

## Cultivation Techniques and Medicinal Properties of *Pleurotus* spp.

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### Summary

The genus *Pleurotus* (oyster mushroom) comprises some of the most popular edible mushrooms due to their favourable organoleptic and medicinal properties, vigorous growth and undemanding cultivation conditions. It can be cultivated on log and a wide variety of agroforestry (by-)products, weeds and wastes for the production of food, feed, enzymes and medicinal compounds, or for waste degradation and detoxification. Many different techniques and substrates have been successfully utilized for mushroom cultivation and biomass production by means of solid-state and submerged liquid fermentation. However, in contrast to submerged liquid fermentation, solid-state fermentation is not often used in large scale due to severe engineering problems. Various *Pleurotus* species have been shown to possess a number of medicinal properties, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities. These therapeutic activities are exhibited by extracts or isolated compounds from *Pleurotus* spp. fermentation broth, mycelia and fruiting bodies. In particular, polysaccharides appear to be potent antitumour and immuno-enhancing substances, besides possessing other beneficial activities. However, the biochemical mechanisms of these therapeutic activities still remain largely unknown. This review focuses on recent advances in the biotechnology of *Pleurotus* spp., with emphasis on the production of fruiting bodies, the production of mycelium and bioactive compounds by solid-state and submerged liquid fermentation. The medicinal properties of this mushroom are also outlined.

*Key words:* *Pleurotus*, mushroom cultivation, biomass production, solid-state fermentation, submerged liquid fermentation, medicinal properties

### Introduction

Cultivation of the oyster mushroom, *Pleurotus* spp., has increased greatly throughout the world during the

last few decades (1,2); in 1997 it accounted for 14.2 % of the total world edible mushroom production (). Its popularity has been increasing due to its ease of cultivation, high yield potential and high nutritional value (3). Al-

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though commonly grown on pasteurized wheat or rice straw, it can be cultivated on a wide variety of lignocellulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic.

New technologies and production techniques are being constantly developed as the number of required controllable environment parameters increases (4). Currently, solid-state fermentations, other than fruiting body production with *Pleurotus* spp., are used either in the transformation of wastes into animal feed or for enzyme production. Submerged liquid fermentation can, on the other hand, provide more uniform and reproducible biomass and can prove interesting for valuable medicinal products or for enzyme production because of uncomplicated downstream processing (5). Current research on *Pleurotus* spp. related to solid-state and submerged liquid fermentation is mainly concerned with substrate composition and optimization of culture parameters.

*Pleurotus* species have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumour growth and inflammation, have hypoglycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities (6). Recent studies of the medicinal properties of oyster mushrooms have focused on isolated bioactive compounds; however synergistic effects of the constituents of mushroom extracts may be possible.

### *Pleurotus* spp. Fruiting Body Production

#### Substrates for oyster mushroom cultivation

*Pleurotus* spp. cultivation is a very simple procedure in the case of log cultivation because it does not involve sophisticated equipment. However, despite its simplicity, large-scale cultivation on natural logs is not often used due to long incubation periods, low yields and environment-dependent production if conducted outdoors. Yields of *P. ostreatus* fruiting bodies vary with the species of trees used and range from 21 % biological efficiency (BE) for beech wood to 3 % BE for alder wood (7).

Broadleaf hardwood sawdust and straw-based substrates with added supplements are more often used in commercial production. In this case, these artificial substrates must be pretreated, mainly for elimination of contaminants, and handled in a clean environment. There are different methods of cultivation like shelf, bag, bottle, tray, jar, grid-frame, wall-frame and others (8). In practice, the most used are bag, bottle and shelf cultivation (9). Evaluation of *P. columbinus* cultivation in different bagging systems, in which partially pasteurized office papers were used as a growing substrate, revealed that polyethylene bags resulted in 109 % BE, followed by pottery (86 %), plastic trays (72 %) and polyester net (56 %) (10). Removal of the bottom half of the plastic cultivation bag and embedding artificial logs vertically

in soil resulted in a BE of up to 123 % and proved to be the optimal method for *P. nebrodensis* cultivation (11).

*Pleurotus* spp. can also colonize and produce mushrooms on pretreated conifer (*Pinus* spp.) wood chips but they do not always readily colonize non-pretreated conifer wood, due to the presence of inhibitory components (12). Some strains can, however, be adapted for cultivation on conifer-sawdust-based substrates (13). *Pleurotus* spp. can also be cultivated on wood waste or unused wood residues associated with harvesting or thinning operations, which can enhance economic returns needed to support ecosystem management (14).

Some pretreatment or supplementation with nutrients may be necessary. *P. ostreatus* BE is much lower when it is cultivated on fresh sawdust than on composted sawdust/bran mixture (15). Rodriguez Estrada and Roysse (16) reported that *P. eryngii* fruiting body yields were significantly higher in substrates containing Mn (50 µg/g) and soybean than in the basal cottonseed hull/sawdust substrate.

