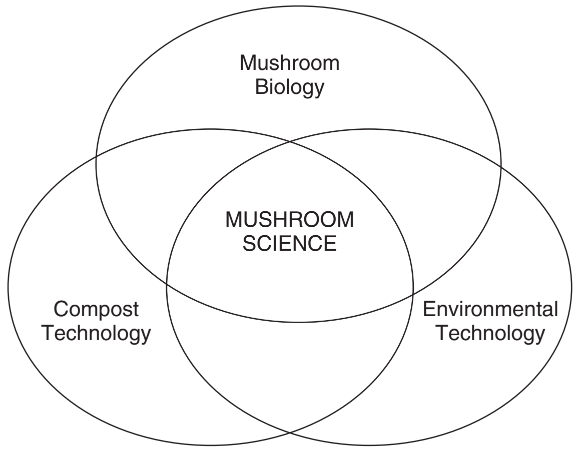

Figure 1.3 Mushroom science: concerned with mushroom cultivation and production.

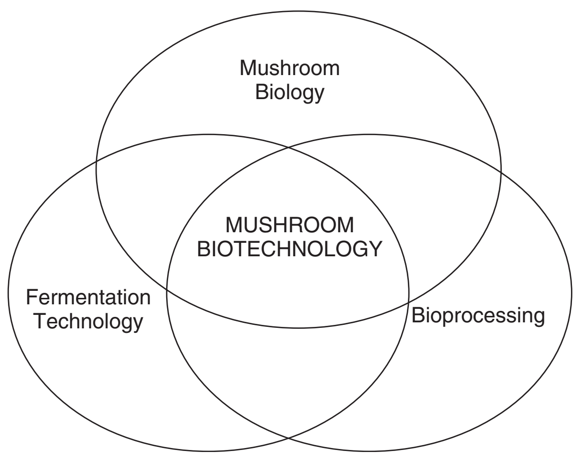

Figure 1.4 Mushroom biotechnology: concerned with mushroom products (mushroom nutriceuticals/dietary supplements).

industries, operates at the cutting edge of science and involves numerous regulatory issues. The third component of applied mushroom biology has been developed in recent years. This is *mushroom bioremediation* and is concerned with the beneficial impacts of mushrooms on the environment (from mushroom mycelia) and encompasses principles of mushroom biology/microbiology, ecology, and bioconversion technology (Figure 1.5).

Therefore, the aims of the discipline of applied mushroom biology (Figure 1.2) are to tackle the three basic problems—shortage of food, diminishing quality of

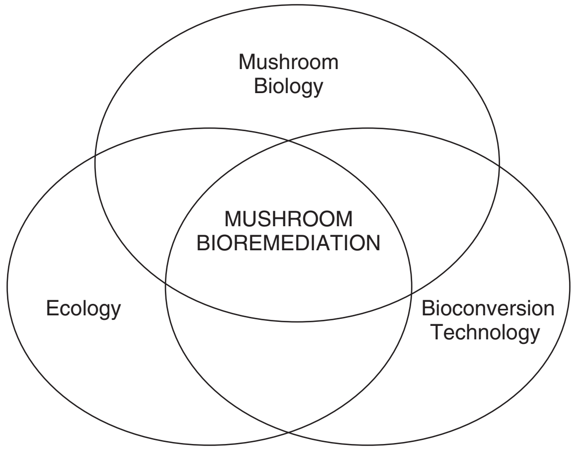

Figure 1.5 Mushroom bioremediation: concerned with beneficial impacts of mushrooms on environment.

human health, and pollution of the environment—which human beings still face, and will continue to face, due to the continued increase of the world population. The twentieth century began with a world population of 1.6 billion and ended with 6 billion inhabitants. The world's population is likely to reach 9.2 billion in 2050 from the current 6.7 billion with most of the growth occurring in developing countries. The growing world population is increasing by about 80 million people per year. At present, about 900 million people in the world are living in poverty. On the other hand, it has been observed that over 70% of agricultural and forest products have not been put to total productivity and have been discarded as waste. Applied mushroom biology not only can convert these huge lignocellulosic biomass wastes into human food but also can produce notable nutriceutical products that have many health benefits. Another significant aspect of applied mushroom biology is using the biota in creating a pollution-free and beneficial environment. The three components of applied mushroom biology are closely associated with three aspects of well-being—food, health, and pollution.

The discipline that is concerned with the principles and practice of mushroom cultivation is known as *mushroom science* (Chang and Miles, 1982). The establishment of principles requires facts arrived at through systematic investigation. The systematic investigation must involve the practical aspects of mushroom cultivation as well as scientific studies. The consistent production of successful mushroom crops necessitates both practical experience and scientific knowledge.

1.3.3 Impact of Applied Mushroom Biology

1.3.3.1 Nongreen Revolution The world population has reached over 6 billion now. It is expected to continue increasing in the twenty-first century.

The amount of food and the level of medical care available to each individual, especially those in less developed countries, will decrease. Environmental pollution and greenhouse gas effects will also become a more serious problem. However, the world has an immense amount of lignocellulosic material resource that, like solar energy, is sustainable. Lignocellulosic material is a kind of biomass which is estimated to amount to 1.09×10^{11} t dry matter on land annually (Chang, 1989), which consists of mainly three components: cellulose, hemicellulose, and lignin. Lignocellulose is a major component of wood and other plant materials. The world's annual yield of cereal straws in 1999 is estimated to be 3570×10^6 t. Since such a large amount of energy is in lignocellulosic biomass (3020 EJ solar energy fixed in biomass per year), it can constitute principal objects for conversion into useful products by man's activities. Note that E is the metric prefix for exa (10^{18}) and joule is the unit of energy.

