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MINI-REVIEW

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Fruiting body production in basidiomycetes

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Abstract Mushroom cultivation presents an economically important biotechnological industry that has markedly expanded all over the world in the past few decades. Mushrooms serve as delicacies for human consumption and as nutriceuticals, as "food that also cures". Mushrooms, the fruiting bodies of basidiomycetous fungi, contain substances of various kinds that are highly valued as medicines, flavourings and perfumes. Nevertheless, the biological potential of mushrooms is probably far from exploited. A major problem up to now is that only a few species can be induced to fruit in culture. Our current knowledge on the biological processes of fruiting body initiation and development is limited and arises mostly from studies of selected model organisms that are accessible to molecular genetics. A better understanding of the developmental processes underlying fruiting in these model organisms is expected to help mushroom cultivation of other basidiomycetes in the future.

The biotechnological importance of mushroom production

History of mushroom production

World-wide, commercial mushroom (fruiting body) production comprises about 5×10^6 tonnes fresh weight year^{-1}, although at present only a few basidiomycetes (*Agaricus*, *Lentinus*, *Pleurotus*, *Auricularia*, *Volvariella*, *Flammulina*, *Tremella* and a few others) can be cultivated (Table 1). Mushroom growing has a long tradition in Eastern Asian countries, especially in China where it started around 600 A.D. with *Auricularia auricula*, the Wood Ear. In Europe, cultivation of *Agaricus bisporus*, the Button Mushroom, was first achieved in France during the seventeenth century. Nevertheless, the world production of cultivated mushrooms expanded by magnitudes over just the past few decades, following strain and technical improvements, coupled with better acceptance of mushrooms as a foodstuff and a spreading of commercial mushroom production over all continents (Table 2; Chang 1993; Royse 1997). Along with the increase in the gross mushroom production, the species used for mushroom cultivation diversified in a manner influenced by the traditional preferences of the producing countries (Table 1; Chang 1996; Yamanaka 1997). In 1994, the value of the world mushroom crop was estimated to be worth U.S. $9.8 billion; a further U.S. $3.6 billion was probably raised from mushroom products (Chang 1996).

There are about 10,000 known mushroom species (fleshy macrofungi, including some ascomycete species). Half of them in principle are edible and many are ectomycorrhizal, i.e. associated with tree roots (Chang 1993). Precious fruiting bodies of mycorrhizal fungi such as *Cantharellus* species (Chanterelles) and *Boletus edulis* (King Bolete), favourites in Western cultures, and *Tricholoma matsutake* (Matsutake), a favourite in Japan, still have to be collected from the wild with variable and unpredictable success from year to year (Redhead et al. 1997; Yamanaka 1997). According to interviews with local and professional mushroom pickers and brockers, the total 1992 harvest of Chanterelles in Washington, Oregon and Idaho was estimated at 500 tonnes, that of *T. magnivelare* (Pine Mushroom, a close relative of Matsutake) 380 tonnes and that of Boletes 222 tonnes, comprising a value of U.S. $14 million (Schlosser and Blatner 1995). Following the recorded exports to Japan, the 1994 harvest of *T. magnivelare* in Canada was at least 500 tonnes, corresponding to a value of U.S. $18.5 million (de Geus and Besch 1997). Due to the high prices of these fungi, efforts to increase the fruiting body yield in the natural habitats are worth undertaking. Covering the plots of growing mycelium by plastic vessels, keeping the tem-

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Table 1 Production of various mushrooms (in tonnes) in different countries in 1994. Data are estimates from various sources (Courvoisier 1994; Schlosser and Blattner 1995; Chang 1996; de Geus and Besch 1997; Yamanaka 1997)

Genus	France	Germany	UK	Italy	Netherlands	Spain	USA	China	Japan	Indonesia	Thailand	World
Agaricus	198,500	58,000	94,000	102,000	210,000	68,000	370,000	359,000		28,000	1,300	1,846,000
Auricularia								385,000	100	200	6,000	420,100
Flamminula								109,000	101,806			229,800
Grifolia									14,103			14,200
Hypsizygus									54,436			54,800
Lentinus	320	500	70	1,500	150	100	2,500	632,000	141,300		300	826,200
Pholiota								4,300	22,638			27,000
Pleurotus	2,000	1,000	150	10,000	450	2,100	900	654,000	20,441	1,000	15,000	797,400
Tremella								156,000				156,200
Tricholoma							≥380		120			≥1,000
Volvariella								115,000		89,000	65,000	298,800

perature within the vessels around 20 °C and watering the plots prolonged the harvesting season up to 30 days and doubled the yield of Matsutake (Redhead 1997). Transplanting studies on the mycelium of *C. lutescens* in Switzerland showed that it is possible to inoculate forest grounds with a desired fungus and obtain fruiting bodies the following year (Ayer and Egli 1997). A recent report of a single *C. cibarius* fruiting body obtained in culture (Danell and Camacho 1997) and the occasional success in producing a fruiting body of *T. matsutake* under artificial conditions (Iwase 1997) raise hopes that mushroom cultivation of ectomycorrhizal species might also be improved in the future to a commercial scale.

Human use of mushrooms and mushroom products

Saprophytic species are cultivated for mushroom production on lignin and cellulose-containing substrates such as wood logs, saw dust, straw and cotton waste (Rajarathnam et al. 1993; Scrase and Elliott 1998), but many other agricultural and industrial wastes can easily be transformed into valuable foodstuff (Poppe and Hofte 1995). The efficiency of fungi in converting substrate to protein is far superior to that of several plants and even animals. Mushrooms are low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals. These nutritional properties make mushrooms a very good dietary food

Table 2 World mushroom production according to the Food and Agriculture Organization of the United Nations http://apps.fao.org/default.htm. Data are mainly for *Agaricus* but may also include *Boletus edulis* and the ascomycetes *Morchella* spp and *Tuber magnatum*. – No production documented

Area	Mushroom production (tonnes year^{-1})				
	1961	1970	1980	1990	1998
Europe	97,120	216,650	467,030	867,750	958,070
Belgium–Luxembourg	–	–	13,600	19,550	30,000
Denmark	4,000	6,090	7,920	8,100	8,690
France	32,340	69,570	152,220	195,700	151,560
Germany	10,530	30,870	47,370	50,200	60,000
UK	20,000	44,910	61,300	123,140	109,500
Ireland	5,000	5,000	6,000	37,000	62,000
Italy	6,460	4,640	41,500	79,380	67,000
The Netherlands	4,000	30,000	60,000	147,000	240,000
Poland	5,090	5,710	26,000	104,000	100,000
Spain	3,000	6,070	33,310	74,480	72,000
South-east Europe[a]	700	1,500	4,500	14,000	30,560
Asia	139,600	266,020	382,900	488,500	733,680
China	102,780	209,020	271,160	363,400	562,160
India	500	1,000	1,300	2,000	35,000
Japan	35,000	48,000	79,900	79,100	75,000
Korea (combined)	100	6,600	25,570	15,280	14,180
South-east Asia[b]	1,100	1,190	4,660	24,900	37,000
North America	57,800	105,560	242,460	376,540	434,500
Canada	7,800	11,760	29,260	52,240	64,500
USA	50,000	93,800	213,200	324,310	370,000
Oceania	300	870	10,730	26,390	47,400
Australia	–	–	8,260	24,390	39,000
Africa	300	500	600	7,720	9,790
South Africa	–	–	–	5,720	6,500
World	295,130	589,600	1,103,730	1,766,920	2,183,450

[a] Bulgaria, Hungary and Romania
[b] Indonesia, Thailand and Vietnam

(Buswell and Chang 1993; Rajarathnam et al. 1993). In addition, consumption of mushrooms has positive effects on the general human health because of a number of special substances, referred to as nutriceuticals (Chang and Buswell 1996). Numerous kinds of mushrooms are collected from the wild for various medical purposes and for use as hallucinogens and psychostimulants; and they have been an element in ethnomedicine throughout living memory (Subramanian 1995; Borchers et al. 1999). Medicinal mushrooms can modulate the immune system, lower blood pressure, blood glucose and lipid concentration and can inhibit tumours, inflammation and microbial growth. Modern science identified and is identifying medically active components of various natures in mushrooms (Jong and Donovick 1989; Table 3) and others are likely to be detected. Currently, polysaccharides such as lentinan and schizophyllan with anti-tumour properties are applied in modern medicine (Chang and Buswell 1996; Waser and Weis 1997). When, as is often the case, a substance of medical interest is specific to the fruiting body stage of the fungus and is not present in the vegetative mycelium, ways of producing mushrooms in culture generates interest. Moreover, mushrooms have a great potential for the industrial production of natural flavours and odours which are often specific to or produced in increased amounts by the fungus at the fruiting stage (Jong and Birmingham 1993a). Although not as important as their contribution to human nutrition and health, the use of mushrooms as ornamen-

