Impact of Developmental, Physiological and Environmental Studies on the Commercial Cultivation of Mushrooms

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Introduction

Fungi are an ideal food because crude protein typically represents 20–30% of fungal dry matter and contains all of the amino acids which are essential to human and animal nutrition. Fungal biomass is digested to leave their chitinous walls as a source of dietary fibre. Fungi contain B vitamins and are characteristically low in fat. An extremely important attribute of all fungal food is that it is virtually free of cholesterol. Fungal protein foods can compete successfully with animal protein foods like meat on health grounds. Since fungal foods can be produced readily, in principle, using waste products as substrates, fungal foods should also be able to compete successfully on grounds of primary cost.

Agaricus bisporus (J.E. Lange) Imbach is by far the most commonly cultivated mushroom, accounting for over 70% of total global mushroom production in the mid-1970s (Moore and Chiu, 2001). Currently, it accounts for something closer to 30% even though production tonnage has more than doubled in the intervening years. A major change during the last quarter of the 20th century was the increasing interest shown in so-called 'exotic' mushrooms. Fresh *Lentinula* (*shiang-gu* in Chinese or *shiitake* in Japanese) and *Pleurotus* (oyster mushrooms) are routinely found alongside *Agaricus* in supermarkets around the world.

One reason for the remarkable increases seen in production of certain mushrooms has been the use of industrial waste products as substrates. Oyster mushrooms are easily grown on a variety of agro-industrial wastes, including cotton wastes. Similarly, while the paddy straw mushroom (*Volvariella volvacea*

 Tropical Mycology, Vol. 1, *Macromycetes* (eds R. Watling, J.C. Frankland, A.M. Ainsworth, S. Isaac and C.H. Robinson)

(Bull.: Fr.) Singer) is traditionally grown in South-east Asia on rice straw, it too can be grown on cotton waste. Indeed, cotton waste (generated by the textile and garment industries) gives higher yields and is also more widely available than is rice straw, so it is far cheaper (rice straw becomes expensive because of the cost of transport). Typically 80–90% of the total biomass of agricultural production is discarded as waste. This is an unacceptable loss of primary production when many mushrooms could be grown on agricultural residues, converting wastes into food, animal feed, pharmaceuticals and other products (Chang, 1998).

This chapter briefly illustrates the wide variety of recent research on the physiology, genetics and morphogenesis of fungi relevant to the commercial cultivation of mushrooms. Some of this research remains to be exploited.

Mushroom Cultivation can be a Profitable and Environmentally Friendly Process

Cultivation of an *Agaricus* crop depends on composted plant litter. Similar approaches have been developed for oyster and paddy straw mushrooms in the Far East, although in the Chinese tradition the most important mushroom crop (*Lentinula*) is cultivated on wood logs. Indeed, this traditional technique is still the most frequently used method in China over a growing region which covers a territory about equal to the entire land area of the European Union. Continuation of the traditional use of locally cut logs is likely to devastate the hill forests. For this and other reasons more industrial approaches are being applied to shiitake growing. Hardwood chips and sawdust packed into polythene bags as 'artificial logs' provide a highly productive alternative to natural logs, and the cultivation can be done in houses (which may only be plastic-covered enclosures) in which climate control allows year-round production.

Improved use of substrates depends on a better understanding of the growth physiology of the fungus. A particularly interesting series of experiments on protein utilization by basidiomycete fungi was carried out by Kalisz *et al.* (1986). Protein is probably the most abundant nitrogen source available to organisms in compost in the form of lignoprotein, microbial protein and plant protein. *A. bisporus* has been shown to be able to degrade dead bacteria and to utilize them as the sole source of carbon, nitrogen and phosphorus (Fermor and Wood, 1981), and all three species studied by Kalisz *et al.* (1986), *A. bisporus*, *Coprinus cinereus* (Schaeff.: Fr.) S.F. Gray and *V. volvacea*, were able to use protein as the sole source of carbon, nitrogen and sulphur. Furthermore, protein was utilized as efficiently as was glucose when provided as a sole source of carbon. Supplied together as carbon sources, both protein and glucose were utilized more rapidly, and growth was greater, than when either protein or glucose was supplied separately as a sole source of carbon. Extracellular proteinase of *Agaricus*, *Coprinus* and *Volvariella* was not subject to catabolite repression by glucose or ammonium regulation as described for the ascomycetes *Aspergillus* and *Neurospora*. Rather, basidiomycete proteinase activity is regulated primarily by induction, although it is subject to sulphur-, carbon- and nitrogen-catabolite derepression to different extents in the different organisms (Kalisz *et al.*, 1989). Another important point is that, *in vitro*, loss of substrate protein from the medium was detectable before extracellular proteinase activity was evident in the medium. Thus, proteinase activity is initially localized to the hyphal wall in basidiomycetes; the enzymes are released only when proteinaceous substrates approach exhaustion. These 'basidiomycete types' of regulation and localization are presumably adaptations to the plant litter habitat. Understanding them should help in the design of artificial composts and in the choice of compost supplements.