Although various strategies have been developed to utilize part of the vast quantities of waste lignocellulose generated annually through the activities of agricultural, forestry, and food processing industries, one of the most significant, in terms of producing a higher value product from the waste, is the cultivation of edible mushrooms by solid-state fermentation. More recently, attention has focused on a second area of exploitation following the discovery that many of these mushrooms produce a range of metabolites of intense interest to the pharmaceutical/nutriceutical (e.g., antitumor, immunomodulation agents, and hypocholesterolemic agents), and food (e.g., flavor compounds) industries. Mushrooms, like all other fungi, lack chlorophyll and are nongreen organisms. They cannot convert solar energy through the process of photosynthesis to organic matter as green plants do, but they can produce extensive enzymes that can degrade lignocellulosic materials for their own nutrients for growth and fruiting. Different mushrooms have different lignocellulolytic enzyme profiles (Buswell and Chang, 1994; Buswell et al., 1996b). These are reflected in qualitative variations in the major enzymatic determinants (i.e., cellulases, ligninases) required for substrate bioconversion. 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) (Buswell et al., 1995). Conversely, *V. volvacea*, which prefers high-cellulose, low-lignin-containing substrates, produces a family of cellulolytic degrading enzymes, including at least five endoglucanases, five cellobiohydrolases, and two β-glucosidases (Cai et al., 1994, 1998, 1999). *Pleurotus sajor-caju*, the grey oyster mushroom, exhibits both cellulase and ligninase secretions (Buswell et al., 1996a) and therefore is the most adaptable of the three species. It can grow on a wide variety of agricultural waste materials of differing composition in terms of the polysaccharide–lignin ratio. This demonstrates the impressive capacities of mushrooms for *biosynthesis*, which is different from *photosynthesis* by green plants. The species of mushroom fungi not only can convert the agricultural and forestry lignocellulosic wastes through solid fermentation technology into the high-quality protein consumed directly in the form of the mushroom fruiting body but also can convert food processing biomass wastes (e.g., soybean wastes using submerged culture) into fungal protein (Buswell and Chang 1994) or "mycomeat" (Miles and Chang 1988). Soybean waste materials (slurries) are generated in large quantities during the processing of soybean milk and "tofu" (bean curd), which are popular foods in many countries now and are, in some places, discarded without treatment, thereby constituting an environmental pollutant. In addition, mushrooms and their mycelia can provide nutriceutical and pharmaceutical products. As outlined above, by blending the advances in basic biological knowledge with that of practical technology, a mushroom-related industry based on utilization of the lignocellulosic waste materials that are abundantly available in rural and urban areas can have positive global impacts on long-term food nutrition, health, environmental conservation and regeneration, and economic and social change. Therefore, the significant impact of applied mushroom biology on human welfare has been named a "nongreen revolution" (Chang, 1999).

1.3.3.2 Mushroom Bioremediation This component of applied mushroom biology deals mainly with the aspects of benefits to the earth from the activities of mushroom mycelium. Environmental contamination can be ameliorated by the application of mushroom mycelial technologies. For example:

1. The use of bioconversion processes to transform the polluting substances into valuable foodstuffs, for example, the proper treatment and reutilization of spent substrates/composts in order to eliminate the pollution problems (Beyer, 2005; Noble, 2005). One of the most intriguing opportunities offered by mushroom mycelia in the area of bioconversion is the exploitation of their ability to degrade pollutants, many of which are highly carcinogenic, released into the environment as a consequence of human activity.
2. The use of fungi/mushroom mycelia as tools for healing soil, what Stamets (2005) called "mycorestoration," which is the use of fungi/mushrooms to repair or restore the weakened or damaged biosystems of environment. The processes of mycorestoration include the selective use of mushrooms for mycofiltration to filter water, mycoforestry to enact ecoforestry policy, mycoremediation to denature toxic wastes, and mycopesticides to control insect pests. Mycorestoration recognizes the primary role fungi/mushrooms play in determining the balance of biological populations.

1.4 MUSHROOM CULTIVATION

Mushroom cultivation 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 some technological elements that are in consonance with those exhibited by our common agricultural crop plants. For example, there is a vegetative growth phase, when the mycelia grow profusely, and a reproductive (fruiting) growth phase, when the umbrella-like body that

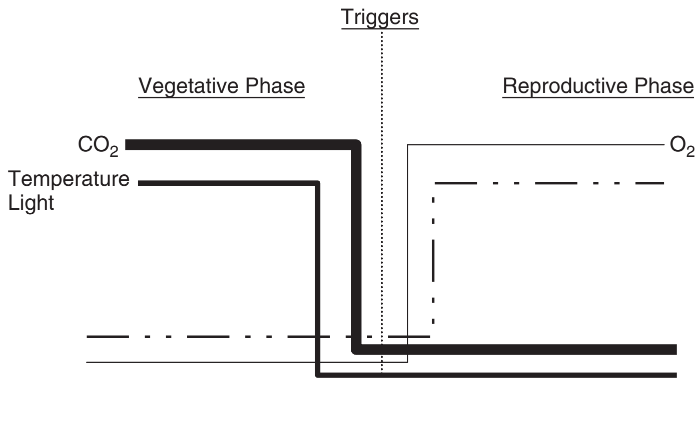

Figure 1.6 Two major phases of mushroom growth and development: vegetative and reproductive. The triggers for the transition from the vegetative phase to the reproductive phase are usually regulated by environmental factors.

we call mushroom develops. In agricultural plants (e.g., sunflowers), when the plants switch from vegetative growth to reproductive growth, retarding tips for further growth (elongation) is an obvious phenomenon of maturity. It is the same principle in mushroom production. After the vegetative (mycelial) phase has reached maturity, what the mushroom farmer needs next is the induction of fruiting. This is the time the mycelial growth tips should be retarded by regulating the environmental factors. These factors, generally called “triggers” or “environmental shocks,” such as switching on the light, providing fresh air, and lowering temperature, can trigger fruiting (Figure 1.6).

1.4.1 Major Phases of Mushroom Cultivation

Mushroom farming is a complex business that requires precision. Indeed, it is not as simple as what some people often loosely stipulate. It calls for adherence to precise procedures. The major practical steps/segments of mushroom cultivation are (1) selection of an acceptable mushroom species, (2) secretion of a good-quality fruiting culture, (3) development of robust spawn, (4) preparation of selective substrate/compost, (5) care of mycelial (spawn) running, (6) management of fruiting and mushroom development, and (7) careful harvesting of mushrooms (Chang and Chiu, 1992; Chang 1998). If you ignore one critical step/segment, you are inviting trouble, which could lead to a substantially reduced mushroom crop yield and mushroom marketing value:

1. Before any decision to cultivate a particular mushroom is made, it is important to determine if that species possesses organoleptic qualities

acceptable to the indigenous population or to the international market, if suitable substrates for cultivation are plentiful, and if environmental requirements for growth and fruiting can be met without excessively costly systems of mechanical control.

2. A "fruiting culture" is defined as a culture with the genetic capacity to form fruiting bodies under suitable growth conditions. The stock culture selected should be acceptable in terms of yield, flavor, texture, fruiting time, and so on.
3. A medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum of "seed" for the substrate in mushroom cultivation is called the "mushroom spawn." Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used. Consideration must also be given to the nature of the spawn substrate since this influences rapidity of growth in the spawn medium as well as the rate of mycelial growth and filling of the beds following inoculation.
4. While a sterile substrate free from all competitive microorganisms is the ideal medium for cultivating edible mushrooms, systems involving such strict hygiene are generally too costly and impractical to operate on a large scale. Substrates for cultivating edible mushrooms normally require varying degrees of pretreatment in order to promote growth of the mushroom mycelium to the practical exclusion of other microorganisms. The substrate must be rich in essential nutrients in forms which are readily available to the mushroom and be free of toxic substances that inhibit growth of the spawn. Moisture content, pH, and good gaseous exchange between the substrate and the surrounding environment are important physical factors to consider.
5. Following composting, the substrate is placed in beds where it is generally pasteurized by steam to kill off potential competitive microorganisms. After the compost has cooled, the spawn may be broadcast over the bed surface and then pressed down firmly against the substrate to ensure good contact or inserted 2–2.5 cm deep into the substrate. Spawn running is the phase during which mycelium grows from the spawn and permeates into the substrate. Good mycelial growth is essential for mushroom production.
6. Under suitable environmental conditions, which may differ from those adopted for spawn running, primordial formation occurs and then is followed by the production of fruiting bodies. The appearance of mushrooms normally occurs in rhythmic cycles called "flushes."
7. Harvesting is carried out at different maturation stages depending upon the species and consumer preferences and market value.

