CHAPTER 4: TECHNOLOGY OF MUSHROOM CULTIVATION

Synopsis

This Chapter deals with the principles and practices of gourmet and medicinal mushroom cultivations. It stresses that successful cultivation involves the interaction of scientific knowledge and practical experience. Annual world production of gourmet and medicinal mushrooms is now estimated in excess of 14 billion US dollars. The operations essential to successful cultivation involve: selection of mushroom spores or strains, maintenance of mycelial cultures, development of spawn or inoculum, preparation of growing medium, inoculation and colonization, and crop management for optimum production. However, for greater pharmaceutical acceptance it is increasingly being recognized that product formation by way of fermenter-grown mycelial biomass will be the preferred option in many cases.

Introduction

Mushroom science is the discipline that is concerned with the principles and practices of mushroom cultivation. As is true in any branch of science, it is essential to establish the facts upon which principles can be derived for future developments of the discipline. Consistent production of successful mushroom crops will be built upon scientific knowledge and practical experience (Chang and Miles, 1989).

Rhere are at least 12,000 species of fungi that can be considered as mushrooms with at least 2,000 species showing various degrees of edibility (Chang, 1999a). Furthermore, over 200 species of mushroom have been collected from the wild and utilized for various traditional medical purposes mostly in the Far East. To date, about 35 mushroom species have been cultivated commercially and of these, about 20 are cultivated on an industrial scale (Table 1). The majority of these cultivate species are both edible and possess medicinal properties. However, two of the major medicinal mushrooms, viz. *Ganoderma lucidum* and *Trametes (Coriolus)* spp. are distinctly inedible. Overall, the world production of cultivated edible and/or medicinal mushrooms was recorded as 4,909.3 x 10^3 tons in 1994 increasing to 6,158.4 x 10^3 in 1997 with an estimated value in excess of 14 billion US dollars (Chang, 1999b).

Mushroom cultivation is a worldwide practice (Table 2). In percentage terms, output yield of the leading 10 species cultivated made up *c* 92% of total world production of these six species, viz: *Agaricus bisporus* (31.8%); *Lentinus edodes* (25.4%), *Pleurotus* spp. (14.2%), *Auricularia auricula* (7.9%), *Flammulina velutipes* (4.6%), and *Volvariella volvaceae* (7.9%), made up 87% of the total production. It can be further observed that by late 1994, of these six species only *Agaricus* and *Pleurotus* were cultivated worldwide to be joined in 1997 by *Lentinus*. The other three of the major six species are grown almost exclusively in Asia (Chang, 1999b).

World production of mushrooms over the last two decades has shown a phenomenal pattern of growth (Table 1), with a 5 times increase in tonnage. While the white button mushroom (*Agaricus bisporus*) still retains the highest overall world production, its relative contribution is decreasing due to the dramatic increase in the other species, viz: *Lentinus* and *Pleurotus* in particular. In 1981, *Agaricus* production represented 72% of world production but by 1997 this had dropped to 32%. Overall, world production of mushrooms is increasingly being dominated by species that are both edible and have medicinal properties.

Table 1 World Production of Cultivated Edible and Medicinal Mushrooms in Different Years (Chang, 1999b)

	1981		1986		1990		1994		1997	
	Fresh Wt X 10^3		Fresh Wt X 10^3		Fresh Wt X 10^3		Fresh Wt X 10^3		Fresh Wt X 10^3	
Species	**Metric tons**	**%**	**Metric tons**	**%**	**Metric tons**	**%**	**Metric tons**	**%**	**Metric tons**	**%**
Agaricus bisporus/ bitorquis	900.0	71.6	1,227.0	56.2	1,420.0	37.8	1,846.0	37.6	1,955.9	31.8
Lentinus edodes	180.0	14.3	314.0	14.4	393.0	10.4	826.2	16.8	1,564.4	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 velutipes	60.0	4.8	100.0	4.6	143.0	3.8	229.8	4.7	284.7	4.6
Tremella spp.	-	-	40.0	1.8	105.0	2.8	156.2	3.2	130.5	2.1
Hypisizygus spp.	-	-	-	-	22.6	0.6	54.8	1.1	74.2	1.2
Pholiota spp.	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	1,357.2	100.0	2,182.0	100.0	3,763.0	100.0	4,909.3	100.0	6,158.4	100.0
Increasing %			73.6		72.5		30.5		25.4	