Despite the fact that the natural habitat of *Lentinula edodes* (Berk.) Pegler is the timber of felled trees, it can be grown effectively, both as mycelium and fruit bodies, in liquid media *in vitro*. Tan and Moore (1992) found that several commercial strains formed good mycelial growth in defined liquid medium in both stationary and shake flask cultures. Supplementation of flask cultures with vermiculite promoted growth and fruiting and the best fruiting *in vitro* was obtained by inoculation on to solid supports. This research demonstrated that liquid inoculum can be produced in quantity by homogenization with no adverse effect on inoculum potential, and it identified convenient media and growth conditions which permitted good yields of mycelial biomass over both long and short incubation periods.

Many agro-industrial wastes are of no apparent use, and are frequently difficult to handle and even more difficult to dispose of or treat in an environmentally acceptable way. They may even be harmful to the environment and potentially hazardous because some of them, such as olive oil waste waters and some cotton residues, are toxic to plants and animals. Disposal of such wastes in soil, water or into urban sewers is illegal in most countries and they can be major pollutants. Many commonly cultivated mushroom fungi, especially *Pleurotus* and *Lentinula* species, are white rot fungi showing high efficiency in lignocellulolytic degradation of a wide range of plant litter (Kerem *et al.*, 1992) which they selectively delignify (Moyson and Verachtert, 1991; Ortega *et al.*, 1992; Hadar *et al.*, 1993). Moreover, they grow well when conventional substrates are supplemented with solutions containing high amounts of phenols and/or tannins (Tomati *et al.*, 1991; Upadhyay and Hofrichter, 1993) and even oil in oil refinery waste waters (S.W. Chiu, Chinese University of Hong Kong, unpublished). Such fungi are able to bioconvert useless agricultural waste into the following:

1. Edible fruit bodies of high organoleptic properties and nutritive value (Chorvathova *et al.*, 1993; Zhang *et al.*, 1994; Bobek *et al.*, 1994). In terms of mushroom cultivation, *Pleurotus* species run second in annual world commercial production, representing the greatest increase (approximately fivefold) over recent years to meet market demands (Chang and Miles, 1991). The black oak mushroom, *L. edodes*, was first cultivated in China and is the most popular dried mushroom in Hong Kong, China and Japan. It holds the third place in the world production table (Chang, 1993). In addition to its unique flavour, a commercially available polysaccharide product extracted from this mushroom named lentinan is a host defence potentiator, improving immunological defence mechanisms against cancer and other diseases (Chihara, 1993). Thus, both mushrooms are important and established commercial products.

2. Good quality fodder. Enriching the waste with fungal protein through the growth of the mycelium improves digestibility by preferentially removing lignin and hemicellulose, leaving cellulose mostly intact as an energy source for ruminants (Martinez *et al.*, 1991; Tripathi and Yadar, 1992).

3. Soil conditioners/fertilizers that benefit overall soil fertility and stability and improve vegetable yield (Saiz-Jimenez and Gomez-Alarcon, 1986; Balis *et al.*, 1991; Flouri *et al.*, 1995).

These advantages have made disposal of an abundant waste coupled with production of a mushroom cash crop a popular model in recent years. *Pleurotus* spp. in particular grow readily on so many lignocellulose agricultural wastes that it becomes an attractive notion to use the fungus to digest the waste while producing crops of mushrooms. *Pleurotus* cultivation may even aid removal of pollutants from landfill or contaminated waste sites because the spent compost contains populations of microorganisms which, since they can digest the natural phenolic components of lignin can also break down chemicals such as polychlorinated phenols. Chiu *et al.* (1998a) demonstrated that the mushroom substrate left after harvesting oyster mushrooms is able to remove the biocide pentachlorophenol (PCP) more effectively than any fungal mycelium. PCP has been the most heavily used pesticide throughout the world, but it is very persistent in the natural environment and a common cause of soil contamination. Bacteria and fungi rapidly, and completely, digested PCP over a wide range of initial concentrations, whereas fungal mycelial incubations left a variety of breakdown products, some of which were also toxic. In many countries spent mushroom substrates are often discarded as wastes. Using them in landfill or contaminated sites would combine soil conditioning with degradation of organopollutants as a prospective bioremediation strategy.

Care must be exercised, however, because *Pleurotus*, like other mushrooms, accumulates metal ions in the basidiome. Wastes gathered from industrial sources for use in mushroom compost may be contaminated by heavy metals to an extent sufficient to render the crop unsuitable for consumption. For example, Chiu *et al.* (1998b) showed that cadmium could be accumulated in *Pleurotus* fruit bodies to such high levels that a single modest serving of mushrooms could cause the consumer to exceed the tolerable food limit recommended for a full week of intake of this metal.