1.4.2 Cultivation of Several Selected Mushrooms

The cultivation of edible mushrooms can be divided into two major stages. The first stage involves the preparation of the fruiting culture, stock culture, mother spawn, and planting spawn, while the second stage entails the preparation of the growth substrates for mushroom cultivation. Cultivation conditions for a few selected mushroom species are briefly described in the following sections.

1.4.2.1 Cultivation of Agaricus Composting is prepared in accordance with well-documented commercial procedures (van Griensven, 1988; Chang and Hayes, 1978; Kaul and Dhar, 2007). In phase I of the process (outdoor composting), locally available raw materials are arranged into piles that are periodically turned and watered. The initial breakdown of the raw ingredients by microorganisms takes place in phase I. This phase is usually complete within 9–12 days, when the materials have become pliable, dark brown in color, and capable of holding water. There is normally a strong smell of ammonia. Phase II (indoor fermentation) is pasteurization, when undesirable organisms are removed from the compost. This is carried out in a steaming room where the air temperature is held at 60°C for at least 4 hours. The temperature is then lowered to 50°C for 8–72 hours depending upon the nature of the compost. Carbon dioxide is maintained at 1.5–2% and the ammonia level drops below 10 PPM. Following phase II composting, the substrate is cooled to 30°C for *Agaricus bitorquis* and to 25°C for *A. bisporus* for spawning. Production of phase III or IV composts for growing *Agaricus* mushrooms has been an advanced technological development in recent years in Western countries. The production of phase III compost is phase II compost spawn run in a bulk tunnel and ready for casing when delivered to the grower. If the phase III compost is then cased and spawn developed into the casing layer before dispatching to the growing unit or delivering to growers, it is named as phase IV compost. The successes of bulk phase III and IV depend a lot on the quality of phase I and II processes. Phase II on shelves produces an average of 4.1 crops per year. Since 1999, growers using Phase III production enjoyed an average of 7.1 crops per year. In recent years, phase IV can generate 10–12 crops per year (Dewhurst, 2002; Lemmers, 2003).

1.4.2.2 Cultivation of Lentinula edodes *Lentinula edodes* (xiang gu in Chinese and shiitake in Japanese) was the second most important cultivated edible mushroom, but since 2002 it has become the world number one cultivated mushroom. It can be cultivated either on wood log or on synthetic substrate logs (Quimio et al., 1990; Stamets, 2000; Chang and Miles, 2004).

1. *Biological Nature* *Lentinula edodes* is a heterothallic mushroom. Its sexuality is controlled by two mating factors, A and B, with multiple alleles, and therefore, its life history is a tetrapolar or bifactorial mating system (Chang and Miles, 1984).

Its life cycle starts the germination of basidiospores. After selected mating between two compatibility germinative mycelium, the dikaryon mycelium or fruiting culture is established. From the fruiting culture, the stock culture, mother spawn, and commercially planting spawn can be made. When the spawn is planted on a suitable substrate, under good climatic conditions the fruiting bodies of the mushroom are developed. Then when the mature stage is reached, the spores are discharged and its life cycle is completed.

Lentinula edodes is kind of wood rot fungus. In nature, it grows on dead tree trunks or stumps. In general, the wood for the mushroom growth consists of crude protein 0.38%, fat 4.5%, soluble sugar 0.56%, total nitrogen 0.148%, cellulose 52.7%, lignin 18.09%, and ash 0.56%. Generally speaking, the carbon–nitrogen ratio in substrate should be in the range of 25 : 1–40 : 1 in the vegetative growth stage and from 40 : 1 to 73 : 1 in the reproductive stage. If the nitrogen source is too rich in the reproductive phase, fruiting bodies of the mushroom are usually not formed and developed.

The optimum temperature of spore germination is 22–26°C. The temperature for mycelial growth ranges from 5 to 35°C, but the optimum temperature is 23–25°C. Generally speaking, *L. edodes* belongs to low-temperature mushrooms; the initial and development temperature of fruiting body formation is in the range of 10–20°C and the optimum temperature of fructification for most varieties of the mushroom is about 15°C. Some varieties can fruit in higher temperatures (e.g., 20–23°C). These high-temperature mushrooms usually grow faster and have a bigger and thinner cap (pileus) and a thin and long stalk (stipe). Their fruiting bodies are easily opened and become flat-grade mushrooms, which are considered to be low quality. The optimum pH of the substrate used in making the mushroom bag/log is about 5.0–5.5.

2. *Culture Media and Preparation* The mushroom can grow on a variety of culture media and on different agar formulations, both natural and synthetic, depending on the purpose of the cultivation. Synthetic media are often expensive and time consuming in preparation; hence they are not commonly used for routine purposes.

The potato dextrose agar, or PDA, is the simplest and the most popular medium for growing the mycelium of the mushroom. It is prepared as follows:

(a) *Ingredients*: Diced potato, 200 g; dextrose (or ordinary white cane sugar), 20 g; powdered agar (or agar bars), 20 g; and distilled water (or tap water), 1 L.

(b) *Procedure*: Peeled potatos are washed, weighed, and cut into cubes. They are boiled in a casserole with at least 1 L of water until they become soft (around 15 minutes). The potatos are removed and water is added to the broth to make exactly 1 L. The broth is returned to the casserole, and dextrose and the agar are added. The solution is heated and stirred occasionally until the agar is melted. The hot solution is then poured into clean flat bottles. For pure or stock cultures, the test tubes are filled with at least 10 mL of liquid agar solution. The bottles or test tubes are plugged with cottonwool. When petri dishes are available, these can be used to produce mycelial plugs for inoculation of mother spawn.

Examples of the different formulas for spawn substrates are described below. Mother grain spawn: (i) Wheat/rye grain + 1.5% gypsum or slaked lime. (ii) Cotton seed hull 40%, sawdust 38%, wheat bran 20%, sugar 1%, and gypsum 1%. (iii) Sugar cane bagasse 40%, sawdust 38%, wheat bran 20%, sugar 1%, and gypsum 1%. Planting spawn: A number of materials, mostly agricultural and forest wastes, can be used to prepare mushroom planting spawn. Three of them are given here as examples: sawdust 78%, rice/wheat bran 16%, sugar 1.5%, corn flour 1.7%, ammonium sulfate 0.3%, calcium superphosphate 0.5%, and gypsum 2%; sawdust 64%, wheat bran 15%, spent coffee grounds 20%, and gypsum/lime 1%; and sawdust 78%, sucrose 1%, wheat bran 20%, and calcium carbonate 1%.