It is pertinent to note that world production of mushrooms is now dominated by China with over 64% of total production. China has become a major producer and consumer of both edible and medicinal mushrooms. Furthermore, China is also the major producer of the non-edible medicinal mushrooms, e.g. *Wolfiporia (Poria) cocos* (10,000 tons) and *Ganoderma lucidum* (4,000 tons) (see Chapter 3). At least 10 new species of edible or medicinal mushrooms have been brought into cultivation in China in recent years and although as yet on a small scale, the potential, especially for mushrooms of medicinal value, is quite significant. Because of their historical background in the use of wild mushrooms, both as food and in Chinese traditional medicines, it is to be expected that China will continue to develop methods for cultivation of an increasing number of, as yet, uncultivatable mushrooms for medicinal exploitation. The traditional acceptance of mushrooms in herbal medicine and in expanding pharmaceutical industries, will ensure that China will continue to be a major exploiter of medicinal mushroom technology (Yamanake, 1997). China is also investing heavily in fermenter technology for growing mushroom mycelium of medicinal species.

Historically, mushrooms were gathered from the wild for consumption and for medicinal use. China has been the source of many early cultivations of mushrooms, e.g. *Auricularia auricula* (600 AD), *Flammulina velutipes* (800 AD), *Lentinus edodes* (1000AD) and *Tremella fuciformis* (1800). *Agaricus bisporus* was first cultivated in France in *c* 1600 while *Pleurotus ostreatus* was first grown in US in 1900. While mushroom cultivation now spans many centuries, it is only over the last 2-3 decades that there have been major expansions in basic research and practical knowledge leading to the creation of a major worldwide industry (Chang and Miles, 1989).

Table 2 World population of cultivated edible and medicinal mushrooms in 1997 (Metric tons) (Chang, 1999b)

	China	Japan	Rest of Asia	North America	Latin Amreica	EU	Rest of Europe	Africa	China	Total	%
Agaricus bisporus	330000	-	68400	425300	51600	875000	115200	36000	54400	1955000	31.8
Lentinus edodes	1397000	115300	47400	3600	300	500	300	-	-	1564400	25.4
Pleurotus spp.	760000	13300	88400	1500	200	6200	5800	200	-	875600	14.2
Auricularia spp.	48000	-	5300	-	-	-	-	-	-	485300	7.9
Volvariella volvacea	12000	-	60800	-	-	-	-	-	-	180800	3.0
Flammulina spp.	15000	10900	25700	-	-	-	-	-	-	284700	4.6
Tremella spp.	13000	-	500	-	-	-	-	-	-	130500	2.1
Hypsizygus marmoreus	2100	7200	100	-	-	-	-	-	-	74200	1.2
Pholioto nameko	3100	24500	-	-	-	-	-	-	-	55500	0.9
Grifola frondosa	2000	3100	-	-	-	-	-	-	-	33100	0.5
Hericium erinaceus	800	-	-	-	-	-	-	-	-		
Coprinus comatus	500	-	-	-	-	-	-	-	-	520800	8.4
Others	514900[a]	2900	800	400	-	200	100	-	200		
Total	3918300	368000	297400	430800	52100	881900	121400	36200	54600		
%	63.6	6.0	4.8	7.0	0.8	14.3	2.0	0.6	0.9	6160800	100

The cultivation of *Agaricus bisporus* is an outstanding example of a biotechnological enterprise that challenges the combined skills of industrial and biological technologies. *A. bisporus* cultivation in Western countries has achieved its current pre-eminence in the mushroom industries because of a solid foundation in basic scientific research in all aspects of *Agaricus* biology (genetics, physiology, biochemistry), bioprocess technology and, above all, the use of modern management principles (Chang *et al*., 1996). This foundation made possible a highly technical approach involving the creation and utilization of specialized equipment and advanced engineering technology. While *Agaricus bisporus* is a highly tasty and nutritious mushroom, it does not appear to have been used for any specific medical conditions. However, much fundamental knowledge has been acquired in recent years which will be of considerable value for other cultivations.

Mushroom cultivation technology

Mushrooms can be cultivated through a variety of methods. Some methods are extremely simple and demand little or no technical expertise. On the other hand, cultivations which require aspects of sterile handling technology are much more technically demanding (Chang and Miles, 1989). In the context of the present report, the simple and advanced methods for the cultivation of the Shiitake mushroom (*Lentinus edodes*) will be highlighted (Stamets, 1993, 2000; Stamets and Chilton (1983). This is a leading medicinal mushroom and, furthermore, many of the other important medicinal mushrooms are wood utilisers and have been easily grown by modifications of these methods. Detailed descriptions of the many growing techniques can be found in the Royce *et al*. (1985), Przbylowicz and Donoghue (1989) and Kozak and Krowczy (1999).