Metabolism and Morphogenesis

In vitro studies of glucose catabolic pathways in *L. edodes* have used radiorespirometry and enzyme analyses (Tan and Moore, 1995). The ^{14}C-1/^{14}C-6 ratios in CO_2 respired from specifically labelled glucose fed to *L. edodes* tissues grown on a chemically defined medium ranged from 2.5 (vegetative mycelium) to 14.9 (young lamellae). This mirrored the relative activity of the pentose phosphate pathway (PPP), very high in the basidiome but low in the mycelium. The highest ratio was recorded in tissues which are biosynthetically most active (young lamellae), which require the reducing power of NADPH generated through the PPP. Extensive conversion of ^{14}C-3,4-labelled glucose to $^{14}CO_2$ in the mycelium underlined the important role of the Embden–Meyerhof–Parnas pathway (EMP) in that tissue. A ^{14}C-1/^{14}C-6 ratio of 14.8 for young lamellae grown on woodchips indicates that the growth medium did not influence the pathway used in the basidiome and showed that the *in vitro* data were truly representative. Ratios for the young pileipellis and stipe were 8.0 and 10.4 respectively. Tan and Moore (1995) used *Coprinus cinereus* as a comparison and found a ^{14}C-1/^{14}C-6 ratio of 3.6 in *C. cinereus* gills, confirming the comparatively lesser importance of the PPP in this organism as suggested by much earlier enzymological analyses (Moore and Ewaze, 1976). Activity of PPP enzymes (glucose 6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase) was three times as high in the basidiome compared with the mycelium, highest activity being in the young pileus (Tan and Moore, 1995). EMP enzymes (fructose 1,6-bisphosphate aldolase (ALD) and glucose 6-phosphate isomerase (PHI)) were more active in mycelium than in the basidiome, and also more active than PPP enzymes within the mycelium itself. Within the basidiome, specific activities of EMP and PPP enzymes were about the same. For comparison, enzymic activity in the mature pileus of *C. cinereus* resembled the pattern in the mycelium rather than that of the basidiome of *L. edodes*. EMP enzymes were very much more active than PPP in *C. cinereus*. *Pleurotus pulmonarius* (Fr.) Kummer (often misnamed *Pleurotus sajor–caju*, Chiu *et al.*, 1998b) also had the PPP as the predominant pathway in hexose metabolism of basidiome tissues (Chiu and Moore, 1999a). Specific activity of the EMP enzyme PHI was twofold higher than that of the PPP enzyme G6PDH in vegetative mycelium, but in basidiomes, specific activity of G6PDH was at least 12-fold greater than that of PHI (Chiu and Moore, 1999a).

A positive correlation between high PPP activity and accumulation of large amounts of mannitol in the basidiome is notable. Quantitative determination of mannitol, using gas–liquid chromatography showed that mycelia of *L. edodes* had a low level of mannitol (about 1% on a dry weight basis) compared with the basidiome stipe and pileus (20–30%). The highest level of mannitol was observed in the pileus of *A. bisporus* (close to 50%), while no mannitol was detectable in the pileus of *C. cinereus* (Tan and Moore, 1994). The contrasts between *C. cinereus* on the one hand and *L. edodes* and *A. bisporus* on the other, serve to emphasize the correlation between mannitol accumulation and high activity of the PPP. Mannitol possibly acts as a store of reducing power and osmoregulatory agent, controlling the influx of water necessary for pileus expansion, although it must be emphasized that basidiome growth in *L. edodes* and *Pleurotus pulmonarius* occurs by hyphal multiplication rather than by hyphal inflation evident in *Agaricus* (see discussion in Tan and Moore, 1994). If the PPP does affect regulation of water influx (through its influence on mannitol content) there could be commercial implications in researching ways to manipulate the PPP in the basidiome (and, consequently, its water content) since 90% of the fresh mushroom consists of water. For commercial production, a mushroom crop is harvested at a particular stage of development to suit the size and shape requirements of the market. It may be possible to manipulate water content of the crop which may shorten the time taken to reach the appropriate morphological stage.

A completely different strategy for linking metabolism to morphogenesis, using urea rather than mannitol as an osmotic metabolite, was evident from earlier work on the enzymology of basidiome development in *C. cinereus*. This emerged from the discovery that the NADP linked glutamate dehydrogenase (NADP-GDH) occurs at high activity in the basidiome pileus, but is absent from the stipe and parental mycelium (Stewart and Moore, 1974). Further work showed that there are several enzymes (glutamine synthetase, ornithine acetyl transferase and ornithine carbamyl transferase) that similarly increase in activity in the developing basidiome pileus. Interestingly, urease activity is virtually absent from the pileus (though constitutive in stipe and mycelia). The whole family of enzymes shows coordinated regulation (Ewaze *et al.*, 1978) and forms a pathway which leads to formation and accumulation of urea. Regulation *in vivo* depends on accumulation of acetyl CoA and absence of ammonia (Moore, 1981). NADP-linked GDH activity is high whether conditions demand amination or deamination, and kinetic analyses also supported the view that the NAD-GDH is equally able to aminate or deaminate (Al-Gharawi and Moore, 1977). Consequently NADP-GDH function is unrelated to general metabolism, but is specifically concerned with cell differentiation. Accumulation of urea has been demonstrated using isotopically labelled precursors (Ewaze *et al.*, 1978): the quantity of urea contained in the pileus increases more than four times (on a dry weight basis) as primordia mature to the stage of spore release. On the other hand, the concentration of urea (on a fresh weight basis) remained essentially unchanged during basidiome development; the specifically amplified enzyme activities drive the urea cycle to form metabolites (mainly urea) which promote the osmotic uptake of water which is needed for the cell expansion involved in basidiome maturation. Interestingly, in *P. pulmonarius* both the PPP and the urea cycle may generate osmoregulators. The PPP predominated in basidiome tissues and urea content remained high and unchanged as cap tissues developed, although it decreased to zero at maturity in the stipe (Chiu and Moore, 1999a).