The *L. edodes* mushroom is produced on both a cottage and a commercial scale. Some issues associated with the different cultivation styles are summarized below:

1. *Cottage-Scale Cultivation* There are many formulas for the composition of the substrate. The ingredients can be variable from place to place and country to country depending upon the raw materials available and local climatic conditions. In general, after mixing the dry ingredients by hand or with a mechanical mixer, water is added to the mixture so that the final moisture content of the substrate is between 55 and 60%, depending on the capacity of the sawdust to absorb water. The ingredients are then packed into autoclavable polypropylene or high-density polyethylene bags. Although they are more expensive, polypropylene bags are the most popular since polypropylene provides greater clarity than polyethylene. After the bags have been filled (1.5–4 kg wet weight) with the substrate, the end of the bag can be closed either by strings or plugged with a cottonwool stopper. Four formulas in the preparation of the substrate for the cultivation of the mushroom are given here as reference. (i) Sawdust 82%, wheat bran 16%, gypsum 1.4%, potassium phosphate, dibasic 0.2%, and lime 0.4%. (ii) Sawdust 54%, spent coffee grounds 30%, wheat bran 15%, and gypsum 1%. (iii) Sawdust 63%, corncob powder 20%, wheat bran 15%, calcium superphosphate 1%, and gypsum 1%. (iv) Sawdust 76%, wheat bran 18%, corn powder 2%, gypsum 2%, sugar 1.2%, calcium superphosphate 0.5%, and urea 0.3%.
2. *Commercial-Scale Cultivation* In general, the operation can use oak or other hardwood sawdust medium to grow the mushroom. The basic steps are (i) mix the sawdust, supplements, and water; (ii) bag the mixture; (iii) autoclave the bags to 121°C and cool the bags; (iv) inoculate and seal the bags; (v) incubate for 90 days to achieve full colonization of the sawdust mixture, in other words, to allow the mycelium to be established for ready fructification; (vi) fruit the colonized and established sawdust logs/bags/blocks 6 times using a 21-day cycle at 16–18°C; and (vii) harvest, clip steps, grade, box, and cold store for fresh market or harvest, dry, cut steps, grade, and dry again before boxing for dry market.

The major equipment used in production consists of a mixer/conveyor, autoclave, gas boiler, cooling tunnel, laminar flow cabinet, bag sealer, air compressor for humidification, and shelves to incubate.

Incubation can be done in two rooms and in two shipping containers. The two shipping containers can be installed near the fruiting rooms. The temperature during incubation is held between 18 and 25°C.

Fruiting can be done in six rooms so that the blocks/logs can be moved as a unit. With compartmentalization, blocks in each room can be subjected to a cycle of humid cold, humid heat, and dry heat.

1.4.2.3 Cultivation of Pleurotus sajor-caju *Pleurotus sajor-caju* (grey oyster mushroom) is comparable to the high-temperature species in the group of *Pleurotus* (oyster) mushrooms, with high temperatures required for fructification. This mushroom has a promising prospect in tropical/subtropical areas. Its cultivation is easy with relatively less complicated procedures (Chang and Miles, 2004; Kaul and Dhar, 2007):

1. *Biological Nature* The temperature for growth of mycelium is 10–35°C. The optimum growing temperature of the mycelium is 23–28°C. The optimum developmental temperature of the fruiting body is 18–24°C. The optimum pH of the substrate used in making the mushroom bag/bed is 6.8–8.0. The C/N ratio in the substrate is in the range of 30 : 1–60 : 1. A large circulation of air and reasonable light are required for the development of the fruiting bodies.
2. *Spawn Substrate* (i) Wheat grain + 1.5% gypsum or lime. (ii) Cotton seed hull 88%, wheat bran 10%, sugar 1%, and gypsum 1%. (iii) Sawdust 78%, wheat bran 20%, sugar 1%, and gypsum 1%. (vi) Sawdust 58%, spent coffee grounds/spent tea leaves 20%, water hyacinth/cereal straw 20%, sugar 1%, and gypsum 1%.
3. *Cultivation Substrate* (i) Cotton seed hull 95%, gypsum 2%, lime 1%, and calcium superphosphate 2%. (ii) Rice straw 80%, cotton waste 18%, gypsum 1%, and lime 1%. (iii) Water hyacinth 80%, cereal straw 17%, gypsum 2%, and lime 1%.

For demonstration purpose, this mushroom can be nurtured to grow into a tree-like shape (Chang and Li, 1982). The cultivation method, which has been tested to be successful, is as follows: Cotton waste or rice straw mixed with water hyacinth is used as the substrate. Tear large pieces of cotton waste into small parts or cut the straw and water hyacinth into small segments. Add 2% (w/w) lime and mix with sufficient water to get moisture content of about 60–65%. Pile the materials up, cover with plastic sheets, and leave to stand overnight. Load the substrate into small baskets or on shelves for pasteurization or cook the substrate with boiled water for 15 minutes. After cooling to approximately 25°C, mix around 2% (w/w) spawn thoroughly with the substrate and pack into columns of 60-cm-long tubes which have hard plastic [polyvinyl chloride (PVC)] tubing of 100 cm (4 cm in diameter) as central support and with plastic sheets as outside wrapping.

Incubate these columns at around 24−28°C, preferably in the dark. When the mycelium of the mushroom has ramified the entire column of substrate after three to four weeks, remove the plastic wrapping and switch on white light. Watering occasionally is needed to keep the surface from drying. In around three to four days white primordia start to appear over the whole surface. After another two to three days, the *Pleurotus* mushrooms are ready for harvesting. During the cropping period watering is very important if many flushes are required.

1.4.2.4 Cultivation of Volvariella The edible straw mushroom *Volvariella volvacea* is a fungus of the tropics and subtropics and has been traditionally cultivated in rice straw for many yeas in China and South East Asian countries. In 1971, cotton wastes were first introduced as heating material for growing the straw mushroom (Yau and Chang, 1972), and in 1973, cotton wastes had completely replaced the traditional paddy straw to grow the mushroom (Chang, 1974). This was a turning point in the history of straw mushroom cultivation because the cotton waste compost through pasteurization brought the cultivation of the mushroom into an industrial scale first in Hong Kong and then in Taiwan, Thailand, and elsewhere in China. Several techniques are adopted for the cultivation of the mushroom, which thrives in the temperature range of 28–36°C and a relative humidity of 75–85%. Detailed descriptions of the various methods are given by Chang and Quimio (1982), Chang and Miles (2004), Kaul and Dhar (2007), and Quimio et al. (1990). Choice of technologies usually depends on personal preference and the availability of substrates and resources. While the more sophisticated indoor technology is recommended for the industrial-scale production of the mushroom, most of the other technologies are low cost and appropriate for rural area development, especially when production is established at the community level.

