Exploitation and Utilization of Wild Fujian *Ganoderma duropora* (Sect. *Phaeonema*) Resource

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In recent years, a strain of wild *Ganoderma duropora* Lloyd. (Sect. *Phaeonema* in Subgen. *Ganoderma*) in the Fujian province, was successfully cultivated; was applied and is popularly known on a wider scale. The micro-morphology was observed by a scanning electron microscope (SEM); the basdiospores were similar to *Ganoderma sinense*, with a size of 6.84–7.37 × 10.26–11.05 μm. In addition, kind of globoid spores (about 2 μm in diameter) were first observed in the hymenium, with a little peduncle (about 1 μm in length) and an acanthoid appendix around the surface. "S2 (Hei)" was officially identified as *G. duropora* with exemplar number HAMS 95275. The strain "S2 (Hei)" was applied and is commonly used for log-cultivation with laburnum on a large scale, in 2004 and 2005. The cultivated yield (in the first year) reached 22.03–24.10 kg/M^3, in the average of 22.03 kg/M^3. The fruit bodies were processed into extract powder by circulating-extraction with ultrasonic wave, concentrating under sub-vacuum conditions and high-speed centrifugal spray-dry. The results showed that the content of moisture, crude protein, total polysaccharide, and ash in the extract powder were 4.4%, 19.1%, 36.2%, and 7.64%, respectively, while the content of heavy metal elements Pb, As, Cd, and Hg were less than 0.5 μg/g, 0.68 μg/g, 0.011 μg/g, and 0.44 μg/g, respectively; hygienic standards complied with *Green Trade Standards of Importing & Exporting Medicinal Plants & Preparations* (WM2-2001). The polysaccharides of the fruit bodies were extracted by ultrasonic circulating technique. Major monosaccharides in the polysaccharide were glucose, mannose, and galactose, with a molar ratio of 0.39: 4.48:1, by gas chromatography analysis.

Artificial Cultivation of *Wolfiporia cocos*

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Wolfiporia cocos Wolf (Polyporaceae), also known as Fu Ling, Tuckahoe, Indian Bread and Hoelen, is a well known medicinal mushroom, the sclerotia of which has been used for centuries in Traditional Chinese Medicine. Varying in color from white to red, *W. cocos* is found growing on the roots of dead pine trees and has traditionally been used as a "tonic" to benefit the internal organs and overall health.

The main chemical constituents of *Poria cocos* include: beta-pachymarose, several organic acids such as tumulos acid, eubricoic acid, pinicolic acid, pachymic acid, 3-betahydroxyanosta-7, 9(11): 24-trien-21-oic acid, chitin, protein, fat, glucose, sterols, lecithin, and choline. This mushroom is commonly used to treat fatigue, diarrhea, dizziness, edema, insomnia, nervousness, urination difficulties, weakness, kidney-related disease, and lack of

energy. It has also shown potential in lowering blood sugar, and has anti-bacterial, immune enhancing, and anti-cancer properties. Given its wide array of chemical constituents, it is of no surprise that such far-reaching therapeutic applications have not only been proposed for the use of *W. cocos*, but in fact, have been already been exploited by both the traditional and modern medical communities of China, Japan, and other Asian countries.

Here, we will be examining the lifecycles of the mushrooms, from spore to fruitbody as well as the potential benefits that it offers to mankind. Furthermore, we will assess methods of artificially cultivating the sclerotia of the mushroom and magnifying the potential benefits of the *W. cocos* mushroom through various means, such as culture parameter modification.

Scaling up of *Schizophyllum commune* Exopolysaccharides: From Shake Flask to Bench Top Bioreactor

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The Egyptians, as far back as 3,000 BC, believed that mushrooms were a sacred food that prolonged life. A mummified 5,000-year old "Ice-man", found in the mountains of Europe, carried a medicine kit of dried mushrooms. Indeed the oldest written record of mushrooms as medicines is in an Indian medical treatise from 3,000 BC. Medicinal mushroom research has focused on the discovery of compounds, mainly polysaccharides that can modulate, positively or negatively, the biological response of immune cells. These compounds, which appear to stimulate the human immune response, are being investigated for the treatment of cancer, immunodeficiency diseases, or generalized immunosuppression following drug treatment. They are also sought for combination therapy with antibiotics and as adjuncts for vaccines. Polysaccharides are regarded as biological response modifiers (BRM). This basically means that they cause no harm and place no additional stress on the body, but help the body to adapt to various environmental and biological stresses.

These polysaccharides are produced by a wide variety of species. However, we have tapped into years of experimentation of *Schizophyllum commune* Fr.: Fr. (higher Basidiomycetes), which is a widely-distributed species that has been studied extensively in genetics and physiology for the past 50 years. It is easily manipulated and contains no known mycotoxins. Among other studies, the liquid cultured mycelium of this mushroom has been reported to contain useful bioactive polysaccharides, which has been identified as glucan in nature. From *Sch. commune* β-glucan (medium product) an antitumor agent (SPG, Schizophyllan) was developed in Japan.

In the present study, *Sch. commune* was scaled up from shake flasks to a bench top bioreactor for production of exopolysaccharides. Morphological and molecular characterizations were accomplished. The biological activity of the EPS (exopolysaccharides) has been determined by testing the direct anti-tumor effect of polysaccharides against leukemia cancer cell lines. This will be the first step towards investigating the possible mechanism of action.

After demonstrating that *Sch. commune* synthesizes exopolysaccha-rides on glucose-rich media, the feasibility of scaling up of polysaccharide production from Petri dishes to shake flasks to a 15 L bioreactor was evaluated. Batch cultivation resulted in maximum yields of 17 g/L of exopolysaccharides. Different concentrations of polysaccharide showed cytotoxic activity against leukemia cancer cell lines. Submerged cultures of fungi have potential application value since some medicinal mushrooms cannot be cultivated using solid state fermentation. In addition, a higher yield of polysaccharides can be obtained using the fed batch culture method. The methods developed in this study can be applied to large-scale production of polysaccharides by a variety of filamentous fungi.

