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Conversion of Wood Waste into Value-Added Products by Edible and Medicinal *Pleurotus* (Fr.) P. Karst. Species (Agaricales s.l., Basidiomycetes)

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ABSTRACT: Unused wood residues can be converted into value-added products such as gourmet and medicinal mushrooms. In this study, wood waste was used to cultivate wood-inhabiting ligninolytic white-rot Basidiomycetes of the genus *Pleurotus.* Ligninolytic Basidiomycetes supplemented with low concentrations of dextrose readily colonized the wood waste; fruiting bodies were produced in 3-8 weeks. After the fruiting bodies were harvested, the basidiomycetes degraded up to 38% Klason lignin and 45% acid-soluble lignin in the spent substrates. Lyophilization of cultures, which stimulated filamentous mycelial growth, accelerated fruiting. The results indicate that growing *Pleurotus* species on wood waste or unused wood residue associated with harvesting or thinning operations can enhance economic returns needed to support ecosystem management. These values include reducing fuels for fire, decreasing pest and disease outbreaks, and increasing biodiversity.

KEY WORDS: *Pleurotus* species, Basidiomycetes, bioconversion, lyophilization, lignin, white-rot

INTRODUCTION

Increased worldwide consumption of wood has led to a growing accumulation of wood wastes in the environment. Other unutilized wood wastes, such as paper products, are also accumulating. The lack of landfill space is a continuing problem (Ince, 1994). Burning waste has become problematic because it releases high levels of carbon dioxide and particulates. The release of carbon dioxide, a greenhouse gas, contributes to global warming. Particulate materials may add to the development of smog in urban areas. Such pollution poses a serious threat to the environment, people, animals, and the sustainability of ecosystems. At the same time, there is an increasing global demand for energy and food and a growing shortage of natural resources. These considerations necessitate the development of environmentally friendly recycling technologies.

Lignocellulosic wastes can be degraded by mushroom-producing white-rot Basidiomycetes. These fungi gain nourishment from the cell wall structural polymers of lignocelluloses and produce edible mushrooms. Wood waste can serve as a valuable resource for the production of nutritious, gourmet, and medicinal mushrooms (Chang and Buswell, 1996; Ishizuki et al., 1997; She et al., 1998; Wasser and Weis, 1999). The *Pleurotus* species: *P. ostreatus* (Jacq.: Fr.) Kumm., *P. populinus* O. Hilber et O. K. Miller, *P. pulmonarius*

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ABBREVIATIONS

BE: biological efficiency; **MEA:** malt extract agar.

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(Fr.) Quél., and *P. sajor-caju* (Fr.) Fr. are wood-inhabiting ligninolytic white-rot Basidiomycetes that grow on hardwoods, wood byproducts (such as wood chips, sawdust, and paper products), and most agricultural wastes. Although oyster mushrooms grow and fruit better on agricultural waste, especially straw, than on wood waste (Croan, unpublished data), wood waste provides adequate nourishment for the fungi, is more readily available, and poses a more pressing problem than does agricultural waste.

Pleurotus mushrooms have been picked in the wild for centuries. Their flavor is typically described as oysterlike, hence the name oyster mushrooms. These mushrooms are very efficient protein producers and are in great demand by the gourmet mushroom industry (Ogundana and Okogbo, 1981). They can be used as antibacterial, antitumor (Cochran, 1978; Gunde-Cimerman, 1999), anticholesterol (Chovot et al., 1997; Gunde-Cimerman and Cimernan, 1995; Gunde-Cimerman, 1999; Wasser and Weis, 1999), antifungal, and antiviral (Gunde-Cimerman, 1999) agents. *Pleurotus* species are fast colonizers that degrade a wide variety of lignin in different wood wastes. They produce extracellular enzymes (lignin peroxidases, manganese peroxidases, lactase) that can modify and degrade lignin. Lignocellulosic materials are made up of polymeric cellulose, amorphous hemicellulose, and complex lignin. The lignin surrounds the hemicellulose and cellulose, protecting them from degradation by hemicellulase or cellulase. As a result of fungal ligninolytic action. the spent wood waste substrates can become a source of available hemicellulose and cellulose that can be used as carbohydrate sources for animal feed (Bisaria et al., 1997) or fertilizers (Stewart et al., 1998).

Aspen waste wood was used in this study because it represents hardwood residue that may be available during harvesting or thinning of stagnant stands and overstocked small-diameter forests. Natural aspen is a pioneer species that grows faster than pines and other hardwoods and softwoods (Fig. 1).

The primary objective of this study was to recycle wood waste into value-added medicinal and gourmet mushrooms using the mushroom-producing white-rot basidiomycetous *Pleurotus* species. A secondary objective was to study the feasibility of using spent substrates to achieve total utilization of wood waste.

MATERIALS AND METHODS

Fungi

Dikaryotic isolates of mushroom-producing white-rot Basidiomycetes, *Pleurotus ostreatus* (FP-101509), (HHB-9790), *P. populinus* (FP-102575), and *P. pulmonarius* (FP-10645) were obtained from the Center for Forest Mycology Research at Forest Products Laboratory (USDA Forest Service, Madison, WI). *Pleurotus* sp. (ASI 2001-2), an undetermined species of winter-fruiting mushroom hybridized from *Pleurotus florida* Eger, nom. nudum (ASI2016) and *P. sajor-caju,* was obtained from the Agricultural Sciences Institute in Suweon, Korea.