Different types of straw are commonly used for *Pleurotus* spp. cultivation. Straw can be composted or pasteurized and extra additives can be used to increase the BE. When using rice and wheat straw for *P. sajor-caju* cultivation, higher yields were obtained on ground than on chopped straw, and yields were 10 % higher on rice than on wheat straw. Higher spawn levels enhanced mushroom yields (17). Rice straw appeared to be the best substrate for *P. ostreatus* mushroom cultivation when compared to banana leaves, maize stover, corn husks, rice husks and elephant grass (15). When cultivating *P. florida*, the incorporation of cotton seed powder into rice straw substrate enhanced mushroom yield, increased net and total protein, free amino acids and total lipids content, while there was a significant decrease in total dietary fibre, free sugars and polymeric carbohydrates (18). Supplementation of rice straw with a residual slurry obtained after production of biogas from manure improved the yield potential and increased protein and mineral contents of *P. sajor-caju* mushrooms (3). Wheat straw supplemented with *Lolium perenne* grass chaff stimulated fructification and mushroom yield of *P. pulmonarius* (19). *P. tuber-regium* strains from Australasian-Pacific regions showed faster mycelium growth rates when cultivated on wheat straw, while wild Nigerian strains performed better in sclerotia yield when cultivated on this substrate (20).

Cultivation of *Pleurotus* spp. on substrates containing added olive mill waste and wastewaters (OMWW) can be a viable alternative for converting these environmentally problematic materials into valuable, highly nutritious food. It has been shown that wetting a wheat straw and bran substrate with OMWW diluted in tap water (25 %) had no significant negative effect on the time required for mycelial colonization, primordium initiation or mushroom yield of *P. sajor-caju* and *P. citrinopileatus*. Application of 50 % OMWW led to a delay in colonization and reduction in yield, and deleterious effects were noted when using 75 % OMWW (21). Substrates with the addition of OMWW up to 30 % did not interfere with mycelial growth of *P. pulmonarius* but they did inhibit fruiting body formation. OMWW up to 10 %, however, did not inhibit pinhead appearance (22). Expe-

riments with wild and commercial strains of *P. ostreatus*, *P. eryngii* and *P. pulmonarius* demonstrated significantly higher colonization rates on wheat straw and cotton waste than on peanut shells. Faster colonization was achieved on non-composted than on composted wheat straw and cotton waste substrates. Cellulose/lignin ratios in substrates were positively correlated to mycelial growth rates and mushroom yields of *P. ostreatus* and *P. pulmonarius*. In addition, there was a positive correlation between the C/N ratio and *P. eryngii* mushroom yield (23). A substrate composed of coffee pulp and *Digitaria decumbens* was also used for *P. ostreatus* cultivation and additional composting of this mixture improved the BE (24). When using a mixture of coffee pulp and wheat straw for *P. djamor*, *P. ostreatus* and *P. pulmonarius* cultivation, the observed decrease in caffeine content of the coffee pulp samples during the fruiting stage suggests that some caffeine accumulates in the fruiting bodies (25).

Much effort has been put into optimizing substrates based on different grass species for *Pleurotus* spp. cultivation. This is an effective way of converting abundant but low value materials into highly nutritional food, especially where wood and straw are scarce (26). *Lolium perenne* grass chaff stimulated fructification and mushroom yield of *P. pulmonarius* (19). Wooden crates were used, in a very simple substrate preparation for *P. ostreatus*, for composting a mixture of grass (*Digitaria decumbens*) and coffee pulp; 60 and 93 % BE were obtained in two harvests. Further composting for two to three days in each case improved the BE (24). When using chopped, pasteurized switch grass (*Panicum virgatum*) and pasteurized cottonseed hulls with wheat straw for *P. cornucopiae* cultivation, higher yields were obtained on cottonseed hulls/wheat straw substrate. Increasing spawn and supplement levels in switch grass/wheat straw substrate stimulated yield in a linear fashion. However, maximum yields were less than 50 % of those obtained with cottonseed hulls/wheat straw substrate (27). When supplementing spent rice straw substrate with oil seed cakes, cottonseed powder proved best in enhancing *P. sajor-caju* mushroom yields. Mushrooms grown on substrate supplemented with cottonseed powder had increased protein and fat content, decreased carbohydrate content and contained no residues of gossypol. In addition, there was a significant reduction in the spawn run period when compared to the use of un-supplemented rice straw (28).

*P. ostreatus* and *P. sajor-caju* exhibited higher ash content when cultivated on rice straw than when cultivated on banana straw, and *P. sajor-caju* also showed higher moisture and fibre content when cultivated on rice straw (29). When cultivating *P. ostreatus* on corn and pumpkin straw, the substrate had no effect on the nitrogen content and amino acid profile of the fruiting bodies; however, the nitrogen content increased from the first harvest to the third harvest (30). Using water hyacinth biomass as substrate, BE was found to be higher with *P. florida* (86 %) than with *P. citrinopileatus* (79 %) (31). When *P. ostreatus* var. *salignus* was cultivated on peanut, soybean, sorghum or wheat straw, the highest and lowest yields were obtained on peanut and sorghum straw, respectively. The highest protein content, pileus/stipe ratio, sporophore mass, percentage of dry material, and nitrogen and carbon contents were obtained with peanut

straw. Sorghum resulted in the lowest mushroom mass and pileus/stipe ratio, whereas the lowest protein and nitrogen content, and dry material mass were obtained with wheat straw (32). Among different agrowastes tested (cotton stalk, coir fibre, sorghum stover and mixtures of these wastes), the maximum yields of *P. sajor-caju* and *P. citrinopileatus* were obtained on cotton stalks, while *P. platypus* yields peaked on sorghum stover (33).