Production of Xylanase by *Flammulina velutipes* Grown on Different Carbon Sources

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Xylan, the most plentiful of the hemicelluloses, is present in the cell walls of all land plants and is particularly abundant in tissues that have undergone secondary thickening. It is composed of 150–200 D-xylonopyranose units joined by β,4-1inkages. Xylanolytic enzymes of microorganisms have received a great deal of attention in the last ten years mainly due to their potential application in the food, feed, and pulp and paper industries. Xylanolytic enzymes can be used in processes where xylan is to be depolymerized, including hydrolysis of xylan-lignin complexes in pulp to facilitate chemical pulp bleaching or the partial hydrolysis of xylans to xylo-oligosaccharides, which can be used as moisture-preserving food additives. Complete enzymatic hydrolysis of plant heteroxylans involves the action of several enzymes, of which l,4-β-xylanase (1,4-β-xylan xylohydrolase, EC 3.2.1.8) is the crucial enzyme for general xylan depolymerization. The purpose of this study is concerned with the effect of carbon sources and their concentrations on the production of xylanase by the edible mushroom *Flammulina velutipes*. Xylanase activity was checked using oat spelt xylan as a substrate, and the reducing group was detected by the dinitrosalicylic acid assay method. Eight different carbon sources (rice husk, wood straw, glucose, sucrose, starch, xylose, and xylan) were used in the range of 0.2, 0.4, and 0.6. Among the carbon sources used, the maximum xylanase production was recorded at 5.3 U/ml when 0.6% xylose was incorporated in the fermentation medium. The level of enzyme activity produced on other carbon sources appeared to be constitutive.

Cultivation of *Pleurotus ostreatus* on Substrates with Grains from Brewery Waste

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Spent brewery grains (SBG) are leftovers from the brewery industry and have no economic advantage for further uses. In the fresh state, grains have approximately 80% water content and therefore cannot be stored for long periods of time and must be used as soon as possible. In Slovenia, used as animal feed, grains can be stored for longer periods of time but only in a dry state. For drying a lot of energy must be used, representing a financial burden to the brewery industry. In this study, fresh SBG containing substrates were used to determine the growth rate and biological efficiency of harvested fruit bodies of *Pleurotus ostreatus* mycelium.

Substrates for cultivation containing different proportions of spent brewery grains, wheat bran (WB), beech sawdust (BS), and lime were prepared. All substrates had 65% water content, were filled into glass jars covered with punctured lid, and sterilized. After inoculation with a wild strain of *P. ostreatus* mycelium culture, the substrate was incubated at 24°C. Data to determine suitability of substrates for mycelium overgrowth was collected. When fully overgrown (after 30 days), they were transferred to a mushroom cultivation room where the yield of harvested fruit bodies was determined.

Of all tested substrates, the highest mycelium growth rate was determined on substrates containing 30% WB, 68% BS, and 2% lime. However, minimum overgrowth rate was determined on substrate containing 40% SBG, 30% WB, and 2% lime. Substrates containing SBG had maximum mycelium growth in combination with 10% SBG, 20% WB, 68% BS, and 2% lime. Visually observed mycelium density was increased with higher portions of WB and SBG, while growth rate was decreasing at higher proportions of WB and SBG. Highest biological efficiency of obtained fruit bodies (51%) was determined on substrate containing 20% WB, 10% SBG, and 2% lime.

According to the results of the experiment, we can conclude that substrates consisting of 20% WB, 10% SBG, and 2% lime are the most appropriate for mushroom cultivation. Testing growth rates with SBG, substrates containing 10% SBG, 20% WB, 68% BS, and 2% lime could be used for animal feed due to its maximum mycelium growth rate. In further experiments different strains should be tested to determine their ability for cultivation on SBG-containing substrates.

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Worldwide Green Mold Disease of the Oyster Mushroom (*Pleurotus ostreatus*) is Caused by Two New Species of *Trichoderma*, *T. fulvidum* and *T. pleurotophilum*

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Certain species of the filamentous fungal genus *Trichoderma* are known as the causative agents of green mold disease resulting in serious losses in *Agaricus* production. The most aggressive strains were originally identified as *T. harzianum* Th2 and Th4 in Ireland and North-America, respectively, but subsequently recognized as the separate species *T. aggressivum* f. sp. *europaeum* and f. sp. *aggressivum*. *Pleurotus ostreatus* is an edible mushroom cultivated on a large scale worldwide. In recent years, severe green mold epidemics of *P. ostreatus,* causing serious crop losses, were reported in South Korea, Italy, Hungary, and Romania (Transylvania). Since Hungary is one of the most important mushroom producers in Europe, and mushroom production and export form significant proportions of Hungarian agriculture and commercialism, the purpose of this study was to identify and characterize the causative agents of the disease and therefore aiming at the development of a method of protection based on biological control.

A large number of *Trichoderma* strains have been isolated from *Pleurotus* substrate samples, derived from substrate producing and growing companies in Hungary, and two *Pleurotus* farms in Romania. *In vitro* confrontation tests were carried out between *P. ostreatus* and *Trichoderma* isolates; *Trichoderma* strains most aggressive towards *P. ostreatus* were characterized by molecular techniques. A PCR-based test, designed previously for the specific identification of *T. aggressivum,* was performed with negative results. Sequence analysis of the internal transcribed spacers (ITS1 and 2) region confirmed that the isolated strains did not belong to the species *T. aggressivum.* The fungal isolates were identified to be co-specific with a still formally undescribed phylogenetic species, *Trichoderma* sp. DAOM 175924. ITS1 and two sequences of the strains belonging to this species proved to be highly similar or identical with those for *Trichoderma* pathogens of *P. ostreatus* in South

Korea, indicating that the green mold disease of *P. ostreatus* in Hungary and Transylvania is due to the same *Trichoderma* species as in South Korea. The group of isolates co-specific to *T.* sp. DAOM 175924 can be divided into two alleles of ITS2 sequences based on a single A/C transversion. Interestingly, all the representatives of this species that were isolated from the winter wheat rhizosphere of South Hungarian agricultural fields contain "A" at this position, while the isolates deriving from Hungarian and Transylvanian *Pleurotus* substrate samples belong almost exclusively to the other, the "C" type. The subsequent sequence analysis of translation elongation factor 1-alpha (*tef1*) and endochitinase *chi18-5* genes showed that these two types of isolates belong to two clearly diverged phylogenetic species. They also differ from each other based on morphological and physiological features, and therefore have recently been described as the new species *T. fulvidum* sp. nov. and *T. pleurotophilum* sp. nov., respectively.