Assessing the Predictability of Mushroom Morphogenesis

The mushroom basidiome (or its equivalent where the cultivated fungus is not a basidiomycete) *is* the crop. Most interest in morphogenesis has concentrated on *Coprinus* (Moore, 1998), although Umar and van Griensven (1995, 1997a, b, c, 1998, 1999) have taken up the challenge of *Agaricus*, and colleagues in Hong Kong and Mexico are now studying *Lentinula*, *Ganoderma* and *Pleurotus*. Unfortunately we are still woefully ignorant of the most basic structural and developmental details of basidiomycete fruit bodies although these are the highest expressions of cellular and tissue organization in the fungal kingdom. One of the most fundamental biological problems which remains unresolved is the way in which cell differentiation is coordinated in the formation of organized tissues. Burnett (1968, p. 144) identified hyphal growth, branching and aggregation as the three fundamental factors involved in fungal differentiation. Thirty years later we are still hypothesizing about hyphal growth and we know virtually nothing about branching and aggregation. Models have been proposed which account for the development of complex morphologies in animals and plants. Growth factors acting as activators and inhibitors are assumed to diffuse through the tissues regulating cell differentiation. Distribution of morphogens depends on adequate communications within the tissue; and this must extend over many cell diameters. Higher plants and animals are well provided with avenues for such communication, via cell processes, desmosomes, gap junctions, plasmodesmata and the like, but electron micrographs show considerable space between fungal cells of young mushroom tissues. Indeed, at about the time that the morphogenetic prepatterning must be taking place, hyphal cells are quite distant and there is no evidence for any lateral cytoplasmic contact between neighbours. However, there is evidence of lateral communication of signals, at least during hymenium differentiation in *Coprinus*. As noted, NADP-GDH occurs at high activity in the basidiome pileus, but is absent from the stipe and parental mycelium. This enzyme can be specifically detected in histological preparations of living tissues using a tetrazolium staining procedure (Elhiti *et al.*, 1979). Application of this technique has shown that an overall increase in enzyme activity in the pileus does not occur through a uniform increase in each constituent cell. Rather, at early stages a scattering of cells show high activity, and it is the proportion of cells showing the enzyme activity which increases as the tissues mature. These scattered cells first appear in narrow stripes across the gill and as the tissues mature the stripes become wider until eventually all the hymenial cells show activity of this enzyme (Elhiti *et al.*, 1979). Evidently, there is a channel for lateral communication, but the relationships between adjacent cells are known in only the vaguest way and evidence for the existence of chemical growth factors or hormones is confused and inconclusive (Novak Frazer, 1996).

Most mycologists will be aware of the description of tissue construction called hyphal analysis introduced by Corner (1932a, b, 1966; Redhead, 1987).

Hyphal analysis has been almost entirely descriptive, and its taxonomic importance is immense (Pegler, 1996). The only *quantitative* study has been done by Hammad *et al.* (1993a, b), who showed that enumerating cell types at different stages of development in the fruit bodies of *C. cinereus* was a powerful way of revealing how basidiome structure emerges during morphogenesis as a result of changes in hyphal type and distribution. Counting and measuring hyphal compartments in different regions of fruit bodies at different stages of development reveal the mechanical generation of the final form of the basidiome. The patterns revealed must be organized by signalling molecules, so these studies raise crucial questions about the nature of such signalling molecule(s), their transduction pathways and the responses they elicit. Attempts are under way to exploit further the numerical approach, coupling it to computer-aided image analysis with the aim of establishing mathematical models describing mushroom morphogenesis. The 'virtual mushroom' may sound rather fanciful, but it is likely to be of value to industry. Relatively simple measurements of how dimensions change during morphogenesis have been used to define the 'normal' mushroom for the *A. bisporus* crop (Flegg, 1996), and image analysis of shape, form and colour of *A. bisporus* can be related to crop development (van Loon *et al.*, 1995) in ways that contribute to defining control programmes for automated harvesting.

The Question of Biodiversity

Biodiversity is the natural resource which can be exploited for breeding new cultivars to satisfy the expanding and diversifying demands of consumers. *L. edodes* has been cultivated in China for over 800 years, but the cultivars used for commercial cultivation throughout China have been found to be genetically homogeneous (Chiu *et al.*, 1996). In addition, traditional production methods pose a number of environmental problems. The traditional log-pile cultivation method is still the one most frequently used. For this, locally felled logs of oak, chestnut, hornbeam, maple and other trees over 10 cm diameter (probably *c.* 20–30 years old) and 1.5–2 m long are normally cut in spring or autumn of each year. Up to 100,000 trees must be felled every year just to maintain current production levels. Traditional usage of natural wood logs has been pursued to the extent that as availability of mature trees has declined, attention has turned to younger trees and other tree species. This, combined with other demands for timber, has contributed to a loss of 87% of the native forests in China (Anonymous, 1997). China now faces the problem that the rate of deforestation is much greater than the rate of reforestation in the remaining 13% forest cover (Mackinnon *et al.*, 1996; Loh *et al.*, 1999). There are planting regulations and prohibitions on felling of young trees, but these are difficult to monitor and 'conservation awareness' is especially low among those living in remote mountainous regions.