Weed plants (*Leonotis* sp., *Sida acuta*, *Parthenium argentatum*, *Ageratum conyzoides*, *Cassia sophera*, *Tephrosia purpurea* and *Lantana camara*) without heat pretreatment were tested for *P. ostreatus* cultivation. *Leonotis* sp. mixed with rice straw was the best substrate for spawn run and mushroom cultivation while *T. purpurea* was the least appropriate. The main problem of *P. ostreatus* cultivation on weed-composed substrates was the low yield that was obtained in the second flush. This problem could be overcome by mixing weed plants with rice straw. The fruiting body protein content was higher when *P. ostreatus* was cultivated on *Cassia sophera*, *Parthenium argentatum* and *Leonotis* sp. than on weeds supplemented with rice straw or on rice straw alone (34).

When hazelnut, *Tilia* spp., European aspen leaves, wheat straw, sawdust and waste paper were used as substrates, the best major component and substrate combination for mushroom productivity were wheat straw and wheat straw in combination with waste paper. Mixtures involving waste paper generally produced higher yields than other combinations. The lowest yield and the smallest fruiting body diameters were obtained from *Tilia* spp. and European aspen leaves in combination with sawdust. The greatest number of fruiting bodies was obtained on a mixture of wheat straw, hazelnut leaves and waste paper (35). Mandeel *et al.* (10) cultivated *Pleurotus* spp. on various lignocellulosic wastes supplemented with fresh chicken manure. The highest BE was noted on cardboard with both *P. columbinus* (134 %) and *P. ostreatus* (117 %). Experiments conducted by Baysal *et al.* (36), which involved cultivation of *P. ostreatus* on waste paper with addition of chicken manure, peat and rice husks, showed that increasing the amount of rice husks added to the substrate accelerated spawn running, pinhead formation and fruiting body formation. Larger proportions of peat and chicken manure had a negative effect on growth. A study on growth and productivity of different *P. ostreatus* strains on sunflower seed hulls supplemented with N-NH<sub>4</sub><sup>+</sup> or Mn, showed that the addition of growth-limiting mineral nutrients increased the mycelial growth rate. BE increased over control values and reached 60–112 %, depending on the strain and the concentration of Mn and N-NH<sub>4</sub><sup>+</sup> (37).

Non-pretreated spent brewery grains were successfully used as a basic substrate material for *P. ostreatus* cultivation in polypropylene bottles. Few fruiting bodies were formed on spent grain alone; however 19 % BE was obtained with the addition of 45 % wheat bran. The chemical analysis of fruiting bodies indicated that *P. ostreatus* cultivated on spent grain substrate had a higher nutritional value than those grown on other types of substrates (38).

### Other factors influencing oyster mushroom development

There have been various reports on other factors that influence the development of oyster mushrooms. None of these factors has been studied in depth; the various observations that have been made are outlined in the paragraphs below.

Cho *et al.* (39) discovered that inoculation of pure *P. ostreatus* mycelium cultures with strains of fluorescent *Pseudomonas* spp., isolated from the mycelial plane of commercially produced mushrooms, promoted the formation of primordia and enhanced the development of the basidiomata. These results strongly suggest that inoculation of the mycelium with specific bacteria may have beneficial applications for mushroom production.

It was reported that the bacterial blotch disease in mushrooms caused by *Pseudomonas tolaasii* was more severe when substrates were amended with Cu in *P. eryngii* cultivation (16). Two compounds from olive mill waste, 4-methylcatechol and catechol, were found to be effective against *P. tolaasii* and supplementation with up to 10 % OMWW reduced bacterium-related symptoms (22).

Qu *et al.* (40) demonstrated the influence of heavy metals in substrates on *P. eryngii* primordial formation, fruiting body development and BE. Heavy metal (As, Hg, and Cd) supplementation decreased average growth yields and BE of *P. eryngii*, whereas Pb supplementation improved both parameters.

Irradiation by red and green light stimulated vegetative growth of *P. ostreatus* mycelium and shortened the substrate colonization and fructification time. The increased fruiting body yield in irradiated cultures reached 36–51 % (41).

The cytolytic protein ostreolysin, isolated from *P. ostreatus* fruiting bodies, was specifically expressed during fruiting initiation, suggesting its involvement in fruiting body formation. When purified ostreolysin was used as a supplement on nutrient media plates inoculated with *P. ostreatus* mycelium, the protein stimulated primordia and fruiting body formation (42).

### Solid-State Fermentation with *Pleurotus* spp.

Most solid-state fermentations (SSF) with *Pleurotus* spp. have been small scale, that is, they have involved solid substrate in Erlenmeyer flasks or in agar plates. Recent studies have been focused on the utilization of lignocellulosic organic waste materials for either lignin degradation, use as animal feed or enzyme production.