1.4.2.5 Cultivation of Agaricus brasiliensis In recent years, *A. brasiliensis*, formerly called *Agaricus blazei* Murill (Wasser et al., 2002), has rapidly become a popular mushroom. It has been proved to be not only a good-tasting and highly nutritious mushroom but also an effective medicinal mushroom, particularly for antitumour active polysaccharides.

Agaricus brasiliensis was a wild mushroom in southeastern Brazil, where it was consumed by the people as a part of their diet. The culture of the mushroom was brought to Japan in 1965 and an attempt to cultivate this mushroom commercially was made in 1978. In 1992, this mushroom was introduced to China for commercial cultivation (Chang and Miles, 2004).

1. *Biological Nature* *Agaricus brasiliensis* belongs to middle-temperature mushrooms. The growth temperature for mycelium ranges from 15 to 35°C and the optimum growth temperature range is 23–27°C. The temperature for fruiting can be from 16 to 30°C and the optimum developmental temperature of fruiting bodies is 18–25°C. The ideal humidity for casing soil is 60–65%. The air humidity in a mushroom house is preferably 60–75% for mycelium growth and 70–85% for fruiting body formation and development. The optimum pH of the compost used in making the mushroom bed is 6.5–6.8. The optimum pH of the casing soil is 7.0. A good circulation of air is required for the development of the fruiting bodies. These conditions are similar to those needed for the cultivation of *A. bisporus*. Under natural conditions, the mushroom can be cultivated for two crops each year. Each crop can harvest three flushes. According to the local climates, the farmer can decide the spawning time in the year in order to have mushrooms for harvest within 50 days after spawning.

2. *Preparation of Mushroom Bed* (Stamets, 2000) *Agaricus brasiliensis* is a kind of mushroom belonging to straw-dung fungi and prefers to grow on substrate rich in cellulose. The waste/by-product agro-industrial materials [e.g., rice straw, wheat straw, bagasse (squeezed residue of sugar cane), cotton seed hull,

corn stalks, sorghum stover, and even wild grasses] can be used as the principal component of the compost for cultivation of the mushroom. It should be noted that these materials have to be air dried first and then mixed with cattle dung, poultry manure, and some chemical fertilizers. The following formulas for making compost are for reference only. They should be modified according to the local available materials and climatic conditions. (i) Rice straw 70%, air-dry cattle dung 15%, cottonseed hull 12.5%, gypsum 1%, calcium superphosphate 1%, and urea 0.5%. (ii) Corn stalks 36%, cottonseed hull 36%, wheat straw 11.5%, dry chicken manure 15%, calcium carbonate 1%, and ammonium sulfate or urea 0.5%. (iii) Rice straw 90.6%, rice bran 2.4%, fowl droppings 3.6%, slaked lime 1.9%, superphosphate 1.2%, and ammonium sulfate/urea 0.3%. (iv) Bagasse 75%, cottonseed hull 13%, fowl droppings 10%, superphosphate 0.5%, and slaked lime 1.5%.

1.4.2.6 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 (Chang and Miles, 2004).

Artificial cultivation of this valuably mushroom was successfully achieved in the early 1970s and, since 1980 and particularly in China, production of *G. lucidum* has developed rapidly. Currently, the methods most widely adopted for commercial production are the wood log, short wood segment, tree stump, sawdust bag, and bottle procedures (Hsu, 1994; Mizuno et al., 1996; Hung, 1996; Mayzumi et al., 1997; Chang and Buswell, 1999; Stamets, 2000).

Log cultivation methods include the use of natural logs and tree stumps which are inoculated with spawn directly under natural conditions. The 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. This method provides for a short growing cycle, higher biological efficiency, good-quality fruiting bodies, and, consequently, superior economical benefit. However, this production procedure is more complex and the production costs much higher than the natural log and tree stump methods. For this production procedure, 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 45–55%. The flowchart for the short-log cultivation method is as follows: selection and felling of the tree, sawing/cutting the log into short segments, transfering segments to plastic bags, sterilization, inoculation, spawn running, burial of the log in soil, tending the fruiting bodies during development from the pinhead stage to maturity, harvesting the fruiting bodies, drying the fruiting bodies by electrical driers, and packaging. It should be noted that the prepared logs/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.

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): (i) sawdust 78%, wheat bran 20%, gypsum 1%, and soybean powder 1%; (ii) bagasse 75%, wheat bran 22%, cane sugar 1%, gypsum 1%, and soybean powder 1%; (iii) cotton seed hull 88%, wheat bran 10%, cane sugar 1%, and gypsum 1%; (iv) sawdust 70%, corn cob powder 14%, wheat bran 14%, gypsum 1%, and cereal straw ash 1%; (v) corn cob powder 78%, wheat/rice bran 20%, gypsum 1%, and straw ash 1%. After sterilization, the plastic bags can be laid horizontally on beds or the ground for fruiting.

1.4.3 Utilization of Mushroom Germplasm

The item of mushroom germplasm is a selective subject only and is not an exhaustive approach to mushroom utilization. It is concerned with the broadening of available mushroom resources in nature in order to conduct successful domesticating and breeding programs, with the aim at developing the cultivation of wild mushrooms and improving all desirable mushroom traits. It cannot be overemphasized that, to fully exploit the opportunities offered by mushrooms which have been properly collected and characterized, it is necessary to ensure a continuous exchange of information between scientists from different disciplines engaged in different areas of mushroom research. Included among the many possible examples that could be quoted are the analytical chemists who analyze the many existing and potentially new growth substrates used for mushroom cultivation in order to certify their suitability from an alimentary standpoint; biochemists who study the fungal enzymes involved in the degradation of the individual components constituting the different substrates; fungal physiologists who focus their attention on the mechanisms underlying carpophore formation; geneticists who are able to comprehend the life cycles of different mushrooms as well as to undertake breeding programs and select strains with desirable characteristics; and growers who transfer to the field scale the knowledge and techniques obtained in the laboratory.

One of the basic requirements for breeding better quality mushrooms in higher yields is the wider availability of a large reserve of phenotypic variation (traits) which can be used for selection purposes by both researchers and the mushroom industry. Since all these phenotypic differences are ultimately under genetic control, mushroom strains with different traits actually possess distinctive gene combinations which can be generated artificially by conventional crossing methods, by protoplast fusion technology, and by transformation with genes cloned using recombinant deoxyribonucleic acid (rDNA) technology. Since the mushrooms themselves are the only source of this genetic material, the genes contained in existing mushroom strains and species represent the total genetic resource, that is, the entire pool of mushroom germplasm. Extinction of a single strain or species would mean the potential loss of many thousands of unique genes that could be used for breeding desirable new strains.