Table 3 Examples of compounds isolated from fruiting bodies of basidiomycetes and their (potential) use in medicine

Species	Product	Applications (potential)	Reference(s)
Agaricus nebularis	Nebularine (purine nucleosides)	Antitumour	Jong and Donovick 1989
Boletus edulis	Polypeptide	Antitumour	Jong and Donovick 1989
Clitocybe illudens	Illudins M and S (sequiterpenes)	Antitumour	Jong and Donovick 1989
Coprinus atramentarius	Coprine	Inhibitor of acetaldehyde dehydrogenase	Michelot 1992
C. cinereus	Galectins	Cell adhesion	Cooper et al. 1997
Flammulina velutipes	Flammulin (polyene)	Antitumour	Jong and Donovick 1989
	Lectin	Haemagglutination	Yatohgo et al. 1988
Ganoderma lucidum	Alkaloids (ergosterol)	Cardiotonic	Jong and Birmingham 1993b
	Ganoderans A, B, C (peptidoglycans)	Blood glucose reduction	
	Ganoderic acids	Reduction of cholesterol, antihepatotoxic	
	Heteroglucans	Antitumour, antiinflammatory	
	Lectin	Haemagglutination	Kawagishi et al. 1997
G. tsugae	Steroid (2β, 3α, 9α-trihydroxy-5α-ergosta-7,22-diene)	Cell cycle inhibition	Gan et al. 1998
	Lanostanoid ester glucoside	Inducer of apoptosis	
Grifolia confluens	Grifolin (2-trans,trans-farnesyl-5-methylresorcinol)	Antibiotic activity	Jong and Birmingham 1990
G. frondosa	Acidic glucans	Antitumour	Jong and Birmingham 1990
	Grifolan (neutral D-glucan)	Antitumour	
	Lipids, peptidoglucan	Antidiabetic	Kubo et al. 1994
Hericium erinaceum	Hericenones C, D and E	Stimulator of nerve growth factor synthesis	Kawagishi et al. 1991
Lentinula edodes	Cortinellin	Antifungal	Jong and Donovick 1989
	Eritadenine	Inhibitor of HMG CoA reductase, lowering of blood pressure, reduction of cholesterol, antithrombotic activities	Waser and Weiss 1997
	Lentinan (polysaccharide)	Antitumour, stimulation of immune systeme, increase of resistance to AIDS, antiviral, antibacterial and antifungal, antidiabetic	
	Thioproline	Antitumour	Kurashima et al. 1990
Lenzites saepiaria	Lenzitin	Antifungal	Jong and Donovick 1989
Oudemansiella mucida	Antibiotic OM-1	Antifungal	Jong and Donovick 1989
Phlebia radiata	Merulinic acids A-C	Antitumour	Jong and Donovick 1989
Pleurotus citrinopileatus	(1–3)-β-D-Glucans	Antitumour	Zhang et al. 1994
P. cornucopiae	Lectin	Haemagglutination	Oguri et al. 1996
P. ostreatus	Lovastin	Inhibitor of HMG CoA reductase	Gunde-Cimerman and Cimerman 1995
Poria cocos	Pachymaran, pachyman, pachymic and tumulosic acids	Antitumour	Jong and Donovick 1989
Schizophyllum commune	Schizophyllan (polysaccharide)	Antitumour	Jong and Donovick 1989
	Scleroglucan	Antitumour	
Sclerotium glucanicum	Scleroglucan	Antiviral	Marchetti et al. 1996
Tremella aurantia	Acidic polysaccharide	Antidiabetic	Kiho et al. 1995
T. fuciformis	Acidic heteroglycans	Antitumour, stimulation of cytokines	Gao et al. 1997
Volvariella volvaceae	Lectins	Immunomodulating actions, antitumour	She et al. 1998

tals in floristic arrangements is another commercial reason for mushroom cultivation (Poppe and Heungens 1991; Chen and Miles 1996).

Biological analysis of mushroom production

A major problem in mushroom cultivation is the poor understanding of the cellular processes and the genetic, physiological and environmental controls that lead to the initiation of fruiting body development. For most basidiomycetes, only limited attention has so far been given to these processes; and/or suitable tools for analysis were missing (De Groot et al. 1998; Stoop and Mooibroek 1999; Umar and Van Griensven 1999). Although not of great edible value, *Coprinus cinereus* (Ink Cap) and *Schizophyllum commune* (Split-Gill) serve as model organisms for the study of developmental processes in the basidiomycetes (Wessels 1993; Moore 1998a; Kües 2000). These fungi have a relatively short life cycle and can easily be manipulated in the laboratory, unlike most other basidiomycetes. Genetic transformation and methods to introduce and screen for mutations are well established (Walser et al. 2000). However, molecular and genetic techniques for *Agaricus* and other saprophytic basidiomycetes are slowly improving (Stoop and Mooibroek 1999).

Cytological events

Mushrooms, the fruiting bodies of basidiomycetes, are produced (for spore production) as specific sexual organs on undifferentiated mycelium generated by vegetative growth. In order to produce a mushroom, hyphal knots (primary nodules) are first formed on the vegetative mycelium by localized intense hyphal branching. In the simplest case, a single hypha locally gives rise to numerous branches with short hyphal compartments. More commonly, hyphal knots originate as bunches of short branches from more than one generative hypha. Branches in the hyphal knots often have a globose and inflated cellular morphology and are embedded in mucilaginous material. Gradually, the branches aggregate with each other to form a fluffy spherical mycelial association of about 1–2 mm (secondary nodules, or initials). Within these small bodies, cell differentiation occurs and establishes the bipolar fruiting body primordia that basically contain all the different tissues present in the mature fruiting body (Fig. 1; Angeli-Papa and Eyme 1978; Van der Valk and Marchant 1978; Williams et al. 1985; Clémençon 1997; Kües 2000). In the typical agaric, a cluster of parallel-orientated hyphae will emerge, forming upon maturation the mushroom stipe above a plectenchymal tissue at the primordial base. The young mushroom cap (pileus) develops from the apical prosenchymal tissue of the initial (pre-pileus) region. The upper part of the pre-pileus region is the origin of the pileus trama; and the lower part forms a ring of glycogen-rich cells that defines the boundary of hymenium (the spore-bearing cell layer) and stipe (Reijnders 1979; Moore 1998a). The young hymenial tissue differentiates into dome-shaped rudiments which, in gill mushrooms such as *Agaricus* and *Coprinus*, eventually become the primary gills (lamellae) that carry the hymenial cell layer on their surface. Primary gill formation proceeds from the edge closest to the stipe towards the cap and from the lower margin of the cap to the apex of the cap (Moore 1998a; Fig. 1). It is accompanied with programmed cell death that helps to establish the hymenial chamber within the primordia (Lu 1991; Umar and Van Griensven 1998). With increasing primordia size, due to growth at the meristemoid tissue at the outer edge of the cap, space for further gills becomes available within the cap. Thus, secondary gills may be generated by splitting the trama of the primary gills in a radially outward fashion. In contrast to primary gills, secondary gills are not connected to the stipe (Clémençon 1997; Moore 1998a).

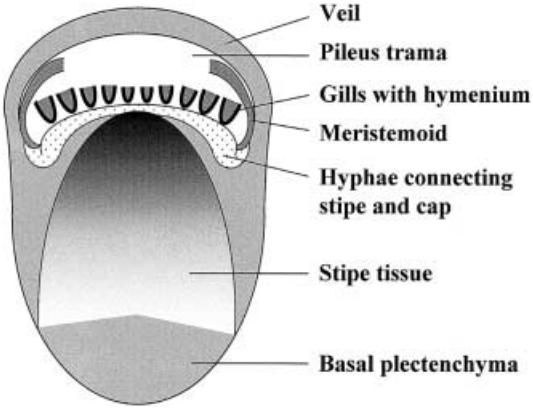

Fig. 1 Schematic representation of a slightly tangential section through the primordium of a typical agaric. The main tissue organization is indicated (modified from Kües 2000)

Mushroom growers call the switch from mycelial extension to the production of mushroom primordia "pinning", the successive development of primordia into mushrooms "fruiting" (Scrase and Elliott 1998). Once the tissues in the primordium are established, i.e. pinning completed, there is usually not much further cell division. In contrast, cells will rapidly elongate in all directions, often several-fold longer than their original length, leading to an increase of volume during the consecutive step of fruiting (Angeli-Papa and Eyme 1978; Williams et al. 1985; Clémençon 1997; Moore 1998a; Kües 2000). In *Coprinus*, the onset and proceedure of fruiting correlates with karyogamy and meiosis; and basidiospores are shed at the point of fruiting body maturation (Kües 2000). In other fungi (including *S. commune*) karyogamy, meiosis and spore formation are less synchronized. Sporulation is completed in the first-established parts of a fruiting body, while there is still cell proliferation and folding of new gills in the younger parts (Wessels et al. 1985).