#### SSF for *Pleurotus* spp. mycelial biomass and mushroom production

When agar-based media are used, medium composition plays an important role as it determines the mycelial growth rate, and correct medium ingredient ratios are crucial for fast colonization. Nasim *et al.* (43) found that malt extract agar (MEA) provided faster *P. ostreatus* mycelial growth rates than did Murashige and Skoog's (MS) medium and potato dextrose agar (PDA). The slowest growth was observed on PDA medium. The most effective carbon, nitrogen and inorganic salt supplements

for vigorous *P. nebrodensis* growth were glucose, peptone and  $MgSO_4$  (11).

Mycelium production on lignocellulosic substrates has also been investigated. Amongst seven mushroom cultivation substrates, the mycelial extension rates were highest on cotton gin-trash, peanut shells and poplar sawdust. Supplemented oak sawdust and olive mill waste were poor substrates for most species examined, while almost all strains performed adequately on corn cobs (44,45). Different substrate combinations were evaluated for *P. ostreatus* mycelial growth and favourable combinations were *Tilia* spp. leaves with wheat bran and *Populus* spp. leaves with wheat straw. The authors also reported that mycelial growth and mushroom yield have different requirements (46), whereas others reported that mushroom yield was directly related to the spread of the mycelium within the substrate (15).

#### SSF waste utilization and enzyme production by *Pleurotus* spp.

Much work has been done recently on waste utilization by *Pleurotus* spp., mostly concerned with the potential of locally available waste and agricultural by-products for either transformation into animal feed or as primary substrates for enzyme production.

Villas-Bôas *et al.* (47) investigated the conversion of apple pomace by *Candida utilis* and *P. ostreatus*, separately and in coculture, in SSF. *C. utilis* was a better candidate for biological treatment of apple pomace as the yeast lowered the residual free sugar concentration more than *P. ostreatus* did. When both organisms were used, apple pomace digestibility decreased while the protein content increased, with the final product being suitable for use as a protein supplement for cattle feed. Fermentation with *P. ostreatus* alone was, however, discarded as a viable treatment of apple pomace. Furthermore, recycling of viticulture waste in SSF with *P. ostreatus* and *P. pulmonarius* also yielded a high-fibre feed for limited use in ruminants (48). Tests on water hyacinth biomass delignification showed that organic matter loss was higher in samples inoculated with *P. citrinopileatus* than *P. florida*. Improvement of delignification and dry matter digestibility was, however, higher with *P. florida*, which also proved to be more effective than *P. citrinopileatus* for the production of highly digestible mycoprotein-rich ruminant feed (31). Intensive *P. ostreatus* mycelial growth was observed in substrates containing leftover hemp seeds, whole ground corn plant or sesame oil press cake. Intermediate growth was observed in substrates containing olive mill waste, rape oil press cake or sunflower oil press cake while slowest growth rates were observed in the substrate containing soy oil press cake (44,45). Ghibom *et al.* (49) introduced a novel approach for utilizing whey permeate with *P. ostreatus* in an SSF system. They concluded that whey permeate could provide a viable substrate for production of *P. ostreatus* mycelium. The optimal growth conditions were 44 g/L lactose, pH=6.0 and 24 °C.

White-rot basidiomycetes are efficient decomposers of lignocellulose, due to their capability to synthesize relevant hydrolytic and oxidative extracellular enzymes. Lignocellulolytic enzymes have significant potential applications not only in the chemical, fuel, food, textile,

laundry and pulp and paper industries but also in agriculture and for animal feed production (50).

Hölker and Lenz (4) introduced a trickle-film fermentation process on sugarcane bagasse with *P. ostreatus* for laccase production, which turned out to be highly suitable for extracellular enzyme production. High enzyme levels were produced when the growth medium was repeatedly changed. When compared to submerged liquid fermentation, the productivity was better and two laccase isoforms were detected.

Banana leaf waste was a better substrate than banana pseudostem waste in the production of extracellular enzymes by *P. ostreatus* and *P. sajor-caju* in SSF and is a potential alternative to other agrowaste substrates. The yields were, however, too low and commercially not viable. It was suggested that a larger surface area of banana leaf waste could be a determining factor for better enzyme production (51). This is in agreement with Zhang *et al.* (17) who reported that *P. sajor-caju* grew faster and provided better yields on ground straw than on chopped straw. There is, however, a substrate particle size limit, as more finely ground straw inhibited growth. Optimal particle sizes should therefore be determined for all applications.

