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Advanced Biotechnological Procedures of Mushroom Cultivation

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The main aim of this work consists in screening the optimal biotechnology of edible and medicinal mushroom growing through the solid-state cultivation and the submerged fermentation by recycling different kind of wastes coming from cereal crop processing as well as winemaking industry. The both fermentation biotechnologies were tested through the controlled cultivation of medicinal mushrooms *Ganoderma lucidum* (Curt.:Fr.) P. Karst (folk name: Reishi or Ling-zhi), *Lentinula edodes* (Berkeley) Pegler (folk name: Shiitake) and *Pleurotus ostreatus* (Jacquin ex Fries) Kummer (folk name: Oyster Mushroom) on different growing substrates made of cereal and grape wastes.

In the first part of this chapter, the laboratory biotechnology of recycling the winery and vineyard wastes by using them as a growing source for edible mushrooms is presented. The biotechnological procedure of continuous controlled cultivation of medicinal mushrooms by submerged fermentation of different sorts of bran and broken seeds resulted from the industrial food processing of wheat, barley and rye seeds is described in the second part of the chapter. Taking into consideration that most of the edible and medicinal mushrooms species requires a specific micro-environment including complex nutrients, the influence of all physical and chemical factors upon fungal biomass production and mushroom fruit bodies formation has been studied by testing new biotechnological procedures.

Comparative results of the chemical investigations regarding some biological active compounds of both fungal pellets from submerged fermentation and mushroom fruit bodies from solid state cultivation are presented. In the last part of this chapter, the biotechnology concerning the controlled cultivation of edible mushrooms in continuous flow by using a modular robotic system is presented.

Keywords: biotechnology, solid-state mushroom cultivation, submerged fermentation, robotic system of mushroom cultivation

Cultivation of *Ganoderma lucidum* and *Grifola frondosa* and Production of their Pharmaceutical Active Compounds

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Basidiomycetes mushrooms comprise a vast but largely untapped source of new pharmaceutical products in fruit bodies, cultured mycelium and culture broth. They are of different chemical composition, such as polysaccharides, glycopeptide-protein complexes, proteoglycans, proteins and triterpenoids, with most scientific attention focussed to the group of non-cellulosic β-glucans with β-(1-3) linkages in the main chain of the glucan, and additional β-(1-6) branch points, that are characteristic for the antitumor and immuno-stimulating action. Mushroom polysaccharides do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host.

Their mechanisms of action involve them being recognized by several immune cells receptors as non-self molecules, so the immune system is stimulated by their presence. Structurally different β-glucans have different affinities toward receptors and thus generate different host responses. Immunomodulating and antitumor activities of these metabolites are related to immune cells such as hematpoietic stem cells, lymphocytes, macrophages, T cells, dendritic cells, and natural killer cells, involved in the innate and adaptive immunity, resulting in the production of biologic response modifiers. Clinical evidence for antitumor and other medicinal activities come primarily from some commercialised purified polysaccharides, such as lentinan from Shiitake - *Lentinula edodes,* krestin from *Coriolus versicolor*, grifolan from *Grifola frondosa*, and schizophyllan from *Schizophyllum commune*, but polysaccharide preparations of some other medicinal mushrooms also show promissing results.

This chapter reviews cultivation possibilities, and focusses on biochemical and medicinal studies of two basidiomycetes with promissing pharmacological effects – *Ganoderma lucidum* and *Grifola frondosa*.

Modern View on Current Status, Future Trends, and Unsolved Problems in Studies of Medicinal Mushrooms

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The target of the present review is to draw attention to many critically important unsolved problems in the future development of medicinal mushroom science in the 21st century. Special attention is paid to mushroom polysaccharides. Many, if not all, higher Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and cultured broth. The data on mushroom polysaccharides are summarized for approximately 700 species of higher Hetero- and Homobasidiomycetes.

The chemical structure of polysaccharides and its connection to antitumor activity, including possible ways of chemical modification, experimental testing and clinical use of antitumor or immunostimulating polysaccharides, and possible mechanisms of their biological action are discussed. Numerous bioactive polysaccharides or polysaccharide-protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses, and exhibit antitumour activities in animals and humans.

Stimulation of host immune defense systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation, and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumour cells.

Whilst the mechanism of their antitumor actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Particularly, and

most importantly for modern medicine, are polysaccharides with antitumor and immunostimulating properties. Several of the mushroom polysaccharide compounds have proceeded through Phase I, II, and III clinical trials and are used extensively and successfully in Asia to treat various cancers and other diseases. A total of 126 medicinal functions are thought to be produced by medicinal mushrooms and fungi including antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, anti-hypercholesterolemia, antiviral, antibacterial, anti-parasitic, antifungal, detoxification, hepato-protective, and anti-diabetic effects.