Although basic points in mushroom development have been defined – hyphal knot formation, formation of an initial aggregate, differentiation of the primordium with distinct cap and stipe tissues, fruiting body maturation by cell extension, basidiospore formation – morphogenesis is still poorly understood and needs far more detailed investigation. Mushroom formation can tolerate much imprecision of the underlying developmental programs without impairing their natural task of spore formation and shedding. Consequently, anatomically malformed fruiting bodies are often still functional (Moore 1998b; Umar and Van Griensven 1999). The borderline between normal and abnormal development remains to be clarified for constant success in mushroom production (Umar and Van Griensven 1999).

Environmental control of fruiting body development

Environmental conditions play a crucial role in the decision whether a fruiting body will be formed. The optimal environmental situation for mycelial growth and the subsequent fruiting is usually very distinct – fruiting body development is often induced after drastically altering the environmental circumstances. There is no universal set of conditions that leads to fructification in all fungi. The conditions for growing and producing fruiting bodies of a given species in culture have to be empirically established; but the natural environment of the fungus may give valuable indications as how to proceed in this determination. Once the situation has been optimized for a certain fungus, this might help when establishing the cultural conditions for related species (Scrase and Elliott 1998).

Production of the vegetative mycelium usually occurs over a wide range of temperatures. Likewise, the formation of hyphal knots and the steps leading to primordia formation might not be restricted to a specific temperature, although such development is usually favoured at certain temperatures. Fruiting is typically induced, after vegetative growth, by reducing the temperature by at least 5 °C, e.g. in *A. bisporus* to 16–18 °C, in *C. cinereus* to 25–28 °C and in *Flammulina velutipes* (Velvet Foot) to 18 °C (Flegg and Wood 1985; Williams et al. 1985; Scrase and Elliott 1998; Kües 2000). Other parameters of fruiting body initiation and maturation include CO_2 concentration, humidity, salinity and pH. Higher CO_2 concentrations may stimulate mycelial growth and inhibit fruiting completely. At lower CO_2 concentrations fruiting bodies might be malformed, with elongated stipes and reduced pilei (Flegg and Wood 1985; Kinugawa 1993; Wessels 1993; Scrase and Elliott 1998). High humidity (90–95%) is favourable for pinning and fruiting (Flegg and Wood 1985; Kinugawa 1993; Kües 2000), but the moisture content of the substrate might be even more critical. The optimal water content for wooden substrates is 35–60% and for other substrates 60–80%. The lower values reflect the oxygen demand of the fungi in the substratum, balanced against their requirement for water (Flegg and Wood 1985; Scrase and Elliott 1998; Ohga 1999a). Basidiomycetes tolerate relatively high levels of salts for growth, but fruiting body development can be more sensitive. Likewise, mycelial growth is less affected by pH, but fruiting body development of several species occurs best at neutral or slightly acidic pH values around 6–7 (Flegg and Wood 1985; Kinugawa 1993) or, in *Lentinus edodes* (Shiitake), at a pH of 4.0 (Ohga 1999b).

Light has been implicated in the fruiting of several *Coprinus* species (Ellis et al. 1999; Kües 2000), *Favolus arcularius*, a Polypore (Kitamoto et al. 1972), *Pleurotus ostreatus*, the Oyster Mushroom (Richartz and MacLellan 1987), *F. velutipes* (Kinugawa 1993), *S. commune* (Yli-Mattila 1991) and some others. However, light is not needed for the fruiting of *A. bisporus* (Wessels 1993). Usually, light positively influences hyphal aggregation and fruiting body maturation. In contrast, light can repress hyphal knot formation. When illumination is too strong or too long, further steps in fruiting body development are also blocked. A short light pulse of low energy is typically enough to induce hyphal aggregation. Fruiting body maturation needs longer exposures of higher energy, but strains of the same species can differ in their light requirements (Richartz and MacLellan 1987; Wessels 1993; Ellis et al. 1999; Kües 2000). Whenever tested, the active wavelengths in controlling fruiting body initiation and maturation were found to be in the blue light/UV range (see above references).

Not surprisingly for ectomycorrhizal fungi, the presence of a symbiontic plant may allow successful fruiting body formation under artificial conditions (Danell and Camacho 1997; Iwase 1997). However, the fruiting of saprophytic species can also depend on the presence of other organisms. Many *Agaricus* strains only fruit when associated with micro-organisms, e.g. pseudomonads. Experiments with charcoal suggest that the micro-organisms do not influence fruiting by producing induction substances, but by eliminating inhibitory compounds (De Groot et al. 1998).

Physiological control of fruiting body development

Fruiting body formation is very much influenced by the physiological condition and nutritional state of the mycelium (Madelin 1956; Flegg and Wood 1985). Compost prepared from straw, horse or chicken manure, calcium sulphate (gypsum), water and some nutritional supplements is a cheap cultural substrate for *A. bisporus* and some other saprophytic basidiomycetes. Manure in the compost serves as N source, straw as C source. Non-composted, chopped and water-soaked straw is sufficient for the cultivation of *Pleurotus*, *Volvariella* and *Flammulina*. Wood-rotting species as *L. edodes* are cultivated on logs or in bags of on moisturized sawdust supplemented with cereal bran. Although not necessarily optimal, since they are low in readily accessible nutrients, these commercially used

substrates satisfy the needs of the fungi for growth and fruiting and, most importantly, help to withstand microbial competitors (Scrase and Elliott 1998). Supplementing compost with protein-rich materials such as soybean meal increases fruiting body yields of *Agaricus* by 25–50% (De Groot et al. 1998). In the laboratory, the dung fungus *C. cinereus* and the wood-rotter *S. commune* fruit on rich, artificial media based on glucose and malt extract (Walser et al. 2000). For fruiting body induction it is important to keep a balance between C and N sources (Madelin 1956; Bottoli et al. unpublished) – hyphal knots in *C. cinereus* will easily form into multicellular duration structures (sclerotia) when the C/N ratio becomes too high (Moore 1998a). In compost, the optimal C/N ratio for the fruiting of *A. bisporus* has been determined to lie between 80:1 and 10:1 (Scrase and Elliott 1998). C/N metabolism and the proteins involved have mainly been studied in *A. bisporus* and *C. cinereus*. Extensive descriptions are found in the literature (De Groot et al. 1998; Moore 1998a; Stoop and Mooibroek 1999).

Since the C sources utilized by basidiomycetes are usually of a lignocellulosic character, the fungi during vegetative growth produce a wide range of enzymes to degrade the lignocellulosic substrates: peroxidases and laccases for lignin degradation and various types of glucanases, cellulases and xylanases for cellulose and hemicellulose degradation (De Groot et al. 1998; Stoop and Mooibroek 1999). Considerable changes in enzyme activities occur during fruiting, indicating a connection to the regulation of fruiting body development. For example in *A. bisporus* and *L. edodes*, laccase activities are highest just before fruiting body initiation and decline rapidly with aggregate formation. Cellulase activities are highest when fruiting bodies develop (Ohga 1992; De Groot et al. 1998; Ohga et al. 1999). With the periodic fruiting (''flushing'') of *Agaricus* in commercial cultures, enzyme activities therefore fluctuate in approximately weekly cycles (De Groot et al. 1998; Ohga et al. 1999).

Substrates offered for mushroom cultivation normally contain organic N sources and are low in free ammonium, since excess can inhibit growth or fruiting of the organisms. A recent study in *A. bisporus* gives evidence for the presence of several high-affinity transport systems for amino acids and NH_4^+. The intracellular pools of NH_4^+ largely regulate uptake (Kersten et al. 1999). When *A. bisporus*, *C. cinereus* and *Volvariella volvacea* (Paddy Straw Mushroom) are grown on protein as C source, excess N gained from protein degradation was found to be excreted as ammonium into the growth medium (De Groot et al. 1998; Moore 1998a). At least in *C. cinereus*, ammonium release is thought to play a positive role for fruiting body initiation as it inhibits the competing process of sclerotia formation from hyphal knots (Moore 1998a).