Stajić *et al.* (52) performed SSF with *Pleurotus* spp. on grapevine sawdust, which was supplemented with synthetic medium to provide nitrogen and trace minerals. Peroxidase activity was detected in all strains evaluated, the highest being with *P. ostreatus* and *P. pulmonarius*. The highest laccase activity was detected at 10 days of fermentation in *P. ostreatus* followed by *P. pulmonarius* and *P. eryngii*. Organic nitrogen sources have been shown to stimulate enzyme production more than inorganic sources. Cyanobacterial biomass was used as a nitrogen supplement and stimulated *P. ostreatus* growth and laccase production in SSF. The authors concluded that dry biomass of diazotrophic cyanobacteria not only helps to maintain an optimal C/N ratio but also confers a good porosity, which sustains the oxygen supply within the matrix of solid particles (53). The nature of the substrate as well as the cultivation method affects the expression of lignocellulolytic enzymes. The study conducted by Elisashvili *et al.* (54) revealed that SSF of tree leaves by *Pleurotus* spp. was favourable for laccase and manganese peroxidase (MnP) production. Furthermore, coculturing can be an effective method for biopulping and improvement of lignin degradation (55,56). Chi *et al.* (56) demonstrated that coculturing *P. ostreatus* with *Ceriporiopsis subvermispora* significantly stimulated lignin degradation when compared to monocultures. Laccase production and MnP activity were stimulated in cocultures of *P. ostreatus* with *C. subvermispora* or *Physisporinus rivulosus* and a change in the isoform composition of those enzymes was also observed.

These studies show that the cultivation method can have drastic effects on the production of valuable substances by *Pleurotus* spp. and its economical feasibility.

### Submerged Liquid Fermentation with *Pleurotus* spp.

Submerged liquid fermentation (SLF) techniques have been developed for a variety of fungi and are used in mycelium propagation for different applications, such

as liquid spawn for fruiting body production on solid substrates; biomass production for food, dietary supplement and pharmaceutical applications; and conversion of waste biomass and enzyme production. SLF offers the possibility of high biomass production in a compact space, shorter time and with fewer chances of contamination (57,58). While SSF will remain the chosen method for mushroom production, there will be a continued increase in the development of SLF technology to produce more uniform and reproducible biomass of medicinal fungi. Western biotechnology companies have yet to recognize the potential in this area of medical bioscience (5).

### SLF for *Pleurotus* spp. biomass and polysaccharide production

The most detailed study of *P. ostreatus* growth in SLF was conducted by Márquez-Rocha *et al.* (59). They studied *P. ostreatus* cultivation in a stirred tank bioreactor and revealed that by varying impeller geometry and speed, and aeration intensity, the growth rate and pellet size changed. A clear tendency was observed for smaller pellet sizes to result in higher specific growth rates. For promotion of mycelium growth the pellets need to be broken down, but on the other hand, a balance between growth and hyphal fragmentation must also be achieved.

The lag phase for *P. tuber-regium* growth and bioconversion efficiency in SLF was shorter with glucose and fructose than with maize starch. In scaled-up fermentations, addition of fructose to basal medium supported higher mycelial yields than the addition of glucose. Yeast extract as the nitrogen source proved better than peptone when monosaccharides were used as the sole carbon source (60).

Recent studies on mushroom polysaccharides have demonstrated many interesting biological activities, which are described later in this review. The production of *Pleurotus* spp. mycelial biomass and valuable polysaccharides in SLF depends on the species used, growth parameters, growth timing and their nutritional requirements (61,62). Response surface methodology, a widely known optimisation procedure, was used to optimise the medium in order to maximize growth and polysaccharide production by *P. citrinopileatus*. The highest polysaccharide yield was obtained with a C/N ratio of 40, an initial pH=5.5 and a cultivation temperature of 25 °C (63). Another parameter that influenced growth and polysaccharide production by *P. ostreatus* in SLF was the initial oxygen transfer rate ( $K_La$ ). Better polysaccharide yields were obtained with a lower initial  $K_La$  (64).

### Waste utilization and enzyme production by *Pleurotus* spp. in SLF

SLF is also suitable for enzyme production and waste bioconversion. Most recent work is focused on substrate optimization for maximal production of hydrolytic and oxidative ligninolytic extracellular enzymes.

Stajić *et al.* (52) performed SLF with *P. eryngii*, *P. ostreatus* and *P. pulmonarius* on different carbon and nitrogen sources. In the medium with the best carbon sources (mandarin peels and grapevine sawdust), both *P. eryngii* and *P. ostreatus* showed the highest laccase activity with

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as the nitrogen source. With *P. ostreatus* and *P. pulmonarius*, the best nitrogen sources for production of peroxidases were peptone and NH<sub>4</sub>NO<sub>3</sub>. The correct composition of the cultivation medium is important for good enzyme production and various inducers have been tested for stimulating *P. ostreatus* enzyme production. Hou *et al.* (65) obtained high levels of laccase activity using cellobiose and peptone as the carbon and nitrogen sources. 2,2'-Azino-di-(3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and Cu had a positive effect on laccase production and the former was shown to be the best laccase inducer in their study. Static cultures were superior to agitated cultures in terms of growth and laccase production, while nitrogen-limited culture media were only beneficial for laccase production. In another study, the Taguchi orthogonal array experimental design was applied for the optimization of laccase production in SLF with *P. ostreatus*. The most influential factor for laccase production was found to be the inducer 2,5-xylydine, followed by glucose concentration, wheat bran content, urea concentration, inoculum size, yeast extract concentration and pH (66). Mikiashvili *et al.* (67) reported that the highest MnP and laccase activities were obtained in xylan-supplemented media, but enzyme activities decreased when supplemented with inorganic nitrogen sources. Peptone, followed by casein, was used as the nitrogen source for the best laccase accumulation; this results being attributable to higher biomass production. MnP and peroxidase secretion was stimulated by supplementation with casein hydrolysate. A liquid medium composed of mandarin peels and leaves inoculated with *P. dryinus* allowed the simultaneous production of hydrolases and oxidases at high levels. Carboxymethyl cellulase and xylanase appeared to be inducible enzymes. Addition of Mn enabled the laccase to MnP ratio to be regulated. For MnP production the presence of lignocellulosic substrates is a prerequisite and shows a positive correlation with their addition (50). This is supported by Hou *et al.* (65), who did not detect any MnP activity in N limited glucose medium with *P. ostreatus*. *Pleurotus* spp. SLF on tree leaves provided better hydrolytic enzyme production than SSF (54).