Keywords: medicinal mushrooms, polysaccharides, beta-glucans, antitumor, immunomodulating, antioxidant activities

Volvariella volvacea – an Oddity Among Cultivated Mushrooms?

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The edible straw mushroom, *Volvariella volvacea*, is cultivated in tropical and sub-tropical regions, particularly in countries of Southeast Asia, and currently ranks sixth in terms of annual production. It is sometimes referred to as the "warm mushroom" because of its relatively high growth temperature (optimum temperatures for vegetative growth, and for the formation of primordia and fruit body development, are 30-35^{O}C and 30-32^{O}C, respectively). However, biological efficiency values (i.e. conversion of growth substrate into mushroom fruit bodies) are still very low (about 10% on rice straw) compared with many of the major cultivated edible mushroom species. Significant improvements in yields (up to 40%) have been achieved following the introduction of high cellulose cotton waste 'composts', and *V. volvacea* is equipped with a consortium of enzymes necessary to hydrolyse cellulose to glucose (i.e. endo-1,4-β-glucanase, cellobiohydrolase and β-glucosidase).

Several different isoforms of these enzymes are synthesized when *V. volvacea* is grown in the presence of a cellulosic substrate in both submerged culture in the laboratory, and in solid-state cultivation systems representative of those used for industrial cultivation. Conversely, the mushroom appears to have a limited ability to degrade the lignin component of lignocellulosic materials commonly used for mushroom cultivation. No lignin peroxidase or manganese-dependent peroxidase activity (two key enzymes in lignin degradation) have so far been detected in *V. volvacea* cultures grown under a variety of conditions. However, two isoforms of laccase, another enzyme long associated with lignin degradation, are produced when the fungus is grown either in submerged culture or on cotton waste "composts", but expression profiles suggest roles in sporophore development and detoxification rather than delignification. More recently, eleven 'laccase' sequences have been identified within a draft *V. volvacea* genomic sequence although, since the encoded proteins have been isolated and the catalytic function established in only two cases, nine of these 'laccase' sequences must, for the present, remain tentative. Another two features associated with *V. volvacea* that are not shared by other cultivated mushrooms is the loss of viability occurring when fungal mycelium is maintained at temperatures below 15°C, and autolysis of the mushroom fruit body when stored at 4°C.

Recent data have indicated that low expression levels of a gene encoding for S-adenosyl-L-homocysteine hydrolase may be linked to the low temperature sensitivity of *V. volvacea* mycelium. In keeping with its other unusual features, *V. volvacea* is reported to be a primary homothallic fungus, a comparatively much less frequent sexuality pattern among basidiomycetes. Here, homokaryotic mycelium, derived from a single meiotic parent, has the potential to undergo transition to the dikaryotic morphology and complete the sexual cycle.

However, unlike the other defined sexuality patterns, the genetic determinants controlling sexuality in *V. volvacea* have yet to be identified and their relationship to heterothallic counterparts clarified. Finally, although *V. volvacea* has traditionally been assigned to the family Pluteaceae *(*Agaricales*,* Basidiomycota*)*, in a more recent reassessment of the taxonomic status of the genus *Volvariella* using data based on nuclear large subunit (nLSU), nuclear small subunit (nSSU) and internal transcribed spacer (ITS) data, most representatives of this genus fell outside the Pluteoid clade.

Cultivation of Medicinal Almond Mushrooms, *Agaricus subrufescens*, in Europe

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In Europe there is a new interest for the cultivation of almond mushrooms (*Agaricus blazei*, or *A. brasiliensis* or *A. subrufescens*) and their use as nutraceutical food or source of bio products and substances of interest in diseases prevention or treatment. One of the challenges is to adapt the cultivation method used for the (*A. bisporus*) for a rapid development of the new crop using the same facilities than the conventional one.

The lecture present some progresses in the field of (i) the selection of strains adapted to button mushroom composts, (ii) the identification of the parameters for fruiting induction and fruiting body development, (iii) the nature and composition of the metabolites produced by the almond mushrooms under these conditions and their toxicological or pharmacological effects.

Natural Biodiversity and Molecular Genetics for Breeding *Agaricus sp.*

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Cultivation of edible mushrooms is the premier agricultural application of mycology. The use of the natural biodiversity and molecular genetics for breeding might contribute significantly to win several challenges on management of fruiting induction, fungal diseases, or food quality which growers and mushroom industry actors are faced with. This lecture deals mainly with the saprophytic edible mushrooms belonging to the genus *Agaricus* and highlights the button mushroom *Agaricus bisporus*.

The importance of wild germplasm in *Agaricus* breeding is discussed by stressing the interest of phylogeny for identifying new interesting species or varieties, and the lack of diversity in the cultivated strains whereas a genetic and phenotypical diversity is now available in *Agaricus* collections. Breeding strategies adapted to their life cycles and using molecular markers and quantitative genetics are proposed for genetic improvement of *Agaricus* strains in the era of genomics.