A large-scale sampling program has been initiated in order to identify the possible sources of the green mold infection. The closest environment of a Hungarian substrate-producing and *Pleurotus*-growing company has been examined. This included sampling of the wheat straw derived from different locations, the soil within the area of the company, the substrate bags inoculated with different strains of *P. ostreatus* as well as the "flow-down" water, which is the by-product of the pre-fermentation phase of wheat straw. Further progress of the project will be presented and discussed.

Several bacterial strains were isolated from wheat straw used for producing substrates for *P. ostreatus* cultivation. They were tested in *in vitro* confrontation assays against *T. pleurotophilum* and *P. ostreatus*. Out of the 28 bacterial isolates tested, 15 were found to inhibit the growth of *Trichoderma* without significantly affecting *Pleurotus*. These strains might be potential candidates of biological control strategies for the prevention of losses in *P. ostreatus* cultivation.

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Lignin, Cellulose, and Hemicellulose Degrading Enzyme Production by Selected Polypores Grown on Wheat Straw

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In recent years, extensive research on Basidiomycetes has increased markedly, mainly due to the potential use of these fungi in the production of enzymes as well as is the bioconversion of lignocellulose and plant raw materials into food, animal feed, etc.

The present study was conducted to investigate the enzymatic bioconversion properties of wheat straw by selected white rot fungi from the genus *Grifola* and *Polyporus*. *Grifola frondosa* (MBFBL26), *Grifola umbellata* (MBFBL690), and two strains of *Polyporus squamosus* (MBFBL1165 and MBFBL456) were investigated. The aim was to determine the rate and amount of enzyme production as well as measure the degradation of wheat straw over a period of 60 days. Assays for lignin, cellulose, and hemicellulose degrading enzymes, as well as substrate analysis for loss of organic matter (LOM) were conducted at day 16, 30, 44, and 60 from day one of experiment.

Lignin-modifying enzymes: Laccase, manganese-dependent and manganese-independent peroxidase activities were highest in all strains on day 16 of cultivation. The order of laccase activity was *P. squamosus* (MBFBL1165) > *P. squamosus* (MBFBL456), > *Grifola umbellatus* (MBFBL690) > *Grifola fron-*

dosa (MBFBL26), with activity values of 2186, 1672, 1060, and 385 U/I, respectively. Manganese-dependent peroxidase (MnP) activities occurred in each strain in the following order: MBFBL456 > MBFBL1165 > MBFBL26 >MBFBL690, with values of 64, 48, 18, and 12 U/I, respectively. Manganese-independent peroxidase (MnIP) activity occurred in each strain in the following order: MBFBL1165 > MBFBL456 >MBFBL26 >MBFBL690, with values of 37, 29, 13, and 11 U/I, respectively.

Cellulose degrading enzymes: Carboxymethylcellulase (CM-cellulase), exo-1,4-β-glucanase (Cellobiosidase), and 1,4-β-glucosidase were assayed. CM-cellulase and Cellobiosidase activities were higher in the *P. squamosus* strains than in the *Grifola* spp. tested. The activities of these enzymes peaked at 44 days of cultivation.1,4-β-glucosidase activity was highest in all strains after 60 days of cultivation. *G. umbellata* (690) had the highest enzyme activity (181 U/I), followed by *G. frondosa* 26 (136 U/I). *P. squamosus* strains 456 and 1165 had values of 123 and 116 U/I, respectively.

Hemicellulose degrading enzymes: Xylanase activity was highest after 60 days of cultivation in all strains tested. However, *P. squamosus* strains, MBFBL456 and MBFBL1165, had much higher enzyme activity (890 and 638 U/I, respectively) compared to *G. umbellata* (MBFBL690) and *G. frondosa* (MBFBL26), which showed 572 and 362 U/I activities, respectively. 1,4-β-xylosidase activity was the only enzyme the two *Grifola* strains showed higher enzyme activities (MBFBL690 and MBFBL26 with values 72 and 72 U/I, respectively) compared to the *P. squamosus* strains MBFBL, MBFBL456, and MBFBL1165, with values of 57 and 52 U/I, respectively.

The highest LOM was 36%, detected by *P. squamosus* MBFBL1165, followed by *P. squamosus* MBFBL456 (34%), *G. frondosa* MBFBL26 (20.4%), and *G. umbellat*a MBFBL690 (7.6%). This suggests that *P. squamosus* strains are superior to the *Grifola* species with respect to substrate degradation.

Wheat straw substrate is known to stimulate high production of laccase and other lignin modifying enzymes in different white rot fungi. It is obvious that the two *Polyporus* strains produced more lignin modifying enzymes than the two *Grifola* spp tested. In hemicellulose degradation, the two *Grifola* strains produced more xylosidase than the *P. squamosus* strains. However, the reverse was the case with xylanase, in which *P. squamosus* strains showed activities in the order of 36% (MBFBL456) and 10% (MBFBL MBFBL1165) higher than MBFBL690. An interesting observation here was that *G. frondosa* showed the highest xylanase activity on day 16 of cultivation, while the rest of the fungi tested showed maximum activity on day 60 of cultivation. Our results indicate that high ligninolytic and cellulolytic enzyme activities resulted in a high level of degradation in wheat straw substrate. We continue to research the production of these enzymes and the abilities of these fungi to release fermentable sugars from lignocellulosic materials.

Optimum of Submerged Fermentation Parameters for *Antrodia camphorata*

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Antrodia camphorata, used in traditional medicine in Taiwan, has been identified as a species of the higher Basidiomycetes (family Meripilaceae). Some bioactive compounds of *A. camphorata* have been isolated and characterized including sesquiterpene lactone, steroids, and triterpenoids. Traditionally, it has been used as a remedy for food-, alcohol-, drug-intoxication, diarrhea, abdominal pain, hypertension, skin itching, and liver cancer among the Chinese. In spite of these potential pharmaceutical applications, relatively little information regarding the process of producing these bioactive compounds has been published. This might be partially due to the fastidious nature of *A. camphorata* that grows specifically in the inner wall of the rotting trunk of *Cinnamomum kanehirai,* native only to Taiwan. It is difficult to

cultivate in a green house; thus, it is expensive to obtain fruiting bodies. Little success has been achieved in solid-state culture. Therefore, using a submerged culture method to obtain useful cellular materials or to produce effective substances from cultured mycelia might be a possible way to overcome the disadvantage of retarded growth of fruiting bodies.