*P. ostreatus* mycelium could be cultivated employing coffee pulp wastewater extract supplemented with glucose. The polluting load was reduced by more than 50% at the end of 20 days of fermentation (68). SLF with *P. ostreatus* was also used for removing phenolic compounds from olive oil mill wastewater (OMWW). Laccase was the sole ligninolytic enzyme detected and was produced during primary growth. The phenolic content and toxicity were successfully reduced, but only when high OMWW dilutions were used. This could be a valuable method for problematic OMWW treatment before its release into the environment (69).

### Medicinal Properties of *Pleurotus* spp.

Recent studies on various *Pleurotus* species have shown a number of therapeutic activities, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities.

### Antitumour activity

Much recent research has been carried out on *Pleurotus* spp. extracts and isolated compounds such as polysaccharides, proteins and other substances that possess antineoplastic activities *in vitro* and *in vivo*.

Various crude extracts of *Pleurotus* species have been shown to possess relatively strong antitumour activities. Methanol extracts of *P. florida* and *P. pulmonarius* fruiting bodies significantly reduced solid tumours in mice (70,71). *P. ostreatus* mycelium extract, alone and combined with the chemotherapeutic agent cyclophosphamide, inhibited *in vivo* tumour growth in mice. The combined administration of the extract with cyclophosphamide decreased the degree of leukopenia compared to administration of cyclophosphamide alone (72). A water extract of *P. ostreatus* exhibited the most significant cytotoxicity by inducing apoptosis of human carcinoma cells, when compared to many other mushroom extracts. It has been suggested that the active compounds in the extract were water-soluble proteins or polypeptides (73).

Among the components of such extracts, polysaccharides are well-documented as potent antitumour and immunomodulating substances (74,75). Many polysaccharides from *Pleurotus* spp. have been isolated and identified (76–86). For some of them, important medicinal properties, including antitumour activities, have been shown. *P. tuber-regium* polysaccharides, extracted from mycelium and fruiting bodies, effectively inhibited solid tumour proliferation in mice. Antitumour effects have also been shown on different human tumour cell lines (87,88). Wong *et al.* (89) showed that *P. tuber-regium* polysaccharides exerted antitumour activity, through cytotoxicity and antiproliferative activity, against human leukaemia cells *in vitro*. The polysaccharides induced apoptosis and caused cell-cycle arrest. Compared to native *P. tuber-regium* polysaccharides, their corresponding carboxymethylated or sulphated derivatives showed higher antitumour activity, presumably because of their higher water solubility and relatively extended flexible chains (90–93). A novel  $\alpha$ -glucan from *P. ostreatus* mycelium induced apoptosis of colon cancer cells *in vitro* (94) and water-soluble polysaccharides extracted from *P. citrinopileatus* fermentation broth have been shown to reduce the number of metastatic tumour nodules in tumour-bearing mice (63).

Antitumour properties have also been demonstrated for *Pleurotus* spp. proteins, proteoglycans, and DNA. A lectin isolated from *P. ostreatus* potently inhibited growth of sarcoma and hepatoma in mice and prolonged their lifespan (95). A *P. eous* lectin exerted antiproliferative effects on human tumour cell lines while showing no cytotoxicity (96). Furthermore, two ribonucleases isolated from *P. sajor-caju* and *P. ostreatus* fruiting bodies exhibited antiproliferative effects on tumour and leukaemia cell lines (97,98). Another protein, eryngeolysin, isolated from *P. eryngii* fruiting bodies, exhibited cytotoxicity against leukaemia cells (99). Water-soluble proteoglycans were purified from *P. ostreatus* mycelium and exerted antitumour activity in sarcoma-bearing mice. Proteoglycans injected into mice reduced the number of tumour cells by cell cycle arrest (100). Moreover, DNA isolated from *P. ostreatus* fruiting bodies administered to mice with

solid Ehrlich carcinoma significantly increased the lifespan of mice (101).