Recently the authors have investigated the population biology of *L. edodes* by examining natural populations in several provinces of China (Chiu *et al.*, 1998c, 1999a,b). The research covers a geographical area which is *c.* 1700 km north to south and 700 km west to east, but includes detailed surveys down to individual logs, includes phenotypes varying from morphology and palatability to DNA sequences. If mushroom farming is to become a sustainable industry, causing minimal disturbance to the natural habitats, alternative resources to logs collected from hillside forests must be promoted. Also, since a reproductive cycle for *L. edodes* takes over 6 months using artificial logs, fast fruiters are desirable, and pathogen resistance is an advantage in mass production. To focus our analysis on a sustainable mushroom cultivation industry, isolates from the field were tested for tolerance to a common bacterial pathogen, and for fruiting ability in indoor cultivation on artificial logs made of sawdust wastes from furniture manufacture.

Although a very limited gene pool is exploited in the cultivated strains in China, enormous biodiversity occurs in the wild. Analysis of local populations reveals that *L. edodes* strains show strong somatic incompatibility reactions and individual territories can be small (a few hundred mm^3). The widespread nature of the species and absence of other means of dispersal indicated that basidiospores are the major, even only, method of natural distribution. This is why harvesting *after* initiation of basidiospore release places the natural gene pool under threat of contamination between cultivated and natural populations of *L. edodes*. Protection of the natural environment is still the best strategy for conserving the biodiversity of this important commercial resource, but collection of wild strains for preservation in a culture collection would directly conserve the wild germplasm. Making a gene bank readily accessible to the public and industry would also generate a commercial resource for exploitation in both cultivation and breeding programmes. It might also reduce the pressure caused by non-professional collecting in the wild. The authors' studies suggested that a move to indoor cultivation, less dependence on multispore spawns and exploitation of a wider range of natural genotypes would better safeguard both cultivated and natural populations of the fungus and avoid denuding hillsides of mature trees.

The concept of sustainable management is too novel for the mushroom farmers of a developing country. However, villages in China are accustomed to working cooperatively to establish a shared facility, and the advantages of indoor cultivation to the farmer (consistency of yield, much shorter production cycle, use of solid industrial/agricultural wastes in the substratum) can be readily appreciated. An essential step is to educate the public and introduce these ideas. Morphological and genetic plasticity is another aspect of biodiversity which is especially evident in *Volvariella* spp. Studies of *Volvariella bombycina* (Schaeff.: Fr.) Singer have revealed enormous phenotypic plasticity (Chiu *et al.*, 1989). A wide range of spontaneous basidiome peculiarities, which are not disease symptoms, are within the normal range of development. These can be amplified and encouraged by environmental stress, such as desiccation. In the more widely cultivated *V. volvacea* most commercial spawns are heterokaryotic although the organism is homothallic. Thus, a mycelium grown from a single (haploid) basidiospore is able to produce fruit bodies and this may be seen as a good means of selecting promising commercial genotypes. However, selfed haploids of *V. volvacea* continue to segregate genetically diverse progeny through several generations of selfing (Chiu and Moore, 1999b). The genetic mechanism is unknown, but it threatens the stability of selected cultivated strains and suggests, again, that multispore spawns may be too genetically diverse to be a reliable means of distributing specific strains. Thus, strain degeneration is a serious problem faced by the *V. volvacea* industry. Although the fungus is homothallic, isolates collected from widely separated localities were found to be genetically different (Chiu *et al.*, 1995). Strain degeneration in *L. edodes*, however, is a different natural phenonemon. It arises from the contamination of airborne spores and random mating events between spore germlings (monokaryons) and the resident dikaryon. The resultant somatic incompatibility and the changed genomes account for the decrease in crop yield in later flushes.

In contrast to the edible fungi described above, *Ganoderma* is unique as a mushroom which is cultivated for its medicinal value. Global production of *Ganoderma* in 1997 was about 4300 t (about 3000 t of which were grown in China (Moore and Chiu, 2001). Under the names *lingzhi* (in Chinese) or *reishi* (in Japanese), several *Ganoderma* spp. of the *Ganoderma lucidum* complex provide various commercial health drinks, powders, tablets, capsules and diet supplements. *Ganoderma* is highly regarded as a traditional herbal medicine, and its popularity in China has spread to other Asian countries, and also to the wider world. *Ganoderma* is cultivated by being inoculated into short segments of wooden logs which are then covered in soil in an enclosure (often a plastic-covered tunnel) which can be kept moist and warm. The fruit bodies then emerge in large numbers quite close together and the conditions encourage the fungus to form the desirable long-stemmed basidiome. Like *Volvariella*, *Ganoderma* also expresses considerable developmental and morphological plasticity, but in this case the fruit bodies are very polymorphic in the wild. The outcome is taxonomic confusion; over 70 species have been described, many being invalid because they are simply developmental or morphological variants of the type species. Collected fruit bodies are highly prized, but if the mycologist cannot identify them reliably, the confusion can be used to profit from exaggerated claims or fraudulent products. Detailed analysis, including DNA markers, will provide reliable means of identification of both fruit bodies and, more importantly, the processed products. The safety of *Ganoderma* products, many of which are sold for prophylactic use and long-term consumption, is another significant aspect being researched, including the ability of fungal fruit bodies to accumulate heavy metal ions. *Lingzhi*, unlike other commonly cultivated mushrooms, is a root rot pathogen and collections can be made in urban and rural environments, including roadsides. Thus, although the wild-collected *lingzhi* is popularly thought to have better medicinal properties, bioaccumulation of heavy metals from polluted environments by this fungus has been demonstrated and will definitely pose a hazard. Artificial cultivation instead of collection from the wild would be a better strategy. Public health concerns would then be similar to those affecting other cultivated crops and would be satisfied by random sampling and quality assessment to assure the safety of the food product in the market.