Optimal parameters of submerged fermentation for *Antrodia camphorata* were studied in this paper. According to the yield of intracellular triterpene, the optimal fermentation parameters were obtained as follows: 40 g/L glucose, 6 g/L soybean, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4$ and 100 mg/L VB1. The optimum cultural period was 6 d at 26°C in 250 mL shake flasks at 100 r/min. The best aeration ratio was 1: 2.5 (medium volume: flask volume). The inoculation volume was 20%. The intracellular triterpene of *A. camphorata* was obtained (about 15.25 mg/ 100 mL) in the optimum culture.

The orthogonal test results showed that the best extracting condition of mycelia polysaccharides from *A. camphorata* were as follows: adding water at a ratio of 1:30 for dry mycelia to water, extracting for 2 h at 90°C and 10% of soluble polysaccharides were extracted. The immunocompetence experimental results showed that the proliferation of mouse lymphocytes was increased significantly by sample 6.

Analyzed using HPLC showed that the best extracted mycelia solvent was ethanol. We also extracted mycelia using different concentrations of ethanol and different extracting times, the results showed that the highest production of triterpene was extracted about 25.24 mg per one gram mycelium by 95% ethanol with ultrasonic. Antitumor activity test *in vitro* showed that the extraction from submerged mycelium of *A. camphorata* obtained a prominent antitumor activity on L1210 (murine lymphoid leukemia) and SW620 (adenocarcinoma), at 50 μg/mL, 80% ethnol extracted for 18 hours.

Characteristics of Cultural Conditions on *Phellinus* spp. and Their Artificial Cultivated Fruiting Bodies

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The *Phellinus* genus belonged to Hymenochaetaceae of Basidiomycetes and had been well known as one of the most popular medicinal mushrooms due to high antitumor activity.

This study was carried out to obtain the basic information for mycelial culture conditions of *Phellinus linteus*, *Ph. baumii*, and *Ph. gilvus*. The colony diameter, mycelial density, and the media for suitable mycelial growth of the abovementioned species were investigated in MEA, glucose peptone, and MCM. The optimum temperature for mycelial growth was 30°C. Carbon and nitrogen sources used in the investigation were mannose and malt extract, respectively. The optimum C/N ratio was 10:1 to 5:1 with 2% glucose concentration; the vitamin was thiamine-HCl; the organic acid was succinic acid; and the mineral salt was $MgSO_4 \cdot 7H_2O$.

The present experiments were conducted to determine which sawdusts influenced mycelial growth of *Ph. gilvus*. The pH values were 6.0 for oak sawdust, 6.5 for mulberry sawdust, 6.6 for elm sawdust, 6.3 for acacia sawdust, and 6.1 for apple tree sawdust. Mycelial density on elm sawdust and acacia sawdust were lower than those of oak and apple sawdust. Fresh fruiting bodies (weight in grams) were measured: 179 g on oak tree, 227 g on oak sawdust, 21 g on elm tree, 76 g on elm sawdust, 106 g on apple tree, and 170 g on apple sawdust. Among them, the yield of oak substrates was high whereas acacia sawdust was poor. The weight of fresh fruiting bodies (g) of *Ph. linteus* yielded 50 g on oak tree, but on oak sawdust no fruiting bodies were produced. *Ph. baumii* yielded 68 g on the oak tree, and 36 g on oak sawdust. Green mold of *Ph. baumii* caused by *Penicillium* spp. was

observed in Daegu in August 2000. The causal fungus was identified as *Penicillium citrinum* based on cultural and morphological characteristics. Conidiophores one-stage branched, terminating in a whorl of 3~5 metulae. Metulae mostly 5.2–7.8 μm. Phialides were flask-shaped, 5.7–7.5 × 2.2–2.7 μm. Conidia were subglobose, 1.8–2.3 μm in size. Colonies on Czapek agar with diameter of 23 mm formed within 8 days at 25°C. The optimum temperature for growth of the fungus was about 20–30°C. On the basis of mycological characteristics and pathogenecity test on host mushrooms, the fungus was identified as *P. citrinum.* This is the first report on the green mold of *Ph. baumii* caused by *P. citrinum* in Korea. The present study also investigated the preventive effect of *P. citrinum* by backfill (sand, granite soil, and smash rock). The results showed that the use of granite resulted in 3–10% infection rate and sand in 2–10% infection of *P. citrinum*. However, the smash rocks showed no infection of this fungus. The chemical properties of smash rocks was pH 8.4, showing alkalinity. The sand and granite soil were pH 6.1 and pH 7.5, respectively. No have heavy metals such as Mn, Fe, Pb, and As were found.

To compare the morphology of three *Phellinus* spp. mushrooms, a microscopic analysis was performed on *Ph. gilvus*, *Ph. baumii,* and *Ph. linteus*; they were similar but showed few differences. A comparison of the shape of fruit bodies revealed *Ph. linteus* and *Ph. baumii* were similar. The structure of the basidiospores was investigated also.

1) *Ph. gilvus* KCTC 6653*,* hymenial pores are 8–9 pores per 1 mm, round-shaped and 0.05 mm in size. Basidiospores, egg-shaped and 3.3 × 2.5 μm in size. 2) *Ph. baumii* Nongong*,* hymenial pores are 7–8 pores per 1 mm, round-shaped and 0.07 mm in size. Basidiospores, egg-shaped and 3.8 × 3.3 μm in size. 3) *Ph. linteus* ASI 26099, hymenial pores are 8–9 pores per 1 mm, round-shaped and 0.08 mm in size. Basidiospores, egg-shaped and 4.0 × 3.3 μm in size.