#### *Immunomodulatory and antimutagenic activities*

The antitumour effects of mushrooms are mostly attributed to stimulation of the immune response. Recently, several compounds from *Pleurotus* species with immunostimulatory activities on humoral and cell-mediated immunity have been isolated. Water-soluble polysaccharides extracted from *Pleurotus citrinopileatus* fermentation broth administered to mice resulted in a significant increase in the number of macrophages, T, CD4<sup>+</sup> and CD8<sup>+</sup> cells (63). Glucans isolated from *P. florida* fruiting bodies activated the phagocytic response of mouse macrophages *in vitro* (83) and significantly induced the proliferative response as well as phagocytic activity of fish leukocytes *in vitro* (102). Moreover, proteoglycans from *P. ostreatus* mycelia exerted immunomodulatory effects by elevating mouse natural killer cell cytotoxicity and by macrophage stimulation (100). DNA isolated from *P. ostreatus* fruiting bodies stimulated mouse natural killer cytotoxic activity *in vitro* (101).

Antimutagenic effects of *Pleurotus* spp.-derived compounds on immune cells have also been reported. A ribonuclease isolated from *P. sajor-caju* fruiting bodies exerted antiproliferative effect on murine splenocytes (97), while eryngeolysin from *P. eryngii* inhibited the stimulated mitogenic response of murine splenocytes (99). Furthermore, *P. flabellatus* lectin did not exhibit any mitogenic activity towards mouse T cells (103).

#### *Antioxidant and gene protective activities*

Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in humans (104).

Of 89 mushroom species tested, an extract from *P. cornucopiae* possessed the most effective antigenotoxic and bio-antimutagenic activities when tested on *Salmonella typhimurium* and *Escherichia coli* (105). Furthermore, *P. cornucopiae* extracts significantly reduced H<sub>2</sub>O<sub>2</sub>-induced DNA damage in Chinese hamster lung cells (106) and *P. ostreatus* extract mitigated genotoxicity, as shown by the fact that it suppressed DNA damage induced by various mutagens in the *Drosophila* DNA repair test (107). On the other hand, a water extract of *P. sajor-caju* fruiting bodies had no genoprotective effects since it did not prevent H<sub>2</sub>O<sub>2</sub>-induced oxidative damage to cellular DNA (108).

Methanol extracts of *P. ostreatus* and *P. cystidiosus* fruiting bodies possessed antioxidant, reducing power, radical scavenging and iron chelating activities that were higher than those of other commercial mushrooms (104). On the other hand, Elmastas *et al.* (109) and Dubost *et al.* (110) reported that oyster mushroom extracts possessed only moderate antioxidant activities compared to other edible mushrooms. The antioxidant activity was positively correlated with total polyphenol content. Furthermore, Lee *et al.* (111) showed that *P. citrinopileatus* extracts prepared from fruiting bodies were more effective than those from mycelium and fermentation broth fil-

trate, presumably due to a higher amount of total phenols. Methanol extracts of *P. florida* and *P. pulmonarius* fruiting bodies showed similar antioxidant activities (70,71), and an ethanol extract from *P. citrinopileatus* fruiting bodies had antioxidant activities comparable to those from *P. eryngii*, *P. ferulae* and *P. ostreatus* mushrooms (111,112).

*P. citrinopileatus* fruiting body extracts have shown antioxidant activities *in vitro* and in hyperlipidaemic hamster rats. Extracts added to a high-fat diet increased the activities of antioxidant enzymes in rats (113). *P. ostreatus* mushroom extracts had antioxidant properties in aged and CCl<sub>4</sub>-induced liver damaged rats, as indicated by significant increases in concentrations of antioxidants and antioxidant enzymes (114,115). Pleuran, a β-glucan isolated from *P. ostreatus*, had a positive effect on the antioxidant status of rats and decreased precancerous lesions induced in rat colon (116). A polysaccharide-peptide complex isolated from *P. abalonus* fruiting bodies prolonged the lifespan of senescence-accelerated mice. Gene expression of antioxidant enzymes was up-regulated and consequently their activities were increased (117).

#### *Anti-inflammatory activity*

Jose *et al.* (71,118) showed that methanol extracts of *P. pulmonarius* and *P. florida* fruiting bodies decreased induced paw oedema in mice and ameliorated acute and chronic inflammation, respectively. Pleuran has also been shown to possess anti-inflammatory activity by exerting antioxidant and immunomodulatory effects on rats with induced colitis (119,120).

Hypersensitive immune responses, such as inflammation in delayed allergy, were suppressed by an ethanol extract of *P. eryngii*. It exhibited anti-allergic activity after oral or percutaneous administration to mice with oxazolone-induced type IV allergy (121).

#### *Cardiovascular disease protection and antihyperglycaemic activities*

Oyster mushrooms possess bioactive compounds with hypocholesterolaemic activities, such as polysaccharides, mevinolin and other statins (122). It has recently been reported that *P. citrinopileatus* fruiting body extracts exerted antihyperlipidaemic effects. Serum triglyceride and total cholesterol levels were lowered in hyperlipidaemic rats supplemented with the extracts, while high-density lipoprotein levels were significantly increased (113). Similar effects were noted when powdered *P. ostreatus* fruiting bodies or a water-soluble polysaccharide extracted from *P. citrinopileatus* fermentation broth were fed to hypercholesterolaemic or diabetic rats, respectively (123,124).