Mushroom cultivation involves several different operations each of which must be performed properly if the enterprise is to be successful. Failure of any phase will result in a decreased harvest or total loss (Table 3).

Table 3 Operations involved in mushroom cultivation

Selection of mushroom spores or strains
Maintenance of mycelial cultures
Development of spawn/inoculum
Preparation of growing medium
Spawn inoculation and colonisation of substrate
Crop management for mushroom production

Strain selection and maintenance:

The first stage in any mushroom cultivation process is to obtain a pure mycelial culture of the specific mushroom strain. Such cultures are now readily purchased from mushroom specialists, mushroom enterprises or mushroom institutes (Stamets, 1993). Such cultures have originally been derived from single or multi-spore cultures or by tissue culture from a mushroom of a high yielding and vigorous strain. Many strains have been developed by considerable genetic breeding programmes. Each type of mushroom culture generally requires unique substrate formulation for propagation and maintenance of purity. This information is normally freely available in the literature. Most growers will obtain spawn cultures from reputable production centres – ensuring purity, vigour and supply when required.

Spawn production:

For large scale production of mushrooms, large quantities of the specific inoculum are required (often 1-5% of the final mushroom production medium). In mushroom growing technology the inoculum is known as the “spawn”. Spawn is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain. Spawn production is a fermentation process in which the mushroom mycelium will be increased by growing through a solid organic matrix under controlled environmental conditions. In almost all cases the organic matrix will be sterilised grain, e.g. millet, rye or wheat. The purpose of the grain spawn is to boost the mycelium to a state of vigour such that it will rapidly colonise the selected bulk growing substrate. The grain is an important nutrient support as well as a vehicle for the eventual even distribution into the growing medium of the mushroom inoculant. Each individual grain becomes coated with the mycelium and in fact becomes a mycelial capsule (Fig. 1). All operations from pure culture isolation through spawn preparation must be conducted under sterile techniques and performed as rapidly as possible to lessen the possibility for contamination to occur.

An extensive technology has been developed throughout the world to ensure the production of high quality mushroom spawn. Many companies now specialize only in the production of mushroom spawn and can ship active spawn to growers when required. Most spawn is now prepared and shipped in autoclavable polypropylene bags with breathing patches. For natural log production of mushrooms the inoculum or spawn can also be in the form of wood chips coated with the specific mushroom strain.

Fig. 1 Colonisation of grain at 3 and 8 days after inoculation (Stamets and Chilton, 1983)

Mushroom production – Log Culture:

Most medicinally important mushroom species can grow as saprophytes on dead wood – primary decomposers. For this reason, *Lentinus edodes* will be used as the model system. Log culture for *Lentinus* or Shiitake was developed in Japan and China over 1000 years ago and is still widely used by small growers in Asia for sale in local markets. The advantage of log culture is that it is a simple and natural method but with the disadvantages that the process is labour-intensive and slow in comparison to growing mushrooms in sterilised sawdust mixtures. Log cultivation is not technically demanding and is relatively easy to carry out – but is seasonal and cannot meet demands for high productivity.

The logs (c 3' long x 6-8" diameter) are cut in winter or early spring from fast growing deciduous species, e.g. alder, poplar, oak, cottonwood which have thick outer bark. Logs can be inoculated with spores or with mycelial plugs inserted into drilled holes and then stacked in piles with mild to heavy soaking. Mycelial growth through the log will occur over several months and the logs are then placed in an upright position partly embedded in the soil. Mushroom production will then occur primarily in the cooler spring and autumn months. Since this is an open, non-sterile procedure contamination with other wood rotting mushroom species can also occur (Fig. 2). Increased environmental control can be achieved by having overhead protection or even growth in greenhouses.

This is still a traditional method especially in China employing thousands of people but has only been used to a limited extent in USA and Europe where it is not commercially worthwhile. However, it generally produces high quality mushrooms and can be an interesting amateur method for producing gourmet and medicinal mushrooms. While the original recognition of the medicinal properties of these mushrooms came from natural growing systems in the forests most production for edible and medicinal purposes is now derived from the artificial log method.