In traditional Chinese medicine, *Ganoderma* is known as 'the mushroom of immortality', which is claimed to alleviate or cure virtually all diseases. Current research is focused on purification and characterization of the bioactive components and determination of clinical value, especially putative anti-tumour and anti-ageing properties. Tests with mice showed *Ganoderma* extract to be only a modest dietary supplement. Evidence of genotoxic chromosomal breakage or cytotoxic effects by *Ganoderma* extract has not been found, nor protection against the toxic effects of the radiomimetic mutagen ethyl methanesulfonate. The true medical value of *Ganoderma* extract needs further investigation, though it is essentially safe for consumption (Chiu *et al.*, 2000).

Acknowledgements

The authors thank the UGC for earmarked grants supporting some of the projects dealing with life cycle strategy in *V. volvacea* and diversity of Chinese *L. edodes*. S.W.C. thanks the Chinese University of Hong Kong for providing direct grants which partially supported the research work on use of spent mushroom substrate in degrading pollutants, and carbon metabolism of *Pleurotus*. We are grateful to Miss May Cheng and Miss Annie Law for their contribution to the projects on spent mushroom substrate and heavy metal uptake by *Pleurotus*.

References

Al-Gharawi, A. and Moore, D. (1977) Factors affecting the amount and the activity of the glutamate dehydrogenases of *Coprinus cinereus*. *Biochimica et Biophysica Acta* 496, 95–102.

Anonymous (1997) *The Chinese Forests*. Forestry Department, Beijing.

Balis, D., Chatjipavlidas, J. and Flouri, F. (1991) An integrated system of treating liquid wastes from olive oil mills. In: Balis, C. (ed.) *Proceedings of the Symposium on Treatment of Olive Oil Mills Wastes*. Geotechnical Chamber of Greece and International Council of Olive Oil, Chania, Greece, pp. 66–74.

Bobek, P., Ozdin, L. and Kuniak, L. (1994) Mechanism of hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats – reduction of cholesterol absorption and increase of plasma cholesterol removal. *Zeitschrift für Ernährungswissenschaft* 33, 44–50.

Burnett, J.H. (1968) *Fundamentals of Mycology*. Edward Arnold, London.

Chang, S.T. (1993) Mushroom biology: the impact on mushroom production and mushroom products. In: Chang, S.T., Buswell, J.A. and Chiu, S.W. (eds) *Mushroom Biology and Mushroom Products*. The Chinese University Press, Hong Kong, pp. 3–20.

Chang, S.T. (1998) A global strategy for mushroom cultivation – a challenge of a 'non-green revolution'. In: Lu, M., Gao, K., Si, H.-F. and Chen, M.-J. (eds) *Proceedings of the '98 Nanjing International Symposium, Science and Cultivation of Mushrooms.* JSTC-ISMS, Nanjing, pp. 5–15.

Chang, S.T. and Miles, P.G. (1991) Recent trends in world production of cultivated mushrooms. *Mushroom Journal* 504, 15–18.

Chihara, G. (1993) Medical aspects of lentinan isolated from *Lentinus edodes* (Berk.) Sing. In: Chang, S.T., Buswell, J.A. and Chiu, S.W. (eds) *Mushroom Biology and Mushroom Products.* The Chinese University Press, Hong Kong, pp. 261–266.

Chiu, S.W. and Moore, D. (1999a) Carbon metabolism in the Oyster mushroom, *Pleurotus pulmonarius.* In: Sietsma, J.H. (ed.) *Abstracts, Seventh International Fungal Biology Conference.* University of Groningen, The Netherlands, p. 46.

Chiu, S.W. and Moore, D. (1999b) Segregation of genotypically diverse progeny from self-fertilized haploids of the Chinese straw mushroom, *Volvariella volvacea. Mycological Research* 103, 1335–1345.

Chiu, S.W., Moore, D. and Chang, S.T. (1989) Basidiome polymorphism in *Volvariella bombycina. Mycological Research* 92, 69–77.

Chiu, S.W., Chen, M.-J. and Chang, S.T. (1995) Differentiating homothallic *Volvariella* mushrooms by RFLPs and AP-PCR. *Mycological Research* 99, 333–336.

Chiu, S.W., Ma, A.M., Lin, F.C. and Moore, D. (1996) Genetic homogeneity of cultivated strains of shiitake (*Lentinula edodes*) used in China as revealed by the polymerase chain reaction. *Mycological Research* 100, 1393–1399.

Chiu, S.W., Ching, M.L., Fong, K.L. and Moore, D. (1998a) Spent Oyster mushroom substrate performs better than many mushroom mycelia in removing the biocide pentachlorophenol. *Mycological Research* 102, 1553–1562.

Chiu, S.W., Chan, Y.H., Law, S.C., Cheung, K.T. and Moore, D. (1998b) Cadmium and manganese in contrast to calcium reduce yield and nutritional value of the edible mushroom *Pleurotus pulmonarius. Mycological Research* 102, 449–457.