*Pleurotus* species also possess blood-pressure-lowering activity. Recently, *P. cornucopiae* has exhibited antihypertensive activity; this might be due in part to D-mannitol, which inhibits angiotensin I converting enzyme (125).

A methanol extract of *P. florida* fruiting bodies significantly inhibited platelet aggregation. The antiplatelet-aggregating activity, along with the anti-inflammatory activities discussed above, suggest its potential therapeutic

utic use against vascular disorders, but the exact mechanism of these activities is unknown (118).

Antihyperglycaemic activity was demonstrated with a water-soluble polysaccharide from *P. citrinopileatus* fermentation broth. The polysaccharide was effective in lowering blood glucose levels in diabetic rats (124).

#### Antimicrobial activity

Antibacterial and antifungal activities have been observed in *Pleurotus* spp. extracts and isolated compounds, presumably produced as a defence mechanism against other organisms. Table 1 (126–129) summarizes recently reported antimicrobial activities of *Pleurotus* spp.

#### Antiviral activity

Mushrooms contain substances that exert direct or indirect antiviral effects as a result of immunostimulatory activity (130). Inhibitory activity against human immunodeficiency virus (HIV)-1 reverse transcriptase has recently been demonstrated for *P. sajor-caju* and *P. pulmonarius* hot water extracts (131). Anti-HIV activity was also demonstrated for a ubiquitin-like protein isolated from *P. ostreatus* fruiting bodies (132). Moreover, Zhang *et al.* (133,134) demonstrated that, in contrast to water-insoluble  $\beta$ -glucans isolated from *P. tuber-regium* sclerotia, their corresponding water-soluble sulphated derivatives exert antiviral activities against herpes simplex virus type 1 and type 2. The effect is presumably elicited by the binding of sulphated  $\beta$ -glucans to viral particles, thus preventing them from infecting the host cells.

#### Concluding Remarks

Much work has been carried out on *Pleurotus* spp. mushroom cultivation, biomass production by means of solid- and liquid-state fermentation, and medicinal properties. Studies on mushroom cultivation have been focused on optimization of alternative substrates. It has been shown that a wide variety of agricultural (by-)products, weeds and wastes can be successfully used to produce food, feed, enzyme and medicinal compounds and to degrade and detoxify wastes. Due to an increasingly negative human impact on the environment, these techniques, together with others, constitute a very important tool for converting abundant quantities of waste materials, which often cause environmental pollution, into

food and valuable compounds. These and many other materials have been successfully used for biomass production.

Solid- and liquid-state fermentations can be successfully applied for *Pleurotus* spp. cultivation. Each one has its advantages and shortcomings and the decision as to which method should be used for a specific application must be carefully evaluated, as the outcomes are influenced by several factors. Solid-state fermentation is still the preferred method for waste utilization. Different approaches can be used for enzyme production, as an example, the trickle film process for laccase production developed by Hölker and Lenz (4). On the other hand, submerged liquid fermentations can be more uniform and reproducible, which is interesting for obtaining products with medicinal properties, although this potential has yet to be recognized by western biotechnology companies (5). Different substrates for solid- and liquid-state fermentations with *Pleurotus* spp. have been evaluated and nutritional requirements and culturing parameters were established. The authors point out that the correct selection of medium composition and environmental parameters is crucial if optimal biomass, enzyme or metabolite production is required.

*Pleurotus* species possess a number of beneficial medicinal properties, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, anti-allergic, hypocholesterolaemic, antihypertensive, antihyperglycaemic, antimicrobial and antiviral activities. These activities have been reported for various extracts and isolated compounds, such as polysaccharides, polysaccharide-protein complexes, proteoglycans, proteins and DNA from oyster mushroom fermentation broth, mycelia or fruiting bodies. In particular, polysaccharides appear to be potent antitumour and immunomodulating substances, besides possessing other beneficial activities. However, the biochemical mechanisms of these therapeutic activities still remain largely unknown.

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Table 1. Reported antimicrobial activities of *Pleurotus* spp.

Species	Effective against	Reference
	Crude extracts from fermentation broth	Gram-positive, Gram-negative bacteria and <i>Aspergillus niger</i> (126)
<i>P. ostreatus</i>	Hexane-dichloromethane extract containing <i>p</i> -anisaldehyde	<i>Bacillus subtilis</i> , <i>Pseudomonas aeruginosa</i> , <i>Aspergillus niger</i> and <i>Fusarium oxysporum</i> (127)
	Various extracts; two main unidentified compounds	<i>Bacillus</i> spp., <i>Escherichia coli</i> , <i>Vibrio cholerae</i> and <i>Salmonella typhi</i> (128)
<i>P. eryngii</i>	Eryngin – an antifungal peptide	<i>Fusarium oxysporum</i> and <i>Mycosphaerella arachidicola</i> (129)
	Eryngeolysin – a haemolysin	<i>Bacillus</i> spp. (99)
<i>P. sajor-caju</i>	12 kDa ribonuclease	<i>Fusarium oxysporum</i> , <i>Mycosphaerella arachidicola</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> (97)



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