Onset of fruiting body development correlates with nutritional exhaustion of the growth substrates. Fruiting body development for commercial mushroom production is thus often induced by covering compost colonized by vegetative mycelium with a layer of moist peat and chalk, which have only limited nutrients (Scrase and Elliott 1998). Typically, mycelia of basidiomycetes are not uniformly competent to differentiate; and only young hyphae can be induced to initiate fruiting body development (Ross 1982a). Mechanical injury of established mycelium locally stimulates fruiting body development, because wounding causes outgrowth of fresh hyphae (Leonard and Dick 1979; Granado et al. 1997; Scrase and Elliott 1998). The molecular principles triggering differentiation are not known. Various substances with fruiting inducing activity in specific or several basidiomycetes have been described: cerebrosides (Wessels 1993; Kües 2000), sucrose esters of fatty acids and other surfactants (Oita and Yanagi 1993; Magae and Itoh 1998; Magae 1999), cAMP and AMP (Wessels 1993; Kües 2000), anthranilic acid and indole (Wessels 1993; Samadder et al. 1997) and other substances of yet unknown nature present in fungal extracts (Butler and Pearce 1999). The variety of these activating substances probably reflects a very complex physiological control of fruiting body initiation. So far, signalling by cAMP has found most attention (Wessels 1993). In *C. cinereus*, cAMP levels were shown to rise with light-induced initial formation due to increased adenylate cyclase activity; and a high cAMP protein kinase activity is associated with the fruiting response. The levels of cAMP decrease again with proceeding primordia development (Moore 1998a; Kües 2000). Transcript analysis of the cloned adenylate cyclase gene *cac* suggests that regulation of protein activity for cAMP production occurs at the post-translational level (Bottoli et al. unpublished).

At the competent stage upon environmental induction, numerous hyphal knots, initials and primordia appear on a colony but only a few of these come to maturation. Relocation of nutritients is thought to occur throughout the mycelium to favour one specific or a few selected primordia. It is unknown what determines these preferences in maturation. In *C. cinereus*, during the transition from vegetative growth to fruiting body development, a breakdown of storage polysaccharides (glycogen) in the substrate mycelium is observed. Instead, glycogen accumulates first in the base of the stem in the young primordium, from which it subsequently disappears and is translocated into the gill tissues during further development. Accumulation of glycogen in the gill tissues is highest upon karyogamy in the early stages of fruiting body maturation, but deposits are absent from the mature fruiting bodies. Glycogen translocation is accompanied by increased glycogen synthase and glycogen phosphorylase activities. It obviously provides the metabolic energy for mushroom development. Per fruiting body, this metabolic energy is estimated at 25 J (Moore 1998a). Glycogen concentrations also fluctuate in the vegetative mycelium of *A. bisporus* with the periods of fruiting. Moreover, synthesis of trehalose increases before hyphal aggregates arise. Upon translo-

cation into emerging aggregates, trehalose is degraded by the developmentally regulated trehalose phosphorylase to glucose and glucose-1-phosphate for energy and the C supply. Unlike *C. cinereus*, mannitol is accumulated in the developing fruiting bodies of *A. bisporus* and probably acts as an osmoticum during growth of the mushroom. It is synthesized from fructose by mannitol dehydrogenase, using NADPH as cofactor. This enzyme is also developmentally regulated, together with a specific glucose 6-phosphate dehydrogenase responsible for increased NADPH production (De Groot et al. 1998; Wannet et al. 1999).

Genetic control of fruiting body development

Fruiting body formation is part of the usual life cycle of basidiomycete fungi, leading to karyogamy, meiosis and sexual spore formation in specific cells within the fruiting body, called basidia. The majority of the basidiomycetes are heterothallic, i.e. two haploid "homokaryotic" mycelia of different mating type specificity have to fuse in order to give rise to a "dikaryotic" mycelium. Such a dikaryon is capable of sexual reproduction. It has two distinct haploid nuclei present in each hyphal segment, one from each parental homokaryon (Fig. 2A; Kües 2000). Other fungi, including many strains of *A. bisporus*, are secondarily homothallic. In these cases, two haploid nuclei with different mating type genes co-migrate into each basidiospore after meiosis. Basidiospores of secondarily homothallic species germinate directly into a fertile dikaryon without the need for mycelial fusions (Fig. 2B; Raper et al. 1972). In contrast, basidiospores of heterothallic organisms receive only one type of haploid nuclei. Consequently, sterile homokaryons arise upon spore germination (Fig. 2A).

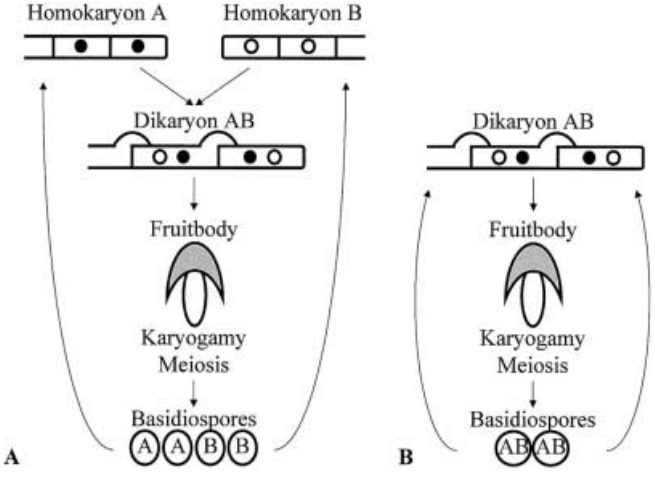

Fig. 2A, B Typical life cycles of basidiomycetes. **A** The basic life cycle of heterothallic species. **B** The basic life cycle of a secondary homothallic species. *Filled* and *open circles* indicate nuclei with different mating type-specificity. In most but not all basidiomycetes, the monokaryotic mycelia have simple hyphal septa and the dikaryotic mycelia have specialized clamps at the hyphal septa (indicated by *curved lines*). The cellular program of clamp cell formation ensures that each hyphal segment in the dikaryon receives a haploid nucleus from each parental type (Kües 2000)

Fruiting bodies usually develop on the dikaryotic mycelium. Since the mating type loci regulate dikaryon formation, these genes ultimately also control fruiting body initiation. Typically, basidiomycetes have two complex mating type loci (haplotypes). The *A* mating type locus codes for two types of homeodomain transcription factors (termed HD1 and HD2) and the *B* mating type locus codes for pheromones and pheromone receptors. In some species, the genes for these functions are so closely linked that they appear as one genetic trait in crosses between compatible strains. In order to elicit dikaryon formation and sexual development, compatible products of different mating type specificity (haplotype) have to interact. A HD1 protein and a HD2 protein from different *A* haplotypes have to dimerize to form an active transcription factor complex. Similarly, a pheromone has to recognize and obviously has to bind to a pheromone receptor from a different *B* haplotype (Casselton and Olesnicky 1998; Hiscock and Kües 1999; Kothe 1999). Homokaryons of *C. cinereus* were found to initiate fruiting body development upon transformation with cloned genes of another *A* mating type specificity, indicating participation of the *A* genes in fruiting body development (Tymon et al. 1992; Kües et al. 1998). Heterologous *A* mating type gene products play a role in at least two stages. The first is in induction of hyphal knot formation occurring in the dark. Successively, it needs compatible *A* genes and a blue light pulse to convert hyphal knots into fruiting body initials (Kües et al. 1998). Ongoing studies with cloned *B* mating type genes suggest that these too induce at least hyphal knot development (Kües and Klaus unpublished). Further indications for the involvement of mating type genes in fruiting come from special mutants of *C. cinereus* and *S. commune* with defects in their mating type loci. Such *Amut Bmut* mutants overcome the normal self-sterility of homokaryons; and fruiting bodies may be formed on mutant mycelium (Raper et al. 1965; Swamy et al. 1984). Work in *C. cinereus* showed that the defects in the *A* mating type loci are based on DNA deletions, leading to in-frame fusions of a set of *HD1* and *HD2* genes whose products normally do not form an active transcription factor complex (Kües et al. 1994; Pardo et al. 1996). Analysis of a mutated *B* mating type locus revealed that loss of self-sterility is due to a constitutively active pheromone receptor caused by a single amino acid exchange (Olesnicky et al. 1999). A similar mutant phenotype in *S. commune* obviously is based on an amino acid exchange in a pheromone making it compatible to a pheromone receptor coming from the same *B* haplotype (Fowler et al. 1998; Kothe 1999).