Chiu, S.W., Yip, M.L., Leung, T.M., Wang, Z.W., Lin, F.C. and Moore, D. (1998c) Genetic diversity in a natural population of shiitake (*Lentinula edodes*) in China. In: *Abstracts of the Sixth International Mycological Congress, Jerusalem, Israel, 23–28 August.* IMC, Jerusalem.

Chiu, S.W., Wang, Z.M., Chiu, W.T., Lin, F.C. and Moore, D. (1999a) An integrated study of individualism in *Lentinula edodes* in nature and its implication for cultivation strategy. *Mycological Research* 103, 651–660.

Chiu, S.W., Yip, P. and Moore, D. (1999b) Genetic diversity of *Lentinula edodes* collected from Hubei Province, China. In: Sietsma, J.H. (ed.) *Abstracts, Seventh International Fungal Biology Conference.* University of Groningen, The Netherlands, p. 47.

Chiu, S.W., Wang, Z.M., Leung, T.M. and Moore, D. (2000) Nutritional value of *Ganoderma* extract and assessment of its genotoxicity and antigenotoxicity using comet assays of mouse lymphocytes. *Food and Chemical Toxicology* 38, 173–178.

Chorvathova, V., Bobek, P., Ginter, E. and Klvanova, J. (1993) Effect of the oyster fungus on glycemia and cholesterolemia in rats with insulin-dependent diabetes. *Physiological Research* 42, 175–179.

Corner, E.J.H. (1932a) A *Fomes* with two systems of hyphae. *Transactions of the British Mycological Society* 17, 51–81.

Corner, E.J.H. (1932b) The fruit-body of *Polystictus xanthopus* Fr. *Annals of Botany* 46, 71–111.

Corner, E.J.H. (1966) A monograph of the cantharelloid fungi. *Annals of Botany Memoirs* 2, 1–255.

Elhiti, M.M.Y., Butler, R.D. and Moore, D. (1979) Cytochemical localization of glutamate dehydrogenase during carpophore development in *Coprinus cinereus*. *New Phytologist*, 82, 153–157.

Ewaze, J.O., Moore, D. and Stewart, G.R. (1978) Co-ordinate regulation of enzymes involved in ornithine metabolism and its relation to sporophore morphogenesis in *Coprinus cinereus*. *Journal of General Microbiology* 107, 343–357.

Fermor, T.R. and Wood, D.A. (1981) Degradation of bacteria by *Agaricus bisporus* and other fungi. *Journal of General Microbiology* 126, 377–387.

Flegg, P.B. (1996) The shape and form of mushrooms. *Mushroom Journal* 560, 12–13.

Flouri, F., Soterchos, D., Ioannidou, S. and Balis, C. (1995) Decolourization of olive oil liquid wastes by chemical and biological means. In: *Proceedings of the International Symposium on Olive Oil Processes and By-Products Recycling, Granada, Spain.*

Hadar, Y., Kerem, Z. and Gorodecki, B. (1993) Biodegradation of lignocellulosic agricultural wastes by *Pleurotus ostreatus*. *Journal of Biotechnology* 30, 133–139.

Hammad, F., Ji, J., Watling, R. and Moore, D. (1993a) Cell population dynamics in *Coprinus cinereus*: co-ordination of cell inflation throughout the maturing fruit body. *Mycological Research* 97, 269–274.

Hammad, F., Watling, R. and Moore, D. (1993b) Cell population dynamics in *Coprinus cinereus*: narrow and inflated hyphae in the basidiome stipe. *Mycological Research* 97, 275–282.

Kalisz, H.M., Moore, D. and Wood, D.A. (1986) Protein utilisation by basidiomycete fungi. *Transactions of the British Mycological Society* 86, 519–525.

Kalisz, H.M., Wood, D.A. and Moore, D. (1989) Some characteristics of extracellular proteinases from *Coprinus cinereus*. *Mycological Research* 92, 278–285.

Kerem, Z., Friesem, D. and Hadar, Y. (1992) Lignocellulose degradation during solid state fermentation – *Pleurotus ostreatus* versus *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology* 58, 1121–1127.

Loh, J., Randers, J., MacGillivray, A., Kapos, V., Jenkins, M., Groombridge, B., Cox, N. and Warren, B. (1999) *The Living Planet Report*. WWF International, Gland, Switzerland.

Mackinnon, J., Sha, M., Cheung, C., Carey, G., Zhu, X. and Melville, D. (1996) *A Biodiversity Review of China*. WWF International, Hong Kong.

Martinez, A.T., Gonzalez, A.E., Valmaseda, M., Dale, B.E., Lambregts, M.J. and Haw, J.F. (1991) Solid-state NMR studies of lignin and plant polysaccharides degradation by fungi. *Holzforschung* 45, 49–54.

Moore, D. (1981) Evidence that the NADP-linked glutamate dehydrogenase of *Coprinus cinereus* is regulated by acetyl-CoA and ammonium levels. *Biochimica et Biophysica Acta* 661, 247–254.

Moore, D. (1998) *Fungal Morphogenesis*. Cambridge University Press, New York.

Moore, D. and Chiu, S.W. (2001) Fungal products as food. In: Pointing, S.B. and Hyde, K.D. (eds) *Bio-Exploitation of Filamentous Fungi*. Fungi Diversity Press, Hong Kong, pp. 237–265.