Mushroom germplasm can be preserved by in situ conservation and ex situ preservation. The maintenance of mushrooms in natural preserves as part of a strategy for protecting an ecosystem constitutes in situ conservation. Although this approach is clearly important, it will not be considered here. Mushroom germplasm can also be preserved ex situ as fungal spores or tissue in the form of a culture collection or gene bank. The collection and classification of information pertaining to the morphological, physiological, biochemical, and genetic characteristics of individual mushroom strains can be stored in computer databases called *germplasm databases*. Such databases would provide valuable and readily accessible information for future breeding programs and academic research (Chang et al., 1995). This emerging mushroom germplasm science will address aspects relating to the collection, identification, characterization, utilization and preservation of mushroom germplasm.

Mushrooms have the potential for multipurpose usages ranging from protein enrichment of the human diet, the selective delignification of lignocellulosic materials as part of the recycling process and reinsertion into the food chain and dietary supplements markets, and a contribution to environmental decontamination. The realization of this potential depends upon the availability of mushrooms which possess the characteristics necessary to achieve specified objectives. The pool of available selective mushrooms from nature through the processes of collection, identification, and utilization should be as large as possible in order to ensure that the most appropriate choice of mushroom germplasm can be caught and utilized.

1.5 WORLD MUSHROOM PRODUCTION

The world market for the mushroom industry in 2001 was valued at over U.S.$40 billion (Chang, 2006a). The mushroom industry can be divided into three main categories: edible mushrooms, medicinal mushroom products, and wild mushrooms (Chang, 2006b). International bodies/forums have developed for each of these segments of the mushroom industry that have helped to bring them to the forefront of international attention: (1) International Society of Mushroom Science (ISMS) for edible mushrooms, (2) World Society for Mushroom Biology and Mushroom Products (WSMBMP) for mushroom biology and medicinal mushroom products, and (3) International Workshops on Edible Mycorrhizal Mushrooms for some wild mushrooms. The three international bodies/forums have done much to promote each of their respective fields, not the least of which is bringing scientists together for useful discussions, encouraging research and the dissemination of valuable information. The outlook for many of the known mushroom species is bright. Production of mushrooms worldwide has been steadily increasing, mainly due to contributions from developing countries, such as China, India, Poland, Hungary, and Vietnam. There are also increasing experimentally based evidence to support centuries of observations regarding the nutritional and medicinal benefits of mushrooms. The value of mushrooms has recently been promoted to tremendous levels with medicinal mushrooms trials conducted for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients in Africa, generating encouraging results (Chang and Mshigeni, 2000). However, harvests of highly prized edible mycorrhizal mushrooms are continuously decreasing. This has triggered research into devising methods for improved cultivation of wild mushroom. It is hoped that there will be even more research into this area, so that larger quantities of wild mushrooms can be massively harvested through semicultivation methods. Technological development in the mushroom industry in general has seen increasing production capacities, innovations in cultivation technologies, improvements to final mushroom goods, capitalizing the nutritional and medicinal properties of mushrooms, and utilizing the natural qualities of mushrooms for environmental benefits. However, there is always the need to maintain current trends and to continue to seek out new opportunities. The challenge is to recognize opportunities such as increasing consumption capabilities with the increase in world population and to take advantage of this by promoting the consumption of more mushrooms.

Generally, cultivated mushrooms should play a greater role in the endeavor to increase food protein. This is especially true in developing countries, since growth substrates for mushrooms are basically agricultural and industrial discards that are inedible for humans (Chang and Miles, 1984). Biological (bioconversion) efficiency, that is, the yield of fresh weight mushrooms in proportion to the spawning compost in *Agaricus* or to the air-dried substrates in other noncomposting mushrooms, can reach 60–100% for *Agaricus* and 15–100% depending on the cultivation conditions for other species.

The statistics in Table 1.1 illustrate the dramatic increase in the production of farmed mushrooms during the period 1960–2002 (Chang 1999, 2006b; Delcaire, 1978).

TABLE 1.1 World Mushroom Production, 1960–2002

Year	World Production (× 1000 t)
1960	170
1965	301
1970	484
1975	922
1978	1,060
1981	1,257
1983	1,453
1986	2,182
1990	3,763
1994	4,909
1997	6,158
2002	12,250

Whereas in 1997, Asia contributed 74.4% of the total world mushroom tonnage, Europe 16.3%, and North America 7.0%, both Africa and Latin America's shares were less than 1%. This is largely due to lack of know-how, lack of understanding that mushrooms can play vital roles toward enhancing human health when used as dietary food supplements, lack of reliable sources of good-quality mushroom spawn for supporting the efforts of local mushroom growers, lack of venture capital to support mushroom farming entrepreneurs, and absence of systematic government support toward promoting mushroom farming as a valuable nontraditional new food and cash crop (comparable to coffee, tea, cotton, tobacco, etc.).

1.6 MUSHROOM BIOTECHNOLOGY

It has been pointed out that mushroom biotechnology is concerned with mushroom products and encompasses the principles of fermentation technology, mushroom biology/microbiology, and bioprocess. The products have a more generalized or tonic effect, which in some cases may act prophylactically by increasing resistance to disease in humans from the balancing of nutrients in the diet and the enhancing of the immune systems.

1.6.1 Nutritional and Medicinal Value of Mushrooms

The greatest difficulty in feeding humans is to supply a sufficient quantity of the body-building material protein. The other three nutritional categories are the source of energy (carbohydrates and fats); accessory food factors (vitamins); and inorganic compounds which are indispensable to good health. Of course, water, too, is essential.

The moisture content of fresh mushrooms varies within the range 70–95% depending upon the harvest time and environmental conditions, whereas it is about 10–13% in dried mushrooms. The protein content of cultivated species ranges from 1.75 to 5.9% of their fresh weight. It has been estimated that an average value of 3.5–4.0% would be more representative. This means that the protein content of edible mushrooms in general is about twice that of onion (1.4%) and cabbage (1.4%) and 4 and 12 times those of oranges (1.0%) and apples (0.3%), respectively. In comparison, the protein content of common meats is as follows: pork, 9–16%; beef, 12–20%; chicken, 18–20%; fish, 18–20%; and milk, 2.9–3.3%. On a dry-weight basis, mushrooms normally contain 19–35% protein, as compared to 7.3% in rice, 12.7% in wheat, 38.1% in soybean, and 9.4% in corn. Therefore, in terms of the amount of crude protein, mushrooms rank below animal meats but well above most other foods, including milk, which is an animal product. Furthermore, mushroom protein contains all the nine essential amino acids required by humans.