This study investigated comparative anti-tumor activity of water extracts of *Ph. gilvus* KCTC 6653 (PGE), *Ph. linteus* ASI 26099 (PLE), and *Ph. baumii* Nongong (PBE) *in vitro.* The anti-tumor activity was evaluated by sulphorhodamine B (SRB) and microtetrazolium (MTT) assay in terms of cell survival level. The tumor cells (sarcoma 180 and P 388) were treated with PGE, PLE, and PBE (7.5, 15, and 30 μg/ml) and Doxorubicin (DOX, 0.001–10 mM). The results showed that DOX, PGE, and PLE inhibited proliferation in a dose-dependent manner against both tumor cells. However, PBE inhibited the cell proliferation in both cell lines by only 30 μg/ml. Among the three mentioned above, PLE was the most effective in anti-tumor activity against sarcoma 180 and PGE was effective against P 388 in the SRB assay. By SRB assay, *Ph. gilvus,* grown on sawdusts and logs of oak, mulberry, elm, apple, and acacia, showed similar anti-tumor activity. In conclusion, all of PGE, PLE, and PBE used in this study showed anti-tumor activity against both Sarcoma 180 and P 388.

Influence of Some Mineral Rows on the Growth of Mycelia of Some Medical Mushrooms

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Mineral rows and products based on zeolits, bentonites, apatites, and kaolinites have very successful application in modern food production technologies. Current investigations showed that minerals due to their crystal-chemical properties, together with adsorptive, ionic exchange and catalytic abilities, can affect the growth of mycelium of different kinds of mushrooms. In this work, we investigated the growth of mycelia of mushrooms *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.*, Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm., *P. eryngii* (DC.: Fr.) Quél., *Lentinus edodes* (Berk.) Singer*, Trametes versicolor* (L.: Fr.) Pilát, and *Morchella conica* Pers. var. *costata* Vent. in the presence of minerals, which were added to the medium in the concentrations 0.25%, 1%, and 2%. Results showed different influences of minerals on the growth of the mycelia cultivated on malt agar and on appropriate substrates. With the addition of 0.25% of minerals to

malt agar, the development of observed mycelia was already accelerated, and with the addition of the 1% of minerals, the growth was even faster. The best results showed *Morchella conica* var. *costata* and *Trametes versicolor,* whose mycelia growth rates were accelerated 2–10 times by the addition of 2% of minerals to the malt agar when compared with growth rates under the same conditions but without minerals. *Trametes versicolor* was growing faster by the addition of different concentrations of some minerals in the substrate, while the growth of *Morchella conica* var. *costata* depended on time, type, and concentration of minerals. *Ganoderma lucidum* was growing faster on almost all substrates, which contained different concentrations of minerals. Genus *Pleurotus* showed very different growth on fortified malt agar and substrates, too. In some cases, growth rate was faster, but in the presence of some minerals, growth was inhibited.

We find these results could be very interesting in industrial mushroom production for shortening the time needed for the growth in spawning and thus in increased production of mushrooms. Medical and edible mushrooms can be produced faster by adding specific minerals in precisely defined concentrations.

The SCFA-Production after *in Vitro* Fermentation of Various Grains and Mushroom Materials

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Short-chain fatty acids (SCFAs), e.g., acetic, propionic, and butyric acids are known to be important energy sources both locally (colonicytes) and systemically (liver, striated muscle, and brain). Butyric acid has delayed the onset of malignant carcinoma in colon cells in *in vitro* animal and clinical trials. The suitable effect of butyrate is based on several co-existing mechanisms like hyperacetylation of histones regulating gene expression and downregulation of EGF-receptors (epidermal growth factors). These phenomena are further accelerated by the fermentative bacteria whose viability is induced so far with an unknown mechanism in the granulocyte and monocyte synthesis of the host. Simultaneously, the interferon-γ and -α production as well as the activation of TNF-α (tumor necrosis factor) take place. Furthermore, the butyric acid enhances the function of CDK-inhibitors (cyclin-directed kinase) in colonicytes. CDK-inhibitors arrest the mitotic activity in malignant carcinoma cells and favor the normal differentiation process of colonic cells. Also, without identifying the underlying mechanisms, it was discovered that SCFAs and probiotic microbial strains can statistically and significantly alleviate the symptoms of acute inflammatory bowl disease (IBD) and diminish the transfer of intestinal bacteria into the hepatic circulation. SCFAs also lower the luminal pH in the colon thus making the environment unsuitable for toxic producing microbial species.

Particularly important is the molar ratio of butyric acid to the total volatile acid concentration produced during the anaerobic fermentation. So far, there are no published data on the suitability of fungal material for the substrate of normal fermentative bacteria. It is also unknown if the level of SCFA produced from mushrooms is high enough to compete with amounts produced by more traditional dietary fiber sources, such as wheat or oat.

In the present work, we analyzed the SCFAs produced after fermentation of eight grain and mushroom raw materials *in vitro*. Chemical measurements were done using gas chromatography coupled with a mass selective detector (GC-MSD). The main SCFAs produced by the fermentation process were acetic, butyric, and propionic acids. In addition, four other SFCAs were found in minor amounts.

The detected SCFA profiles and the quantitative proportions of the main SCFAs differed markedly depending on the tested material. It is noteworthy that the fermentation of mushroom materials, such as oyster mushroom (*Pleurotus ostreatus*) and shiitake (*Lentinus edodes*), resulted rather equal butyric acid levels with the fermentation of rye (*Secale cerelae*), which in this respect was superior among all tested cereals. Our results indicate that some but not all mushroom species should be regarded as highly recommended food stuffs and should be included in the daily diet to substantially promote colonic health.

Effects of Environmental Factors on Bacterial Disease in *Pleurotus ostreatus* Cultivation

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The blotch on *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. (oyster mushroom) cap is a symptom of a bacterial disease caused by *Pseudomonas tolaasi.* This disease is problematic in *P. ostreatus* cultivation and can lead to total loss of yield.

In this study, effects of several environmental factors in *P. ostreatus* cultivation have been investigated to reduce symptoms caused by this bacterial disease. Yellow-brownish blotches were observed on *P. ostreatus* caps, after 24 hours of inoculation of *P. tolaasii* ATCC33618. Infected oyster mushrooms were grown in two different environmental conditions, uncontrolled and controlled, and the significant recovery from the disease was observed in the physically controlled environmental conditions. The symptom on infected mushroom caps has been reduced compared to the initial symptom in the controlled environmental condition and has been sharply increased under the condition of non-air circulation and high temperature. Bacterial blotch disease was epidemic under the unfavorable mushroom growth conditions, whereas the disease was confined under the favorable environmental conditions, controlling physical factors such as temperature, humidity, and ventilation. In the field test, high-quality *P. ostreatus* mushrooms could be obtained from the treatment with ventilation inducing variable humidity but not from the unventilated, where mushrooms were wilted and withered. Ventilation with variable humidity is important for the cultivation of the *P. ostreatus* mushroom. Temperature is an additional environmental factor. The longer stem and the smaller cap of the *P. ostreatus* mushroom have better quality in Korea. Mushroom development was delayed, but they produced better quality mushrooms at low temperature; when the growth rate was high, mushrooms were of a poorer quality at high temperatures (20–23°C), and no oyster mushroom was developed at higher than 30°C.