Spontaneous switching of mating types has occasionally been observed in *Agrocybe aegerita* and *Agaricus bitorquis*, leading to fruiting body formation on mycelia originally homokaryotic but then evidently dikaryotic.

The mechanism for how mating type-switching in basidiomycetes occurs is not yet clear (Labarère and Noël 1992; Martínez-Carrera et al. 1995). Outside of the mating type loci, mutations in more than 40 basidiomycetes are known to cause fruiting body formation on a homokaryon (Stahl and Esser 1976; Martínez-Carrera et al. 1995). Only one mutated gene, *pcc1* in *C. cinereus*, has so far been analyzed. *pcc1* encodes a HMG box transcription factor that functions somewhere in the *A* mating type pathway. Defects in *ppc1* release repression of fruiting body formation (Murata et al. 1998). Another interesting gene, *frt1* from *S. commune*, was identified during a search for *B* mating type genes by its ability to induce fruiting in certain homokaryotic transformation recipients. The Frt1 protein has a P-loop motif at its N-terminal end, suggesting it belongs to the ATP-binding proteins. Frt1 is dispensable for fruiting in the dikaryon and is believed to play a role in negative regulation of dikaryon-expressed genes in vegetatively growing homokaryons (Horton et al. 1999).

Other functions contributing to fruiting body development

Mutants in isolation of genes for fruiting body development

In the first instance dikaryons and later *Amut Bmut* homokaryons of *C. cinereus* have extensively been employed to induce mutations in the fruiting pathway (Takemaru and Kamada 1972; Granado et al. 1997; Cummings et al. 1999; Muraguchi et al. 1999). Use of the mutant collections is made in cloning genes acting in fruiting body development. An interesting gene cluster possibly involved in membrane metabolism and vesicle formation has recently been identified by a transformation that restores the switch from hyphal knot to initial formation in an UV mutant blocked at this step (Liu et al. unpublished). Mutants defective in gene *ich1* are disturbed in pileus development at the primordial stage. The product of the gene, identified by mutant complementation, contains a sequence resembling nuclear localization sites. Ich1 has thus been suggested to act in the nucleus (Muraguchi and Kamada 1998). However, the protein has also a potential *S*-adenosyl-methionine binding domain typical for non-DNA methylases and ca 20% identity to *O*-methyltransferases from *Aspergillus* species, enzymes acting in aflatoxin biosynthesis (Kües 2000). Complementation of a third mutant, defective in stipe elongation, identified that gene *eln2* encodes a new type of P450 cytochrome (Muraguchi and Kamada 2000). Moreover, gene-tagging by restriction enzyme mediated insertion mutagenesis enabled the isolation of gene *spo11*, encoding a putative topoisomerase II-like protein, from a sporulation-defective mutant (Cummings et al. 1999).

These initial examples of cloning genes with the help of mutants promises that the process of fruiting body development in *C. cinereus* will be unravelled in the future. Due to missing effective transformation systems, this is not yet possible in most other basidiomycetes (Stoop and Mooibroek 1999). Gene cloning is restricted to those molecular techniques identifying genes by their transcripts, by sequences deduced from isolated proteins and by known sequences from other organisms.

A potpourri of genes linked to fruiting body development and some speculations on their functions

Some fruiting body-specific or fruiting body-induced genes have been obtained by molecular techniques from *Agaricus* (cytochrome P450 gene *CYPA*, septine gene *SEPA*, gene *ATPD* for the α-subunit of ATP-synthase, and the hydrophobin genes *HYPA*/*ABH1*, *HYPB*, *HYPC*/*ABH2*). In addition, a number of genes acting in C/N metabolism and in substrate degradation are available (De Groot et al. 1998; Stoop and Mooibroek 1999). Amongst them are two genes for laccases (Ohga et al. 1999). Laccases have repeatedly been implicated in fruiting body initiation in basidiomycetes, but their role in fruiting remains controversal (Ross 1982b; Ohga 1992; Ikegaya et al. 1993; Das et al. 1997; Ohga et al. 1999). Partially, this might be because basidiomycetes contain several different laccase genes – *C. cinereus* for example has at least six (Bottoli et al. 1999; Yaver et al. 1999). In *L. edodes*, two laccase genes (*lac1*, *lac2*) were shown to be active at later stages of fruiting body formation, especially in the cap. A function in the formation of extracellular pigments, coupled to oxidative polymerization of cell wall components to strengthen cell-to-cell adhesion, is anticipated for proteins Lac1 and Lac2 (Zhao and Kwan 1999).

Hyphal aggregation has been shown to be mediated by a short peptide containing an Arg-Gly-Asp motif, part of the 2,140 amino acid protein MFBA from *L. edodes*. However, gene *mfbA* is expressed in late fruiting body development, mostly within the gill-less regions of the pileus at a stage when most tissues of the mushroom have already been established, making it unlikely to be a general aggregation factor (Yasuda et al. 1997). Moreover, the protein contains several domains (e.g. a cysteine-rich SET domain and a Phd finger) typical for transcription factors affecting chromatin structure, which sheds doubt on the function of MFBA as a cellular aggregation factor (Kües and Boulianne, unpublished observation).

The product of the gene *Aa-Pri1* of *Agrocybe aegerita*, highly similar to a haemolysin of *Aspergillus fumigatus*, has been proposed to allow hyphae to aggregate, following the protein binding to a specific membrane receptor (Fernandez Espinar and Labarère 1997). However, in view of the occurrence of programmed cell death during primordia development, one might also speculate that the primordia-specific protein Aa-Pri1 has a cytolytic activity like other haemolysins.

The primordia-specific product of gene *priA* from *L. edodes* contains cysteine-rich motifs resembling zinc

fingers typified by transcription factor TFIIIA, zinc clusters observed in methallothionins (Ishizaki et al. 1999) and – revealed by Blast search – epidermal growth factor (EGF)-like domains found in extracellular matrix proteins and integral membrane glycoproteins (Stone et al. 1999). PriA has a C-terminal CAAX motif known to promote membrane interactions of proteins. The protein increases heavy metal sensitivity in *Escherichia coli* when heterologously expressed (Ishizaki et al. 1999). Although the PriA function is currently obscure, the primordia-specific protein PriB of *L. edodes* has been shown to bind to a specific DNA sequence and thus probably functions as a transcription factor (Miyazaki et al. 1997).

Fruiting body specific lectins, carbohydrate-binding proteins probably acting in hyphal aggregation, have been isolated from many species (Wang et al. 1998; Table 3). So far, only the lectin gene *ABL* from *Agaricus* and the galectin (β-galactoside-binding lectin) encoding genes *cgl1* and *cgl2* of *C. cinereus* have been identified (Crenshaw et al. 1995; Cooper et al. 1997). Consistent with a function in hyphal aggregation, onset of expression of *cgl2* correlates with hyphal knot formation and onset of *cgl1* expression coincides with initial formation. The secreted proteins are preferentially found in the veil and the outer portion of the stipe, tissues that have to resist strong forces during maturation of the fruiting body (Boulianne et al. 2000).

Other proteins shown to contribute to the structure of fruiting bodies are the hydrophobins, small cysteine-rich secreted proteins that assemble into a hydrophobic rodlet layer on the surface of fungal hyphae. First identified in *S. commune* (Wessels 1997), fruiting body-specific hydrophobin genes are now known from *Pleurotus ostreatus* (Penas et al. 1998), *A. aegerita* (Santos and Labarère 1999) and *A. bisporus*, where different tissues were shown to express different hydrophobins (De Groot et al. 1999). Hydrophobins have been shown to line air channels in the fruiting bodies of *S. commune* and *A. bisporus* (Lugones et al. 1999).