Mushroom production – enriched sawdust culture:

While many wood utilising gourmet and medicinal mushrooms have traditionally been cultivated on hardwood logs outdoors in the natural environment an alternative, more intensive and regulated cultivation technique has been developed in several Asian laboratories over the last 2-3 decades. The success of this new approach largely reflects the major increase in world production of wood utilising mushrooms (Table 1).

In this innovative approach various hardwood sawdust or wood chips supplemented with nitrogen-rich additives such as rice bran (though other cereal brans work adequately) are mixed together and then compacted into special autoclavable polypropylene bags of various dimensions (Stamets, 1993, 2000; Yamanake, 1997). The bags are then autoclaved to ensure complete internal

Fig. 2 ***Lentinus edodes*** **fruiting on oak logs (S**tamets and Chilton, 1983)

sterility, allowed to cool to c. 20°C and then aseptically inoculated with the desired amount of spawn. This stage demands complete sterile handling techniques and any relaxation of standards allows microbial contamination with concomitant financial losses. The inoculated bags – sometimes known as space bags or artificial logs, can then be moved to growing rooms with computer controlled environments giving accurate humidity and temperature conditions.

While formulations of the sawdust/supplement media are easily obtained from the literature, successful producers retain a strong element of secrecy with the exact composition of the supplements. Following inoculation the bags are stacked on trays or suspended from wires for several weeks during which time the mycelium grows through the sawdust mix secreting enzymes which degrade the complex macromolecules of the substrate – lignin, cellulose, hemicellulose – the breakdown products being absorbed by the advancing mycelium. When the mycelium has reached maturity the log is given a cold temperature shock for 12-24h, restacked and the bag opened and within a few days the mushrooms develop (Fig. 3).

Overal, this new method greatly shortens the production time and gives much higher yields. Using natural log cultivation the time from spawning to harvesting of mushrooms can be between 8 months to one year with complete exhaustion of the log up to 3 years. 100 kg natural log can produce c. 10-15 kg fresh mushrooms over this period. In contrast, with the synthetic log, mushrooms can be harvested about 80 days after spawning and completion of economic production from then takes less than 6-8 weeks. 100 kg sawdust plus supplement can produce c. 80 kg fresh weight of mushrooms, with even higher yields regularly possible. This represents at least an 8-10 fold greater production than the natural log method.

Fig. 3 Production of *Lentinus edodes* from artificial logs (Stamets, 2000)

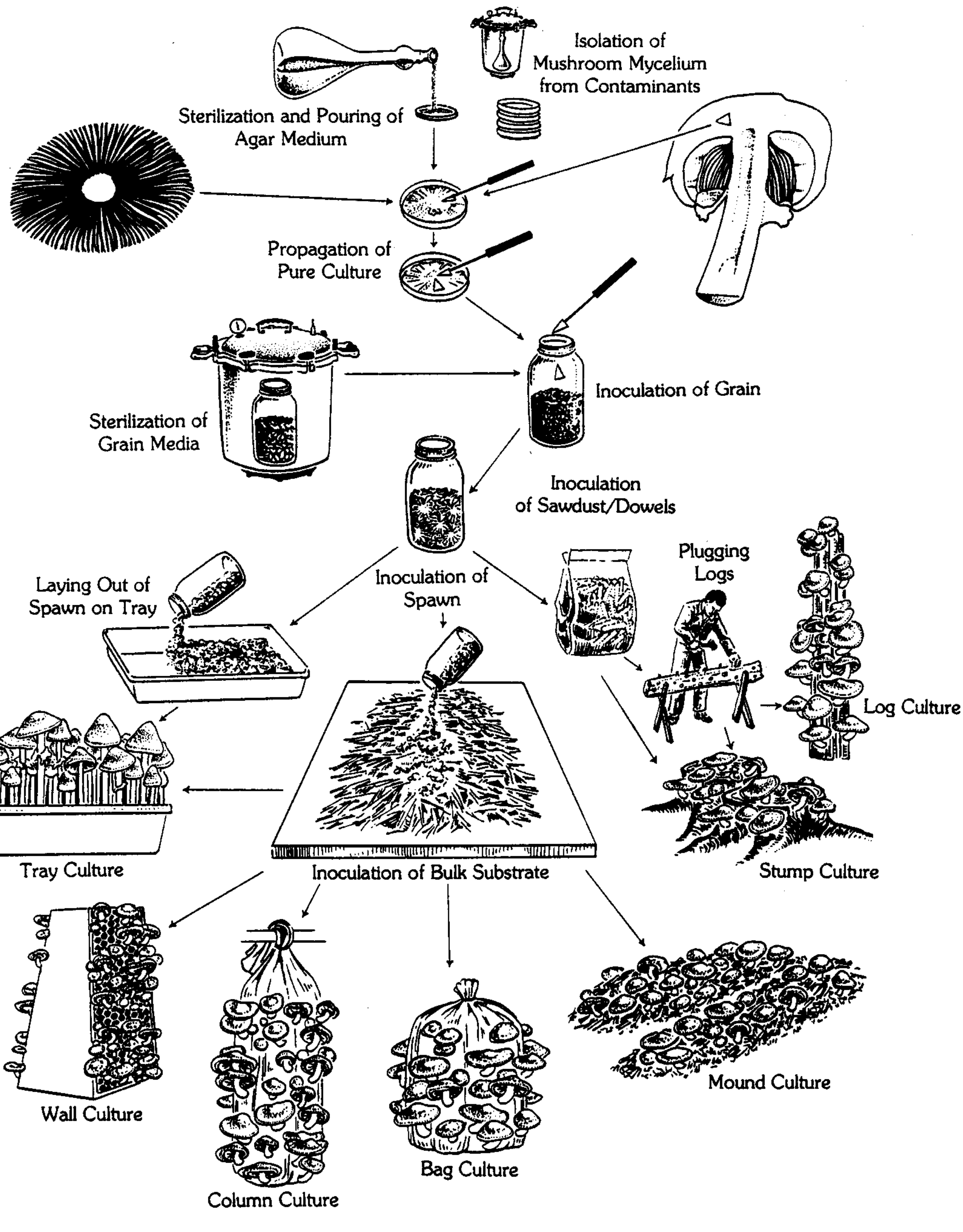
Crop management for production:

The whole process of mushroom production requires continued and careful attendance. Correct control of ambient temperature and humidity are highly critical together with a full understanding of potential microbiological contamination especially from other fungi. In many parts of the world pesticides will be employed – especially fungicides. However, the high premium paid for organic mushrooms necessitates that good management practices must be in operation to avoid the need for pesticides. This is especially important when concentrated powdered mushrooms are consumed as nutriceuticals.

At present most wood utilising gourmet and medicinal mushrooms are imported from Europe and the Far East especially China. In many cases, quality standards are dubious. However, a new production facility has now been established in Kent, which is now producing high quality fresh *Lentinus edodes* to the market and a wide range of other gourmet and medicinal mushrooms are planned.

At present the medicinal mushrooms are available worldwide as fresh mushrooms produced by either of the two main growing methods. Such mushrooms can be dried or extracted in various ways to obtain concentrated extracts of the potent and unique health enhancing medicinal products. Further purification has produced several pharmaceutical grade products now used for cancer therapy procedures in Japanese hospitals. An overview of the various techniques for growing mushrooms is shown in Fig. 4.

Fig. 4 Diagram illustrating overview of general techniques for the cultivation of mushrooms (Stamets and Chilton, 1983).



Mycelial production – liquid tank fermentation

In this approach the need for the mushroom fruitbody is bypassed with the mycelium of the medicinal mushroom being cultivated in deep-tank liquid fermentation culture. This is a relatively new approach and if the important medicinal compounds can be produced in this way it will lead to major innovations and product diversity. Furthermore, the ability to use pure substrates and controlled growth environments will aid in the final purity of the products. How essential the formation of the complex fruitbody is in the final determination of product type and variety has still to be shown. However, it has already been shown that the medicinally important polysaccharides can be produced in this way as seen with PSK and PSP from *Trametes versicolor*. The growth of filamentous fungal mycelium in fermenters is well understood especially in the antibiotic industry. However, Basidiomycetes do have slower growth rate and lower yields when compared with, for example, *Penicillium* and *Streptomyces*.

A further advantage of this approach would be the mycelial cultivation of medicinal mushroom species that so far have defied axenic culture, e.g. many mycorrhizal species. It is now clear that liquid fermentation methods are becoming an important means of producing more uniform mycelial biomass from several types of medicinal mushrooms for product extraction and purification. This will generate nutraceutical and pharmaceutical products from medicinal mushrooms that can achieve higher quality standards and safety (see Chapter 9 for full details).

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