According to these results, temperature may be another key factor, which is closely related to mushroom development. The results in this study indicated that hygiene, relative humidity, temperature, and ventilation may be key environmental factors affecting development, growth, and bacterial blotch disease in oyster mushroom cultivation. In summary, multiple environmental factors rather than a single factor must be considered for good production of *P. ostreatus*. Development of bacterial disease during cultivation of *P. ostreatus* does not seem to be a sudden phenomenon but seems to be a phenomenon resulted from deteriorated defense mechanisms of the mushroom affected by environmental stresses. It is difficult to produce the oyster mushroom during summer in most local areas of Korea, and undesirable effects of the environment on disease, mushroom development, and growth are increased unless high temperature is controlled during the summer season.

Therefore, most oyster mushrooms are produced during early spring and late fall, however, controlling environmental factors such as ventilation, temperature, humidity, etc., is still needed for the production of high-quality oyster mushrooms in Korea. Furthermore, as

indicated by the results from this study, environmental factors play important roles in the development of *P. ostreatus*. For the stable production of oyster mushrooms and the high income of farmers, environments for the germination, development, and growth of *P. ostreatus* should be considered important factors for cultivation.

Mycotechnology for Optimal Recycling of Winery and Vine Wastes

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The main aim of this work was to study the ecological mycotechnology of recycling the winery and vine wastes by using them as appropriate substrata for edible and medicinal mushroom growing. Thus, a few technological variants of total recycling of wine-producing industry and vineyard wastes were planned to be used as suitable composts for enhanced growing of mushrooms. According to the main purpose of this work, two fungal species of Basidiomycetes group, namely, *Lentinus edodes* (Berk.) Singer (Shiitake) as well as *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. (Oyster Mushroom) were used as pure mushroom cultures in experiments. They were taken from the main fungal culture collection of NRDIBH – Stefanesti – Arges. The stock cultures were maintained on malt-extract agar (MEA) slants. Then, the pure mushroom cultures were expanded by growing in 250-ml flasks containing 100 ml of liquid multi-grain-extract medium.

The experiments of inoculum preparation were set up under the following conditions: constant temperature, 23°C; agitation speed, 90–120 rev min^{-1}; pH level, 5.0–6.0. All these seed mushroom cultures were incubated for 120–168 h. After the inoculum preparation, the experiments were focused on getting the spawn of *P. ostreatus* and *L. edodes*. In this respect, the seed cultures of these mushroom species were inoculated in liquid culture media at a pH of 6.3, previously distributed into rotary shake flasks of 1000 ml. During the incubation time period, all spawn cultures were maintained in special culture rooms, designed for optimal incubation at 23°C.

In the next stage of experiments, the culture composts for mushroom growing were prepared from the lignocellulosic wastes resulting from vineyard cuttings and marc of grapes in order to be used as substrates for mushroom development and fruit body formation. In this respect, three variants of culture composts made of marc grapes and vineyard cuttings were prepared in the following ratios: 1:1, 1:2, and 1:4 (w/w). All the vineyard cuttings were mechanically treated by bulky grinding and after that they were hydrated in water basins.

The research experiments were achieved by growing all these fungal species inside of special cultivation rooms, where the culture parameters were kept at optimal levels in order to get the highest production of mushroom fruit bodies. The effects of compost composition (carbon, nitrogen, and mineral sources) as well as other physical and chemical factors (such as temperature, inoculum size, pH level, incubation time, etc.) on mycelia growing and especially on fruit body formation were investigated. During the whole stage of fruit body formation and development, the culture parameters were set up and maintained at the following levels, depending on each mushroom species: air temperature, 15–17°C; the air flow volume, 5–6 m^3/h; air flow speed, 0.2–0.3 m/s; the relative moisture content, 80%–85%, light intensity, and 500–1000 lux for 8–10 h/d.

Studying the comparative effects of physical and chemical factors that could influence mycelia growth as well as mushroom fruit body formation and development of *Pleurotus ostreatus* and *Lentinus edodes*, the following representative results were registered: (1) maltose, as one of all tested carbon sources, had shown the highest influence upon mycelia growing and fresh fungal biomass producing around 28–35 g%; (2) among the five nitrogen sources examined, wheat bran was the most efficient for mycelia growth and fungal biomass production of *Lentinus edodes* and *Pleurotus ostreatus*, at 35–40 g% fresh fungal biomass weight, which is closely followed by malt extract at 25–30 g%;

(3) $CaCO_3$ yielded the best mycelia growth as well as fungal biomass production at 28–32 g% and was registered as the best mineral source; (4) the optimal pH levels for mushroom fruit body production were 6.5–7.0 for both mushroom species, registered during cultivation cycles at the constant temperature level of 17°C; (5) concerning the influence of spawn age upon mycotechology productivity, in the case of *P. ostreatus* species, the spawn age of 144 h and its volume of 5% (v/w) were the best variants and for *L. edodes* species the highest fruit body production was registered at the spawn age of 192 h and the volume of 7% (v/w).

The final fruit body productions of these two mushroom species were registered between 1.5–2.8 kg relative to 10 kg of composts made of vine and winery wastes. Using this kind of mycotechnology all these wastes from the winery and vineyard areas could be totally recycled as suitable substrates for edible mushroom growth. The final products of this biotechnology are the mushroom fruit bodies and the spent composts of the mushroom cultures, which could be used as fodder supplements having a protein content of 12–15 g% dry weight registered at the end of culture cycles.

Mushrooms: Production Prospects and Commercialization

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Once upon a time mushrooms were regarded as 'Food of Gods' and were revered as a culinary delight only for the rich and elite classes. But for thousands of years they have been valued throughout the world as a health food and medicine and are believed to be the elixir of life. And now they are considered 'the precious pearls of cookery' or 'poor man's meat'. Mushrooms spring up in fantastic shapes and a plethora of colors and enhance the aesthetics of the nature. Thomas Carlyle had aptly summed up the mushroom beauty, 'Nature alone is antique and the oldest art a mushroom'. Their fascinating beauty and mystique have attracted the poets and the painters equally through the eras. Due to greater awareness of health, nutritionally rich and health promoting foods are gaining importance in recent times.