Conclusions

Basidiomycetes offer a great biotechnological potential for efficiently converting vast amounts of low-cost lignocellulosic wastes from agriculture, forestry and plant-processing industries into mushrooms and mushroom products highly valuable for human consumption and health. Due to our limited knowledge of the organisms, this potential is far from being fully exploited. Fruiting body production of basidiomycetes is in no aspect well understood to date. There are many observations regarding fruiting body formation in basidiomycetes, but currently it is mostly impossible to connect them with each other. In order to achieve a better insight, it will be necessary to intensify studies on just a few model organisms. As the example of hydrophobins in *S. commune* nicely demonstrated, once we have a clue on a specific aspect of fruiting body formation, the information gained can be applied to species less accessible to experimental studies. Progress has now been made in cloning genes contributing to fruiting body initiation and formation, some being specific for the fruiting body. Mutant analysis will help to unravel their role in fruiting. Where mutants are not directly available, analysis will be assisted by construction of gene ''knock-outs'', as has recently been shown for a number of genes in the model organisms *S. commune* (Robertson et al. 1996; Horton et al. 1999; Lengeler and Kothe 1999) and *C. cinereus* (Pardo 1995). Understanding the process of fruiting body development will, in the long term, positively affect the procedures of mushroom cultivation as well as mushroom breeding programs to improve strain characters for better yield, taste, appearance, disease resistances and shelf-life.

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References

Angeli-Papa J, Eyme J (1978) Ultrastructural changes during development of *Agaricus bisporus* and *Agaricus sylvicola*. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushrooms. Academic Press, New York, pp 53–82

Ayer F, Egli S (1997) Propagation d'une colonie de *Cantharellus lutescens* Fr. PAR transplantation de moussses colonisées par le mycélium du champignon. Doc Mycol 104: 9–12

Borchers AT, Stern JS, Hackman RM, Keen CL, Gershwin ME (1999) Mushrooms, tumors, and immunity. Proc Soc Exp Biol Med 221: 281–293

Bottoli APF, Kertesz-Chaloupková K, Boulianne RP, Granado JD, Aebi M, Kües U (1999) Rapid isolation of genes from an indexed genomic library of *C. cinereus* in a novel *pab1*$^+$ cosmid. J Microbiol Methods 35: 129–141

Boulianne RP, Liu Y, Aebi M, Lu BC, Kües U (2000) Fruiting body development in *Coprinus cinereus*: regulated expression of two galectins secreted by a non-classical pathway. Microbiology, in press

Buswell JA, Chang ST (1993) Edible mushrooms: attributes and applications. In: Chang ST, Buswell JA, Miles PG (eds) Genetics and breeding of edible mushrooms. Gordon and Breach, Y-Parc, Switzerland, pp 297–324

Butler GM, Pearce RB (1999) Fruiting inducing activity amongst isolates and single spore progeny of *Phellinus contiguus*, other species of *Phellinus* and certain other wood-rotting fungi. Mycol Res 103: 482–486

Casselton LA, Olesnicky NS (1998) Molecular genetics of mating recognition in basidiomycete fungi. Microbiol Mol Biol Rev 62: 55–70

Chang ST (1993) Mushroom biology: the impact on mushroom production and mushroom products. In: Chang ST, Buswell JA, Chiu SW (eds) Mushroom biology and mushroom products. The Chinese University Press, Hong Kong, pp 3–20

Chang ST (1996) Mushroom research and development – equality and mutual benefit. In: Royse DJ (ed) Mushroom biology and

mushroom products. Proc 2nd Int Conf, University Park, Pa. Penn State University Press, pp 1–10
Chang ST, Buswell JA (1996) Mushroom nutriceuticals. World J Microbiol Biotechnol 12: 473–476
Chen AW, Miles PG (1996) Cultivation of *Ganoderma* bonsai. In: Royse DJ (ed) Mushroom biology and mushroom products. Proc 2nd Int Conf, University Park, Pa. Penn State University Press, pp 325–333
Clémençon H (1997) Anatomie der Hymenomyceten. Eine Einführung in die cytologie und Plectologie der Krüstenpilze, Porlinge, Keulenpilze, Leistlinge, Blätler pilze und Röhrlinge. Anatomy of the hymenomycetes. An introduction to the cytology and plectology of crust fungi, bracket fungi, club fungi, Chantarelles, Agarics and Boletes. F. Flück-Wirth, Teufen, Switzerland
Cooper DNW, Boulianne RP, Charlton S, Farrell EM, Sucher A, Lu BC (1997) Fungal galectins, sequence and specificity of two isolectins from *Coprinus cinereus*. J Biol Chem 272: 1514–1521
Courvoisier M (1994) Production de champignons cultives de l' union Europeene 1994, prévision 1995. Bull Fed Nat Synd Agric Cult Champig 66: 6–16
Crenshaw RW, Harper SC, Moyer M, Privalle LS (1995) Isolation and characterization of a cDNA clone encoding a lectin gene from *Agaricus bisporus*. Plant Physiol 107: 1465–1466
Cummings WJ, Celerin M, Crodian J, Brunick LK, Zolan ME (1999) Insertional mutagenesis in *Coprinus cinereus*: use of a dominant selectable marker to generate tagged, sporulation-defective mutants. Curr Genet 36: 371–382
Danell E, Camacho FJ (1997) Successful cultivation of the golden chanterelle. Nature 385: 303
Das N, Sengupta S, Mukherjee M (1997) Importance of laccase in vegetative growth of *Pleurotus florida*. Appl Environ Microbiol 63: 4120–4122
de Geus N, Berch S (1997) The pine mushroom industry in British Columbia. In: Palm ME, Chapela IH (eds) Mycology in sustainable development: expanding concepts, vanishing borders. Parkway Publishers, Boone, N.C., pp 55–67
De Groot PWJ, Visser J, Van Griensven LJLD, Schaap PJ (1998) Biochemical and molecular aspects of growth and fruiting of the edible mushroom *Agaricus bisporus*. Mycol Res 102: 1297–1308
De Groot PWJ, Roeven RTP, Van Griensven LJLD, Visser J, Schaap PJ (1999) Different temporal and spatial expression of two hydrophobin-encoding genes of the edible mushroom *Agaricus bisporus*. Microbiology 145: 1105–1113
Ellis RJ, Bragdon GA, Schlosser BJ (1999) Properties of the blue light requirements for primordia initiation and basidiocarp maturation in *Coprinus stercorarius*. Mycol Res 103: 779–784
Fernandez Espinar MT, Labarère J (1997) Cloning and sequencing of the *Aa-Pri1* gene specifically expressed during fruiting initiation in the edible mushroom *Agrocybe aegerita*, and analysis of the predicted amino-acid sequence. Curr Genet 32: 420–424
Flegg PB, Wood DA (1985) Growth and fruiting. In: Flegg PB, Spencer DM, Wood DA (eds) The biology and technology of the cultivated mushroom. Wiley, London, pp 141–177
Fowler TJ, Mitton MF, Raper CA (1998) Gene mutations affecting specificity of pheromone/receptor mating interactions in *Schizophyllum commune*. In: Van Griensven LJLD, Visser J (eds) Proc 4th Meet Genet Cell Biol Basidiomycetes. Mushroom Experimental Station, Horst, The Netherlands, pp 130–134
Gan KH, Fann YF, Hsu SH, Kuo KW, Lin CN (1998) Mediation of the cytotoxicity of lanostanoids and steroids *of Ganoderma tsugae* through apoptosis and cell cycle. J Nat Prod 61: 485–487
Gao, Q, Killie MK, Chen, H, Jiang R, Seljelid R (1997) Characterization and cytokine-stimulating activities of acidic heteroglucans from *Tremella fuciformis*. Planta Med 63: 457–460
Granado JD, Kertesz-Chaloupková K, Aebi M, Kües U (1997) Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. Mol Gen Genet 256: 28–36
Gunde-Cimerman N, Cimerman A (1995) *Pleurotus* fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase – lovastin. Exp Mycol 19: 1–6
Hiscock SJ, Kües U (1999) Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. Int Rev Cytol 193: 165–295
Horton JS, Palmer GE, Smith WJ (1999) Regulation of dikaryon-expressed genes by FRT1 in the basidiomycete *Schizophyllum commune*. Fungal Genet Biol 26: 33–47
Ikegaya N, Goto M, Hayashi Y (1993) Effect in phenolic growth and fruit-body formation of *Lentinus edodes*. Trans Mycol Soc Jpn 34: 195–207
Ishizaki T, Kanno T, Kajiwara S, Shishido K (1999) Increased heavy metal sensitivity of *Escherichia coli* producing the expression product of *priA* derived from the basidiomycete *Lentinus edodes*. FEMS Microbiol Lett 171: 141–146
Iwase K (1997) Cultivation of mycorrhizal mushrooms. Food Rev Int 13: 431–442
Jong SC, Birmingham JM (1990) The medicinal value of the mushroom *Grifola*. World J Microbiol Biotechnol 6: 227–235
Jong SC, Birmingham JM (1993a) Mushrooms as a source of flavor and aroma compounds. In: Chang ST, Buswell JA, Chiu SW (eds) Mushroom biology and mushroom products. The Chinese University Press, Hong Kong, pp 345–366
Jong SC, Birmingham JM (1993b) Medicinal benefits of the mushroom *Ganoderma*. Adv Appl Microbiol 37: 101–127
Jong SC, Donovick R (1989) Antitumor and antiviral substances from fungi. Adv Appl Microbiol 34: 183–262.
Kawagishi H, Ando M, Sakamoto H, Yoshida S, Ojima F, Ishiguro Y, Ukai N, Furukawa S (1991) Hericenones C, D and E, stimulators of nerve growth factor (NGF)-synthesis, from the mushroom *Hericium erinaceum*. Tetrahedron Lett 32: 4561–4564
Kawagishi H, Mitsunaga S-I, Yamawaki M, Ido M, Shimada A, Kinoshita T, Murata T, Usui T, Kimura A, Chiba S (1997) A lectin from mycelia of the fungus *Ganoderma lucidum*. Phytochemistry 44: 7–10
Kersten MASH, Arninkhof MJC, Op den Camp HJM, Van Griensven LJLD, van der Drift C (1999) Transport of amino acids and ammonium in mycelium of *Agaricus bisporus*. Biochim Biophys Acta 1428: 260–272
Kiho T, Morimoto H, Sakushia M, Usui S, Ukai S (1995) Polysaccharides in fungi. XXXV. Anti diabetic activity of an acidic polysaccharide from the fruiting bodies of *Tremella aurantia*. Biol Pharm Bull 18: 1627–1629
Kinugawa K (1993) Physiology and the breeding of *Flamminula velutipes*. In: Chang ST, Buswell JA, Miles PG (eds) Genetics and breeding of edible mushrooms. Gordon and Breach, Y-Parc, Switzerland, pp 87–109
Kitamoto Y, Suzuki A, Furikawa S (1972) An action spectrum for light-induced primordium formation in a basidiomycete, *Favolus arcularius* (Fr.) Ames. Plant Physiol 49: 338–340
Kothe E (1999) Mating types and pheromone recognition in the homobasidiomycete *Schizophyllum commune*. Fungal Genet Biol 27: 146–152
Kubo K, Aoki H, Nanba H (1994) Anti-diabetic activity in the fruit body of *Grifolia frondosa* (Maitake). Biol Pharmaceut Bull 17: 1106–1110
Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64: 316–353
Kües U, Göttgens B, Stratmann R, Richardsson WVJ, O'Shea SF, Casselton LA (1994) A chimeric homeodomain protein causes self-incompatibility and constitutive sexual development in the mushroom *Coprinus cinereus*. EMBO J 13: 4054–4059
Kües U, Granado JG, Hermann R, Boulianne RP, Kertesz-Chaloupková K, Aebi M (1998) The *A* mating type and blue light regulate all known differentiation processes in the basidiomycete *Coprinus cinereus*. Mol Gen Genet 260: 81–91
Kurashima Y, Tsuda M, Sugimura T (1990) Marked formation of thiazolidine-4-carboxylic acid, an effective nitrite trapping agent in vivo, on boiling of dried shiitake mushroom (*Lentinus edodes*). J Agric Food Chem 38: 1945–1949
Labarère J, Noël T (1992) Mating type switching in the tetrapolar basidiomycete *Agrocybe aegerita*. Genetics 13: 307–19