In addition to their good 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. They are low in calories, carbohydrates, and calcium. It has also been reported that a total lipid content varying between 0.6 and 3.1% of the dry weight is found in the commonly cultivated mushrooms. At least 72% of the total fatty acids are found to be unsaturated in all the four tested mushrooms (Huang et al., 1985). It should be noted that unsaturated fatty acids are essential and significant for our diet and our health.

In recent years, there has been a trend toward discovering ways of treating mushrooms so as to give them added value. For example, Wermer and Beelman (2002) have reported on growing mushrooms enriched in selenium. By adding sodium selenite to compost over a range of 30–300 PPM, they found that the mushrooms increasingly absorbed selenium according to the amount in the compost, so that it is possible to grow mushrooms containing a desired concentration. Selenium is an essential micronutrient that has generated much recent interest in nutritional and medical research—and more recently within the food industry (Beelman and Royse, 2006). Selenium has numerous physiological functions but is best known as a necessary cofactor for the glutathione peroxidase enzyme system. This system is responsible for removing free radicals from the body, thus reducing oxidative damage.

The desirability of a food product does not necessarily bear any correlation to its nutritional value. Instead, its appearance, taste, and aroma sometimes can stimulate one's appetite (preference). In addition to nutritional value, mushrooms have some unique color, taste, aroma, and texture characteristics which attract their consumption by humans.

The second major attribute of mushrooms, their medicinal properties, has also been drawn to our attention for study, for example, for hypotensive and renal effects (Yip et al., 1987), immunomodulatory and antitumor activities of polysaccharide–protein complex (PSPC) from mycelial cultures (Liu et al., 1995, 1996; Wang et al., 1995a, 1996b, c), immunomodulatory and antitumor activities of lectins from edible mushrooms (Wang et al., 1995b, 1996a, 1997), isolation and characterization of a type I ribosome inactivation protein from *V. volvacea* (Yao et al., 1998), and medicinal effects of *G. lucidum* (Chang and Buswell, 1999; Chang and Miles, 2004). For more detailed coverage of this aspect and comprehensive lists of mushrooms used in dietary supplements and in medicines, the reader is referred to later chapters.

1.6.2 Nutriceuticals and Dietary Supplements

There has been a recent upsurge of interest in mushrooms not only as health vegetables (food) but also as a source of biological active compounds of medicinal value, including use as complementary medicine/dietary supplements for anticancer, antiviral, immunopotentiating, hypocholesterolemic, and hepatoprotective agents. These new compounds, termed *mushroom nutriceuticals* (Chang and Buswell, 1996), are extractable from either the fungal mycelium or fruiting body and represent an important component of the expanding mushroom biotechnology industry.

Of the 14,000–15,000 species of so-called mushrooms in the world, around 400 have known medicinal properties. However, it has been estimated that there are about 1800 species of mushrooms with the potential of medicinal properties. Both these mushrooms and their rootlike structure (called mycelium) produce several medicinal or nutriceutical (general immune-enhancing) compounds, central of which are the polysaccharides (high-molecular-weight strings of sugars), triterpenes, and immunomodulatory proteins. Although virtually all mushrooms and many foods have polysaccharides in their cell walls, certain mushroom species have been found to contain polysaccharides which are particularly effective in retarding the progress of various cancers and other diseases and in alleviating the side effects of chemotherapy and radiation treatment (through cell-level regenerative effects). There are now many studies in Asia, and particularly in China and Japan, documenting life-span increases of cancer patients undergoing conventional cancer treatment plus mushroom extract consumption or injection (Mizuno et al., 1995; Liu, 1999). At the same time, due to the enhancement of the immune systems, it can help people reduce the possibility of being infected by other diseases.

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, for example, Lentinan, a high-molecular-weight $(1 \rightarrow 3)$-β-D-glucan from *L. edodes* and various products from *G. lucidum*. Only about 15% of all products are based on extracts from mycelia. Notable examples are PSK (trade name Krestin) of a polysaccharide peptide and PSP (polysaccharide-bound peptide) extracted from *Coriolus versicolor*. A smaller percent of mushroom products are obtained from culture filtrates, for example, schizophyllan, a high-molecular-weight $(1 \rightarrow 3),(1 \rightarrow 6)$-$\beta$-D-glucan prepared from *Schizophyllum commune* Fr., and PSPC (a protein-bound polysaccharide complex) from *Tricholoma lobayense* Hein. However, due to increased quality control and year-round production, mycelial products are the wave of the future.

The market value of medicinal mushrooms and their derivative dietary supplements worldwide was about U.S.$1.2 billion in 1991 and about U.S.$3.6 billion in 1994 (Chang, 1996). In 1999, it was estimated to be U.S.$6.0 billion. The market value of *Ganoderma*-based nutriceuticals alone in 1995 was estimated at U.S.$1628.4 million (Chang and Buswell, 1999). The corresponding monetary values were generated by another famous mushroom, *L. edodes*. Ninety-nine percent of all sales of medicinal mushrooms and their derivatives occurred in Asia and Europe with less than 0.1% in North America. The 1999 U.S. market for dietary supplements based mainly on mushrooms was estimated to be U.S.$35 million. However, in recent years, the North American demand is increasing between 20 and 40% annually, depending upon species.

1.7 DEVELOPMENT OF WORLD MUSHROOM INDUSTRY MOVEMENTS

Although mushrooms have been collected from the wild and cultivated artificially for human food and for medicine uses for hundreds and thousands of years, it is

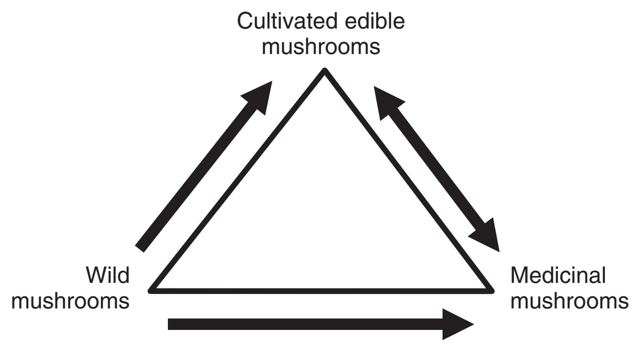

Figure 1.7 Mushroom industry can be considered to be composed of cultivated edible mushrooms, medicinal mushrooms, and wild mushrooms Single arrows indicate that number of both edible and medicinal mushroom species increases from time to time through identification and domestication of unknown and wild mushrooms. Double arrow indicates that most edible species also possess medicinal properties while many medicinal mushrooms can be artificially cultivated.

only recently that the three main segments of the mushroom industry could be identified. These three segments have received international recognition as important interrelated components (Figure 1.7), with each deserving its own special patronage and paths of development: (1) cultivated edible mushrooms (mushroom themselves used directly or indirectly as food), (2) medicinal mushrooms (mushroom derivatives used as nutriceutical therapy/dietary supplements), and (3) wild mushrooms, including edible mycorrhizal, symbiotic, and poisonous mushrooms (collected, up to now, only from the wild). The development of three important international bodies/forums has helped to bring each of these three components of the mushroom industry to the forefront of international attention, showcasing their positive contributions to human welfare (Chang, 2006b).