Moore, D. and Ewaze, J.O. (1976) Activities of some enzymes involved in the metabolism of carbohydrate during sporophore development in *Coprinus cinereus*. *Journal of General Microbiology* 97, 313–322.

Moyson, E. and Verachtert, H. (1991) Growth of higher fungi on wheat straw and their impact on the digestibility of the substrate. *Applied Microbiology and Biotechnology* 36, 421–424.

Novak Frazer, L. (1996) Control of growth and patterning in the fungal fruiting structure. A case for the involvement of hormones. In: Chiu, S.W. and Moore, D. (eds) *Patterns in Fungal Development.* Cambridge University Press, Cambridge, pp. 156–181.

Ortega, G.M., Martinez, E.O., Betancourt, D., Gonzalez, A.E. and Otero, M.A. (1992) Bioconversion of sugarcane crop residues with white-rot fungi *Pleurotus* spp. *World Journal of Microbiology and Biotechnology* 8, 402–405.

Pegler, D.N. (1996) Hyphal analysis of basidiomata. *Mycological Research* 100, 129–142.

Redhead, S.A. (1987) The Xerulaceae (Basidiomycetes), a family with sarcodimitic tissues. *Canadian Journal of Botany* 65, 1551–1562.

Saiz-Jimenez, C. and Gomez-Alarcon, G. (1986) Effects of vegetation water on fungal microflora. *Actas International Symposium on Olive By-Products Valorization.* Food and Agricultural Organization of the United Nations (FAO), United Nations Development Programme, Sevilla, Spain, pp. 61–76.

Stewart, G.R. and Moore, D. (1974) The activities of glutamate dehydrogenases during mycelial growth and sporophore development in *Coprinus lagopus* (*sensu* Lewis). *Journal of General Microbiology* 83, 73–81.

Tan, Y.-H. and Moore, D. (1992) Convenient and effective methods for *in vitro* cultivation of mycelium and fruiting bodies of *Lentinus edodes. Mycological Research* 96, 1077–1084.

Tan, Y.-H. and Moore, D. (1994) High concentrations of mannitol in the shiitake mushroom (*Lentinula edodes*) *Microbios* 79, 31–35.

Tan, Y.-H. and Moore, D. (1995) Glucose catabolic pathways in *Lentinula edodes* determined with radiorespirometry and enzymic analysis. *Mycological Research* 99, 859–866.

Tomati, U., Galli, E., Dilena, G. and Buffone, R. (1991) Induction of laccase in *Pleurotus ostreatus* mycelium grown in olive oil waste waters. *Agrochimica* 35, 275–279.

Tripathi, J.P. and Yadar, J.S. (1992) Optimisation of solid substrate fermentation of wheat straw into animal feed by *Pleurotus ostreatus* – a pilot effort. *Animal Feed Science and Technology* 37, 59–72.

Umar, M.H. and van Griensven, L.J.L.D. (1995) Morphology of *Agaricus bisporus* in health and disease. In: Elliott, T.J. (ed.) *Science and Cultivation of Edible Fungi.* A.A. Balkema, Rotterdam/Brookfield, The Netherlands, pp. 563–570.

Umar, M.H. and van Griensven, L.J.L.D. (1997a) Morphogenetic cell death in developing primordia of *Agaricus bisporus. Mycologia* 89, 274–277.

Umar, M.H. and van Griensven, L.J.L.D. (1997b) Hyphal regeneration and histogenesis in *Agaricus bisporus. Mycological Research* 101, 1025–1032.

Umar, M.H. and van Griensven, L.J. L.D. (1997c) Morphological studies on the life span, developmental stages, senescence and death of fruit bodies of *Agaricus bisporus. Mycological Research* 101, 1409–1422.

Umar, M.H. and van Griensven, L.J.L.D. (1998) The role of morphogenetic cell death in the histogenesis of the mycelial cord of *Agaricus bisporus* and in the development of macrofungi. *Mycological Research* 102, 719–735.

Umar, M.H. and van Griensven, L.J.L.D. (1999) Studies on the morphogenesis of *Agaricus bisporus*: the dilemma of normal versus abnormal fruit body development. *Mycological Research* 103, 1235–1244.

Upadhyay, R.C. and Hofrichter, M. (1993) Effect of phenol on the mycelial growth and fructification in some basidiomycetous fungi. *Journal of Basic Microbiology* 33, 343–347.

Van Loon, P.C.C., Sonnenberg, A.S.M., Swinkels, H.A.T.I., van Griensven, L.J.L.D. and van der Heijden, G.W.A.M. (1995) Objective measurement of developmental stage of white button mushrooms (*Agaricus bisporus*). In: Elliott, T.J. (ed.) *Science and Cultivation of Edible Fungi.* Balkema, Rotterdam, pp. 703–708.

Zhang, J., Wang, G.Y., Li, H., Zhuang, C., Mizuno, T., Ito, H., Suzuki, C., Okamoto, H. and Li, J.X. (1994) Antitumor polysaccharides from a Chinese mushroom, Yuhuangmo, the fruiting body of *Pleurotus citrinopileatus. Bioscience, Biotechnology and Biochemistry* 58, 1195–1201.

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Volume 1, Macromycetes

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