Mushrooms are low-calorie, high-fiber, high-protein, and no-cholesterol food. They possess unique nutritional and therapeutic values and need to be involved in the common man's meal. Besides their antioxidant and anti-microbial activity, they are valued for their flavor, texture, and culinary delicacy and, thus, are becoming an important group of neutriceutical and pharmacological entities. Increasing demand for value-added products has increased the demand for mushrooms. Mushrooms that have been treasured as 'SOMA' in India for thousands of years are incredibly popular foods in most countries.

On the one hand, India is a developing country with globalization in its formative stage. Malnutrition among its rural parts is a matter of concern, where the majority of the population is engaged in agricultural activities. On the other hand, an increase in population and reduction in per capita land, rise in temperature, weather vagaries, and increases in cost of production all put a marginal farmer and the bread earner on the crossroads. At this crucial time, mushroom production technology emerged as a boom to several sections of the society. However, in spite of varied agro-climatic conditions and huge agricultural and industrial wastes available, the rate of growth of the mushroom industry in India is slow as compared to other mushroom growing countries.

Farmers, entrepreneurs, government, semi-government, and non-government organizations should take the initiative in mushroom cultivation and popularize their consumption for successful development of the enterprise. A significant impact can be made for a sustainable rural industry in upgrading the commercial cultivation of mushrooms for socioeconomic development. The huge agro-waste serves as the best resources for mushroom cultivation. With the transfer of technology to the farmers, mushroom production can be adopted at small, medium, and commercial scales. It is of great significance for landless and

marginal stakeholders, as it requires less space. Mushroom production has tremendous scope as an income generating activity. India predominantly has a tropical-to-subtropical climate, so rapid progress can be made in the mushroom industry by cultivating and commercializing species like the button, oyster, and milky mushrooms. There is a lot of scope not only for farmers but unemployed youth, illiterates, and school dropouts, who have no alternative sources of income and can adapt this venture as an economic activity. It is the need of the hour and should receive greater attention of the researchers, growers, and industries.

Enzyme Activities and Growth Rates of *Grifola frondosa* Mycelia on Substrates Composed of Wheat Bran, Wasted Brewery Grains, and Beech Sawdust

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Grifola frondosa, also known as Maitake, is a lignin and cellulose degrading Basidiomycete with excellent nutritional and medicinal properties. Cultivation of this medicinal mushroom on substrates with spent brewery grains presents an alternative to animal feed and/or waste as landfill.

Substrates were prepared with different proportions of waste brewery grains - SBG (0% and 10%), wheat bran - WB (10%, 20%, and 30%), beech sawdust, and 2% $CaCO_3$. The moisture content was adjusted to 65%. Substrates were transferred into racing tubes (25 mm diameter, 175 mm length) with uniform filling of 40 g. They were steam sterilized at 121°C for three hours and inoculated at one side with a 9 mm disk of actively growing *G. frondosa* mycelia on potato dextrose agar and closed with cotton plugs. The tubes were incubated in a dark, controlled environment at 24°C. After 29 days of growth the extracellular enzymes were extracted from 5 g of overgrown substrate with 10 mL of extraction buffer (0.1M sodium phosphate (pH 6.5) with 5% Tween-80) and screened for total peroxidase (TP), manganese-independent peroxidase (MiP), lignin peroxidase (LiP), and laccase (Lac) activity. Lac activities were determined as described in Podgornik et al. (Lett Appl Microbiol, 2001, 32(6):407–411). TP, MiP, and LiP activities were determined as described in Podgornik et al. (Enzyme Microb Technol, 2001, 29(2–3):166–172). Growth and enzyme activity experiments were performed in triplicates. The experiments were performed at the mycological laboratory of the Institute for Natural Science, Ljubljana, and enzyme activities at the Faculty of Chemistry and Chemical technology, University of Ljubljana, Slovenia.

There was no marked difference in mycelia growth rates and enzyme activity levels found in substrates with 10%, 20%, and 30% WB with or without SBG supplementation. At both substrates where 30% of wheat bran was added there was a marked increase in the production of enzymes; however, there was no marked difference if a SBG was added to the substrate or not.

It was found that mycelia growth rates were about 15% slower in the substrate with 30% wheat bran with the addition of spent beer grains than in the substrate with no additional SBG (95 mm/29 days for 30% of WB in the substrate compared to 81 mm/29 day in the substrate with 30% WB and 10% SBG). Lignin peroxidase activity was not detected in any of the prepared substrates.

The correlation of growth rate and enzyme activity was found to be inversely proportional as the concentration of wheat bran supplementation increased. It is interesting that, on the one hand, a sharp increase in the enzyme activities is noted at 30% of wheat bran supplementation even if no spent beer grains were added. This implies that spent beer grains are more a sawdust replacement than a wheat bran replacement. But, on

the other hand, the growth rate was strongly inhibited when 30% of wheat bran with 10% spent beer grains were used in comparison with the substrate with no spent beer grains added. The cause of this effect will be further explored in forthcoming studies.

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Experiments on *Hericium erinaceus* Cultivation in Hungary

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The number of cultivated mushroom species in Hungary is exceptionally narrow and little attention is paid on the posibility of other mushroom species to be grown. We carried out cultural experiments on *Hericium erinaceus* (Bull.: Fr.) Pers. (the monkey head mushroom), which has a singular taste and possess an outstanding content value.

The present work included harvest of the subkingdoms, development of initial spawn, and establishment of the spawn composition and growth conditions.

Wild isolates from domestic places were studied. We have preserved the vegetative mycelium culture of the isolates on malt agar under laboratory conditions. Beech-wood sticks were used for a long time preservation. The production of the initial spawn was carried out on cooked malt, the production of the inoculum was on a mixture of perlite and bran, and the production of the grain spawn was on cooked rye.

Ten kinds of fostering soils, filled into 10 litre polipropilen bags, were used in the growith experiments. The material was sterilyzed and grafted with the spawn (4%). Thereafter, it was incubatied at 25°C in perforated bags and the effect of different environment conditions (CO_2, RH, and T) on the fruiting bodies growth was examined.