1.7.1 International Movement for Edible Mushrooms

The movement mainly concerned with mushroom production (mushrooms themselves) was initiated during the First International Conference on Mushroom Science held in Peterborough, the United Kingdom, May 3–11, 1950. Chairman F. C. Atkins with P. J. Bels, E. B. Lambert, and R. L. Edwards were on the organizing committee. The committee members later formed the International Commission on Mushroom Science, which eventually developed into the ISMS.

The Seventeenth International Congress of ISMS will be held May 21–24, 2008, in Cape Town, South Africa. Traditionally, the focus of the ISMS has been on the *A. bisporus* mushroom industry. In recent years, the interests of the ISMS have become more diversified, but *A. bisporus* is still its main concern.

1.7.2 International Movement for Medicinal Mushrooms

The movement mainly concerned with mushroom products (mushroom derivatives) was instituted during the First International Conference on Mushroom Biology and Mushroom Products held in Hong Kong, August 23–26, 1993. Chairman S. T. Chang with J. A. Buswell, V. E. C. Ooi, K. W. K. Liu, and S. W. Chiu were on the organizing committee.

The WSMBMP was launched in January 1994 in response to strong interest expressed at the conference in Hong Kong the previous year. The object of the WSMBMP is to promote the enhancement and application of knowledge related to the basic and applied aspects of mushroom biology and mushroom products (mushroom derivatives possessing medicinal properties from edible, medicinal, and wild mushrooms) through publications, meetings, and other means deemed appropriate. The WSMBMP holds the International Conference for Mushroom Biology and Mushroom Products (ICMBMP) every three years. The sixth one is to be held in Bonn, Germany, in 2008.

The international movement for the medicinal segment of the mushroom industry was given a further boost with the launch of the *International Journal of Medicinal Mushrooms* (IJMM) in 1999 by Solomon P. Wasser as editor-in-chief with Takashi Mizuno, Shu-Ting Chang, and Alexander L. Weis as editors. This then led to the inaugural International Medicinal Mushroom Conference (IMMC) held in Kiev, Ukraine, September 12–14, 2001. It has been agreed that there is an IMMC after an interval of two years. The second IMMC was held in Pattaya, Thailand, July 17–19, 2003, and the third in Port Townsend, Washington, the United States, October 12–17, 2005. IMMC 4 will be in Slovenia in 2007 and IMMC 5 in China in 2009.

1.7.3 International Movement for Wild Mushrooms

The movement, mainly concerned with edible mycorrhizal mushrooms, was born as a pre-Congress activity during the second International Conference on Mycorrhizas in Uppsala, Sweden, in 1999. Two years later, the second International Workshop on Edible Mycorrhizal Mushrooms (IW-EMM) was held in Christchurch, New Zealand, July 3–6, 2001. The third IW-EMM was hosted by the University of Victoria, Canada, August 16–22, 2003, and the fourth was held in Murcia, Spain, November 29–December 2, 2005. The fifth IW-EMM was held in Yunnan, China, in 2007. It should be noted that edible mycorrhizal mushrooms belong to a special group of wild mushrooms which include other symbiotic mushrooms, for example, termite, hallucinogenic, and poisonous mushrooms.

These three international bodies/forums have done much to promote each of their respective fields, not the least of which is bringing together scientists in international forums for useful discussions, encouraging research and the dissemination of valuable information. These three segments of the mushroom-based industry are not for competition but for complementation.

1.8 CONCLUDING REMARKS

As the population of the world is expected to continue increasing in the twenty-first century, so will the amount of food and the level of medical care required by each individual, especially those living in less developed countries. The level of environmental pollution will also become a serious problem. However, the world has an immense amount of lignocellulosic biomass resource which, like solar energy, is sustainable. Currently, the bulk of the lignocellulosic biomass is, to a large extent, considered insignificant or of no commercial value and certainly of no food value, at least in its original form. It should be noted that large amounts of research funds have been set aside to search for increased productivity of the core product, like the oil in the coconut, the cellulose in the tree, the fiber in the sisal, the coffee in the coffee berry, or the grain in the cereal crop. However, little research funding has been reserved for the search for the reuse of many by-products (wastes) from the core products, which are usually considered waste materials. When they are carelessly disposed to the surrounding environment by dumping or burning, these so-called wastes are bound to lead to environmental pollution and consequently to health hazards. It should be emphasized that these lignocellulosic wastes are actually a kind of new natural resource or new raw material. If they could be properly managed and utilized, then eventually economic growth would be promoted. In other words, the by-products in processing the core products can be used/treated as raw materials for the production of second- or third-core products. For example, cereal straw, coffee pulp, spent coffee ground, and sisal waste can be used to grow mushrooms. After harvesting mushrooms, some spent substrates can be used as feeding materials for animals or used for growing earthworms, and afterward, the residues can be used as soil conditioners or crop fertilizers. In the whole exercise, there is no waste produced. This is the concept of zero emissions or total productivity of raw materials (Pauli, 1996). Therefore, the significant impact of applied mushroom biology on human welfare in the twenty-first century could be considered globally as “nongreen revolution.”

Since mushrooms, like all other fungi, lack chlorophyll and are nongreen organisms, they cannot convert solar energy through the process of photosynthesis to organic matter, as the green plants do. But they can produce a wide range of enzymes which can degrade lignocellulosic materials for their growth and for fruiting. This serves to demonstrate the magnificent capacities of the mushrooms for biosynthesis, which is different from photosynthesis affected by green plants. Mushrooms not only can become nutritious protein-rich food (through mushroom science) but also can provide nutriceutical and pharmaceutical products (through mushroom biotechnology). In addition, through mushroom bioremediation, the recycling of the by-products (wastes) in the course of each stage of mushroom production using mushroom mycelia can create a pollution-free environment. Therefore, mushrooms, with their great variety of species, can constitute a cost-effective means of supplementing the food nutrition of humankind through the production of edible mushrooms; alleviate the suffering caused by certain kinds of illnesses through medicinal mushrooms and their derivatives as nutriceuticals/dietary supplements (Chang and Buswell, 1996, 2003; Chang and Mshigeni, 2001) and mycomedicinals (Stamets and Yao, 1998); and reduce environmental pollution as well as heal the soils (Stamets, 2005) through mushroom mycelial activities.

The implementation of applied mushroom biology through a well-designed package of multidisciplinary technologies has already had an impact on human welfare at national and regional levels in the twentieth century. It is believed that by blending advances in basic biology with practical technology, mushroom-related industries can have a global and positive impact on long-term food nutrition, health benefits, environmental conservation and regeneration, and economic and social changes.

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