Major Diseases Affecting the Button Mushroom *Agaricus bisporus* during Commercial Production and Investigations for their Biological Control

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The button mushroom, *Agaricus bisporus*, is for many decades the most common cultivated mushroom and is consumed throughout the world. *Agaricus bitorquis*, more adapted to hot temperatures, also counts for edible mushroom produced at commercial scale. More recently, cultivation of the Brazilian almond mushroom (formerly *Agaricus blazei*, then renamed *A. braziliensis*, *A. subrufescens*) increased due to the medicinal properties of the mushroom. Agaricus spp. are susceptible to a wide range of virus, bacterial, and fungal diseases.

Interaction between *A. bisporus* and the causal agents responsible for the most severe diseases, namely the bacteria *Pseudomonas tolaasii* and *Pseudomonas reactans* and the fungi *Trichoderma aggressivum* and *Lecanicillium fungicola,* was extensively studied. Several works deal with pathogens affecting A. *bitorquis* and the Brazilian medicinal mushroom. The lecture synthesizes data on biological disorders of Agaricus spp. and present recent work providing tracks for biological control.

Exploitation of biotechnological potential of agro-industrial residues and by-products through mushroom cultivation

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A huge amount of lignocellulosic agricultural crop residues and agro-industrial by-products are annually generated, rich in organic compounds that are worthy of being recovered and transformed. Mushroom cultivation presents a worldwide expanded and economically important biotechnological industry that can use efficiently both liquid or solid-state-fermentation of differing composition (in terms of polysaccharide/lignin ratio) organic waste materials for food protein recovery or/and the production of added-value products (mycelial biomass, mushrooms, enzymes and medicinal compounds).

As the ability of the different mushroom species to utilize various substrates depends on both mushroom-and substrate-associated factors, e.g. examination of the lignocellulolytic enzymes profiles of the important commercially cultivated mushrooms *Agaricus bisporus, Volvariella volvacea, Pleurotus* spp., *Lentinula edodes* and *Ganoderma* spp. demonstrates varying abilities to utilize different lignocellulosics as growth substrates.

Several aspects of mushroom physiology, nutrition and cultivation are outlined, and several of our experiments will be presented in both solid–state and liquid-state cultivation, focusing mainly on their efficiency of substrate conversion into mycelial biomass, metabolites (exopolysacchirides, enzymes etc.) and fruiting bodies. The studies comprise: (a) the production of biomass, extra- and intra-cellular polysaccharides (EPS, IPS) of *Ganoderma lucidum, G. applanatum, Auricularia auricula, Agrocybe aegerita, Pleurotus ostreatus, P. pulmonarius, Flammulina velutipes, Lentinula edodes and Volvariella volvacea* medicinal fungi during their growth on glucose based substrate in liquid cultures, (b) experimental data concerning evaluation of lignocellulosic wastes for production of the important edible and medicinal mushrooms genera *Pleurotus, Ganoderma* and *Lentinula*, through examination of their growth rates and conversion efficacy to fruiting bodies (b) investigations of the production of mycelial biomass and extracellular enzymes (laccase and endoglucanase secretion) during solid-state fermentation (SSF) of agricultural residues by *Ganoderma* and *Lentinula* and of the impact of agro-residues properties on bioconversion process.

Keywords: *Pleurotus* spp., *Lentinula edodes*, *Ganoderma* spp., growth rate, biomass, enzymes, EPs, IPS, fruit-bodies

Spent mushroom substrate for degrading organochlorine pesticides like endosulfan and chlorothalonil

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White rot mushrooms have an excellent biochemical machinery to degrade lignin. The enzyme system involved allows mycelium to colonize agricultural by-products and produce mushrooms. Commercially, once the colonized substrate has produced two or three flushes of carpophores, the spent substrate is discarded or used for land filling.

One alternative use could be bioremediation, due to the important amount of enzymes still able to catalyze important reactions like the breakdown of pesticides. In this case we will explain some results obtained while using *Pleurotus pulmonarius* spent substrate to degrade the insecticide endosulfan and the fungicide chlorothalonil.

Low input technology for pasteurizing substrate for Oyster mushroom production

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Pasteurization is needed to protect substrate used to produce Oyster mushrooms *Pleurotus* spp against antagonistic microorganisms. Normally this protection is given by supplying heat to the substrate in order to raise temperature and kill thermo sensitive microorganisms.

The energy used represents and important part of the mushroom production costs. In this case we will discuss about low input alternatives already employed like alkaline disinfection and self-heating pasteurization. These technologies are well adapted to rural mushroom farms and have a low energy cost.

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