The different subkingdoms produced different kinds of fruiting bodies under same conditions. The subkingdom HER2 produced the most beautifull and biggest fruiting bodies, and the subkingdom HER3 produced the largest quantity (15 kg/q substrate). The claims of the market are fluctuating by countries, therefore, the fruiting body value is variable.

It is noteworthy that *H. erinaceus* was protected in Hungary in 2005 together with other mushroom species. The list consists of 34 protected species among which there are *Volvariella bombycina, Hypsizygus ulmarius,* etc.

Effect of Temperature Regime during Incubation on Yield and Fruit Body Quality in Intensive Cultivation of *Lentinus edodes*

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Depending on temperature optimum, commercial strains of *Lentinus edodes* (Berk.) Singer can be divided into "winter strains" and "summer strains". For thermophylic strains optimal temperature during incubation is around 27°C. Cold-liking mycelia prefer 24°C. Mushroom companies in middle and north

Europe use "winter" strains only. The aim of the research was to investigate the effect of high temperature in substrate blocks during maturation on yield and fruit body quality of "winter" strains of *Lentinus edodes*.

During the incubation phase, decreasing the temperature can lead to pre-fructification. This is a phenomenon when fruit bodies appear before block transportation to the cultivation room. These mushrooms are deformed and cannot be marketed. Even reducing the temperature to 18–20°C in the incubation room can provoke early fruit body formation. However, raising the temperature during incubation can also cause negative changes in substrate blocks. Ventilation and capacity of air-conditioning in incubation rooms in Europe often do not take into account possible long-time increasing of outside air temperature up to 30–35°C, as it was the case in summer 2006. It was observed in the experiment that even short (1–3 weeks) temperature increases (3–6°C) during the incubation of substrate blocks caused negative effects. The following parameters were measured in the experiment: (1) duration of fruit body forming on the blocks (harvesting time in days); (2) yield pro block (%); and (3) correlation of mushrooms belonging to first and second class of quality (%), as well as amount of deformed fruit bodies (%).

Experiments were carried out in a commercial mushroom company in 2006–2007. For this research commercial shiitake strain 3782 was cultivated in sawdust substrate blocks. The influence of three temperature regimes 24, 27, and 30°C was investigated for a time of 10, 17, and 24 days each at the end of total incubation time. The incubation lasted 130 days. As a result of the experiments, the importance of supporting an optimal temperature regime during the whole incubation was confirmed.

A 7-day-increase of the temperature (from 3°Ñ) reduced the yield as well as quality, caused deformed mushrooms and prolonged harvesting time. In industry, duration of harvest plays a very important role. A short period of fruit body formation (less than 5 days) with maximum mushroom production is preferred. This reduces expenses for electricity and water as well as labor costs. Additionally, a short cultivation phase is preferred from a hygienic point of view. It is much easier to provide appropriate conditions in cultivation for a short time. Molds, concurrent microorganisms, and parasites do not have enough time for growth and developing. Also, marketing of mushrooms as a product with a short shelf-life and consumption time, makes growers carefully plan quantity and time of production. Increasing air temperature and prolonging its influence on blocks permits us to observe the intense phenomenon described above. Negative signs became more obvious and achieved their maximum in third variant of experiment, when incubation temperature rose to 30°C for 24 days. However, we found that a relatively short cultivation period (7 days) and an increase in temperature up to 30°C did not have a dramatic effect as a longer cultivation period of 24 days at 27°C.

Strain 3782 demonstrated the highest yield; it had the best mushroom quality with the shortest harvest time when the incubation temperature of substrate blocks was 24°C during the whole incubation period. The best quality mushrooms were collected from blocks with the highest yield. Increased temperature during maturation leads to a prolonged harvest (1.3–5.0 times), yield reduction to 10%–75%, and increase (1.5–10 times) of quality class II fruit bodies.

Genetic Diversity and Self-Fertility for Breeding *Pleurotus ostreatus*

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The production of oyster mushrooms, *Pleurotus*, has increased significantly in the last 10 years. Twelve dikaryotic strains derived from Asia and Europe were used in mating-type analysis of *P. ostreatus*. Mating type testers A1B1, A2B2, A1B2, and A2B1 were obtained from the NIAST, Korea. The dikaryon strains

produced fruiting bodies on cotton waste substrates and a single basidiospore was isolated from each basidiocarp. The individual monokaryons were identified for mating type and on the basis of mating reactions with the master strains on mushroom complete medium. Clamp connections were seen in the contact zone of compatible mating after 10–14 days. Of the 12 strains examined, 11 proved to have the A1A2B1B2 genotype. Strain 2487 had a different genotype.

This study conducted to reveal the phylogenetic relationships and DNA finger printing on 71 commercial strains of *P. ostreatus* that contained different species based on random amplified polymorphic DNA (RAPD) analysis. RAPD analysis was carried out with 12 universal rice primers (URP), operon primer, and FGL17 primer. FGL17 primer was known to have a 600 bp specific band on the *P. ostreatus*. This primer used to be a useful tool for detecting and identifying *P. ostreatus* rapidly. There are some pairs of isolates that have 95%–100% similarity in PCR polymorphic bands.

Pleurotus ostreatus has bifactorial heterothallism. Single basidiospore isolates from fruiting bodies are homokaryotic and self-sterile. However, we found that homokaryons derived from some strains of *P. ostreatus* could develop fruiting bodies of two different types. One hundred and two isolates out of 155 monospore isolates formed fruiting bodies (65.8%). The first group not only had mature or sporulating fruiting bodies but also clamp connections, which the initial isolate also did not present clamp connections (Abortive homokaryotic fruiting, AHF). The second group had developed fruiting bodies with clamp connections even though the initial homokaryotic colony did not form clamp connections (Pseudo-homokaryotic fruiting, PHF). The mycelial colonies derived from PHF by tissue culture formed clamp connections, while mycelial colonies of AHF lacked them.

We obtained 535 PHF and 79 AHF inter-strain hybrids among 8 strains of *P. ostreatus* by hyphal anastomosis. The fruiting body yield of the PHF group is higher than that of the AHF group in bottle cultivation. A preselection of single spore isolates for fertility would save labor in strain improvement of *P. ostreatus*.