Lengeler KB, Kothe E (1999) Mated: a putative peptide transporter of *Schizophyllum commune* expressed in dikaryons. Curr Genet 36: 159–164

Leonrad TJ, Dick S (1979) Monokaryotic fruiting in *Schizophyllum commune*: genetic control of the response to mechanical injury. Mol Gen Genet 175: 5–12

Lu BC (1991) Cell degeneration and gill remodelling during basidiocarp development in the fungus *Coprinus cinereus*. Can J Bot 69: 1161–1169

Lugones LG, Wösten HAB, Birkenkamp KU, Sjollema KA, Zagers J, Wessels JGH (1999) Hydrophobins line air channels in fruiting bodies of *Schizophyllum commune* and *Agaricus bisporus*. Mycol Res 103: 635–640

Madelin MF (1956) Studies on the nutrition of *Coprinus lagopus* Fr, especially as affecting fruiting. Ann Bot 20: 467–480

Magae Y (1999) Saponin stimulates fruiting of the edible basidiomycete *Pleurotus ostreatus*. Biosci Biotechnol Biochem 63: 1840–1842

Magae Y, Itoh Y (1998) Effects of sucroses ester of fatty acids on fruit body formation of *Pleurotus ostreatus*. Nippon Nogei Kagaku Kaishi 72: 631–635

Marchetti M, Pisani S, Pietropaolo V, Seganti L, Nicoletti R, Degener A, Orsi N (1996) Antiviral effect of a polysaccharide from *Sclerotium glucanicum* towards herpes simplex virus type 1 infection. Planta Med 62: 303–307

Martínez-Carrera D, Challen MP, Smith JF, Elliott TJ (1995) Homokaryotic fruiting in *Agaricus bitorquis*: a new approach. In: Elliott TJ (ed) Science and cultivation of edible fungi, vol I. Balkema, Rotterdam, pp 37–44

Michelot D (1992) Poisoning by *Coprinus atramentarius*. Nat Toxins 1: 73–80

Miyazaki Y, Tsunoka O, Shishido K (1997) Determination of the DNA-binding sequences of the Zn(II)2Cys6 zinc-cluster-containing PRIB protein, derived from the basidiomycete *Lentinus edodes* gene. J Biochem 122: 1088–1091

Moore D (1998a) Fungal morphogenesis. Cambridge University Press, New York

Moore D (1998b) Tolerance of imprecision in fungal morphogenesis. In: Van Griensvan LJLD, Visser J (eds) Proc 4th Meet Genet Cell Biol Basidiomycetes. Mushroom Experimental Station, Horst, The Netherlands, pp 13–19

Muraguchi H, Kamada T (1998) The *ich1* gene of the mushroom *Coprinus cinereus* is essential for pileus formation in fruiting. Development 125: 3123–3132

Muraguchi H, Kamada T (2000) A mutation in the *eln2* gene encoding a cytochrome P450 of *Coprinus cinereus* affects mushroom morphogenesis. Fungal Genet Biol 29: 49–59

Muraguchi H, Takemaru T, Kamada T (1999) Isolation and characterization of developmental variants in fruiting using a homokaryotic fruiting strain of *Coprinus cinereus*. Mycoscience 40: 227–234

Murata Y, Fujii M, Zolan ME, Kamada T (1998) Molecular analysis of *pcc1*, a gene which leads to *A*-regulated sexual morphogenesis in *Coprinus cinereus*. Genetics 149: 1753–1761

Oguri S, Ando A, Nagata Y (1996) A novel developmental stage-specific lectin of the basidiomycete *Pleurotus cornucopiae*. J Bacteriol 178: 5692–5698

Ohga S (1992) Comparison of extracellular enzyme activities among different strains of *Lentinus edodes* grown on sawdust-based cultures in relationship to their fruiting abilities. Mokuzai Gakkaishi 38: 310–316.

Ohga S (1999a) Effect of water potential on fruit body formation of *Lentinula edodes* in sawdust-based substrate. J Wood Sci 45: 337–342

Ohga S (1999b) Evaluation of maturity by use of pH indicators in sawdust-based cultures of *Lentinula edodes*. J Wood Sci 45: 431–434

Ohga S, Smith M, Thurston C, Wood DA (1999) Transcriptional regulation of laccase and cellulase genes in the mycelium of *Agaricus bisporus* during fruit body development on a solid substrate. Mycol Res 103: 1557–1560

Oita S, Yanagi O (1993) Stimulation of *Schizophyllum commune* fruit body formation by inhibitor of membrane function and cell wall synthesis. Biosci Biotech Biochem 57: 1270–1274

Olesnicky NS, Brown AJ, Dowell SJ, Casselton LA (1999) A constitutively active G-protein-coupled receptor causes mating self-compatibility in the mushroom *Coprinus*. EMBO J 17: 2756–2763

Pardo EH (1995) Organisation of the *A* mating type locus of *Coprinus cinereus*. PhD thesis, University of Oxford, Oxford

Pardo EH, O'Shea SF, Casselton LA (1996) Multiple versions of the *A* mating type locus of *Coprinus cinereus* are generated by three paralogous pairs of multiallelic homeobox genes. Genetics 144: 87–94

Penas MM, Ásgeirsdóttir SA, Lasa I, Culianez-Macià FA, Pisabarro AG, Wessels JGH, Ramírez L (1998) Identification, characterization, and in situ detection of a fruitbody-specific hydrophobin of *Pleurotus ostreatus*. Appl Environ Microbiol 64: 4028–4034

Poppe J, Heungens K (1991) First commercial growing of ornamental mushrooms and its use in floristry. In: Maher MJ (ed) Science and cultivation of edible fungi, vol 2. Balkema, Rotterdam, pp 821–830

Poppe JA, Hofte M (1995) Twenty wastes for twenty cultivated mushrooms. In: Elliott TJ (ed) Science and cultivation of edible fungi, vol I. Balkema, Rotterdam, pp 171–179

Rajarathnam S, Shashireka MN, Bano Z (1993) Biopotentialities of the basidiomycetes. Adv Appl Microbiol 37: 233–361

Raper CA, Miller RE, Raper JR (1972) Genetic analysis of the life cycle of *Agaricus bisporus*. Mycologia 64: 1088–1117

Raper JR, Boyed DH, Raper CA (1965) Primary and secondary mutations at the incompatibility loci in *Schizophyllum commune*. Proc Natl Acad Sci USA 53: 1324–1332

Redhead SA (1997) The pine mushroom industry in Canada and the United States: why it exists and where it is going. In: Palm ME, Chapela IH (eds) Mycology in sustainable development: expanding concepts, vanishing borders. Parkway Publishers, Boone, N.C., pp 15–54

Redhead SA, Norvell LL Danell E (1997) *Cantharellus formosus* and the pacific golden chanterelle harvest in western North America. Mycotaxonomy 65: 285–322

Reijnders AFM (1979) Developmental anatomy of *Coprinus*. Persoonia 10: 383–424

Richartz G, MacLellan A (1987) Action spectra for hyphal aggregation, the first stage of fruiting, in the basidiomycete *Pleurotus ostreatus*. Photochem Photobiol 45: 815–820

Robertson CI, Bartholomew KA, Novotny CP, Ullrich RC (1996) Deletion of the *Schizophyllum commune Aα* locus: the roles of *Aα Y* and *Z* mating-type genes. Genetics 144: 1437–1444

Ross IK (1982a) Localization of carpophore initiation in *Coprinus congregatus*. J Gen Microbiol 128: 2755–2762

Ross IK (1982b) The role of laccase in carpophore initiation in *Coprinus congregatus*. J Gen Microbiol 128: 2763–2770

Royse DJ (1997) Speciality mushrooms: consumption, production and cultivation. Rev Mex Micol 13: 1–11

Samadder PP, Sen K, Kurosawa S (1997) Isolation and characterization of *Schizophyllum commune* mutants resistant to indole and caffeine. FEMS Microbiol Lett 150: 277–282

Santos C, Labarère J (1999) *Aa-Pri2*, a single-copy gene from *Agrocybe aegerita*, specifically expressed during fruting initiation, encodes a hydrophobin with a leucine-zipper domain. Curr Genet 35: 564–570

Schlosser WE, Blatner KA (1995) The wild edible mushroom industry of Washington, Oregon and Idaho. J For 93: 31–36

Scrase RJ, Elliott TJ (1998) Biology and technology of mushroom culture. In: Wood BJB (ed) Microbiology of fermented food, vol 2, 2nd edn. Blackie, London, pp 543–584

She QB, Ng TB Liu WK (1998) A novel lectin with potent immunomodulatory activity isolated from both fruiting bodies and cultured mycelia of the edible mushroom *Volvariella volvaceae*. Biochem Biophys Res Commun 247: 106–111

Stahl U, Esser K (1976) Genetics of fruit-body production in higher basidiomycetes. I. Monokaryotic fruiting and its correlation with dikaryotic fruiting in *Polyporus ciliatus*. Mol Gen Genet 148: 183–197

Stone AL, Kroeger M, Sang QX (1999) Structure-function analysis of the ADAM family of disintegrin-like and metalloproteinase-containing proteins (review). J Protein Chem 18: 447–465

Stoop JMH, Mooibroek H (1999) Advances in genetic analysis and biotechnology of the cultivated button mushroom, *Agaricus bisporus*. Appl Microbiol Biotechnol 52: 474–483

Subramanian CV (1995) Mushrooms: beauty, diversity, relevance. Curr Sci 69: 986–998

Swamy S, Uno I, Ishikawa T (1984) Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 130: 3219–3224

Takemaru T, Kamada T (1972) Basidiocarp development in *Coprinus macrorhizus* I. Induction of developmental variations. Bot Mag 85: 51–57

Tymon AM, Kües U, Richardson WVJ, Casselton LA (1992) A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain. EMBO J 11: 1805–1813

Umar MH, Van Griensven LJLD (1998) The role of morphogenetic cell death in histogenesis of the mycelial cord of *Agaricus bisporus* and in the development of macrofungi. Mycol Res 102: 719–735

Umar MH, Van Griensven LJLD (1999) Studies on the morphogenesis of *Agaricus bisporus*: the dilemma of normal versus abnormal fruit body development. Mycol Res 103: 1235–1244

Van der Valk P, Marchant R (1978) Hyphal ultrastructure in fruitbody primordia of the basidiomycetes *Schizophyllum commune* and *Coprinus cinereus*. Protoplasma 95: 57–72

Walser PJ, Hollenstein M, Klaus MJ, Kües U (2000) Genetic analysis of basidiomycete fungi. In: Talbot NJ (ed) Molecular and cell biology of filamentous fungi: a practical approach. (Practical approach series) IRL Press, Oxford (in press)

Wang HX, Ng TB, Ooi VEC (1998) Lectins from mushrooms. Mycol Res 102: 897–906

Wannet WJB, Aben, EMJ, van der Drift C, Van Griensven LJLD, Vogels GD, Op den Camp HJM (1999) Trehalose phophorylase activity and carbohydrate levels during axenic fruiting in three *Agaricus bisporus* strains. Curr Microbiol 39: 205–210

Waser SP, Weis AL (1997) Medicinal mushrooms. *Lentinus edodes* (Berk) Sing (Shiitake mushroom). Peledfus Publishing, Haifa, Israel

Wessels JGH (1993) Fruiting in higher fungi. Adv Microb Physiol 34: 147–202

Wessels JGH (1997) Hydrophobins: proteins that change the nature of the fungal surface. Adv Microb Physiol 38: 1–45

Wessels JGH, Dons JJM, de Vries OMH (1985) Molecular biology of fruit body formation in *Schizophyllum commune*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, Cambridge, pp 485–497

Williams MAJ, Beckett A, Read ND (1985) Ultrastructural aspects of fruit body differentiation in *Flamminula velutipes*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, Cambridge, pp 429–450

Yamanaka K (1997) I. Production of cultivated edible mushrooms. Food Rev Int 13: 327–333

Yasuda T, Ishihara H, Amano H, Shishido K (1997) Generation of basidiomycetous hyphal cell-aggregates by addition of the Arg-Gly-Asp motif-containing fragment of high-molecular-weight cell-adhesion protein MFBA derived from the basidiomycete *Lentinus edodes*. Biosci Biotechnol Biochem 61: 1587–1589

Yatohgo T, Nakata M, Tsumuraya Y, Hashimoto Y, Yamamoto S (1988) Purification and properties of a lectin from the fruitbodies of *Flamminula velutipes*. Agric Biol Chem 52: 1485–1493.

Yaver DS, del Carmen Overjero M, Xu F, Nelson BA, Brown KM, Halkier T, Bernauer S, Brown SH, Kauppinen S (1999) Molecular characterization of laccase genes from the basidiomycete *Coprinus cinereus* and heterologous expression of the laccase Lcc1. Appl Environ Microbiol 65: 4943–4948

Yli-Mattila T (1991) Photobiology of fruit body formation in *Schizophyllum commune*. Luonnen Tutkija 95: 116–123

Zhang J, Wang G, Li H, Zhuang C, Mizuno T, Ito H, Suzuki C, Okamoto H, Li JX (1994) Antitumor polysaccharides from a chinese mushroom, "Yuhuangmo", the fruiting body of *Pleurotus citrinopileatus*. Biosci Biotech Biochem 58: 1195–1201

Zhao J, Kwan HS (1999) Characterization, molecular cloning, and differential expression analysis of laccase genes from the edible mushroom *Lentinula edodes*. Appl Environ Microbiol 65: 4908–4913