Media

The dikaryotic mycelial isolates were plated on 1.5% (w/v) malt extract (Bacto, Difco, Detroit, MI) and 2% (w/v) agar (Bacto, Difco). Malt extract agar (MEA) 90-mm diameter plates were inoculated with a mycelium/agar plug (6-mm diameter) of a young, actively growing margin of the colony. Prior to using the mycelium/agar plug as an inoculum for grain spawn, the plug was inoculated at the center of the plate and incubated at 24°C in the dark for 1-2 weeks or until mycelial growth had covered the entire surface of the MEA plates.

Grain Spawn Production

A mixture of 500 g of barley, 5 g of gypsum (calcium sulfate), and 600 ml of water was used for spawn production. Calcium sulfate was used to loosen the substrate for aeration. Each ingredient was individually weighed in a polypropylene autoclavable bag (20.2 × 42 cm) with a microporous filter patch (Sunbag, Santomi Sangyo, LD., Japan). The microporous filter patch allows gas exchange but prevents the passage of contaminant spores. The ingredients of each bag were manu-

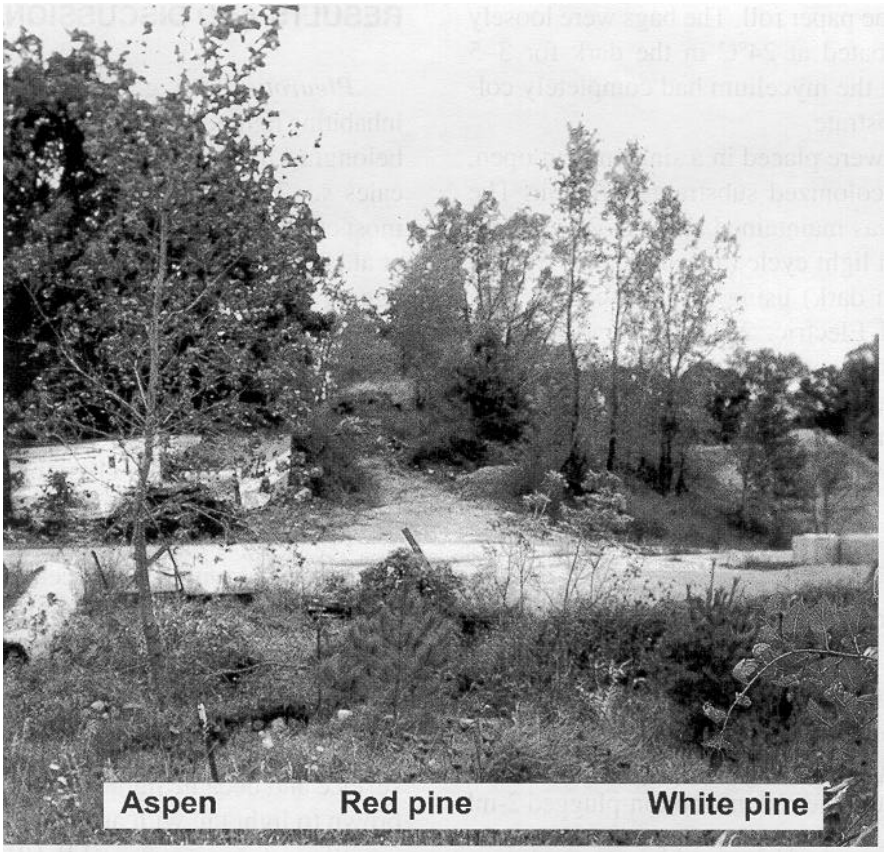

FIGURE 1. Growth characteristics of aspen, red pine, and white pine in Dane County, Wisconsin (Courtesy of H. N. Spelter, USDA Forest Service, Forest Products Laboratory, Madison, WI).

ally mixed and the bags autoclaved at 121°C for 45 min. The autoclaved bags were incubated at room temperature for 2-5 days to allow germination of inherent fungal spores. The bags were then re-autoclaved at 121°C for 20 min. After cooling. each bag was inoculated with actively growing mycelia from one or two MEA plates, depending upon the amount of mycelial growth, and mixed manually. The bags were loosely tied to allow air exchange and incubated at 24°C in total darkness for 2-4 weeks or until mycelial growth had covered the surface of all the grain.

Fruiting Body Production

Wood waste. Seven hundred grams of frozen aspen (*Populus tremuloides*) chips of various sizes (0.5-3.5 cm × 0.2-0.25 cm) and 650 ml of distilled water were placed in autoclavable bags. Each bag was mixed manually, loosely tied, and autoclaved at 121°C for 45 min. The autoclaved bags were incubated at room temperature for 2-5 days and were then reautoclaved at 121°C for 20 min. After the bags were cooled. 50 ml of 40% glucose was added to each bag to give a final concentration of 1.5% glucose. The bags were then inoculated with grain spawn at a level of approximately 15% (wet weight basis). The bags were manually mixed, loosely tied, and incubated at 24°C in the dark for 3-5 weeks or until the mycelium had completely colonized the substrate.

Paper: Rolls of commercial-grade toilet paper were saturated with distilled water (approximately 500-600 ml depending on size of roll), placed in autoclavable bags, and autoclaved at 121°C for 45 min (D. L. Czederpiltz, personal communication). After 2-5 days, the bags were reautoclaved at 121°C for 20 min. After cooling, the bags were inoculated with grain spawn by filling the hole in

the center of the paper roll. The bags were loosely tied and incubated at 24°C in the dark for 3-5 weeks or until the mycelium had completely colonized the substrate.

The bags were placed in a sink and cut open, exposing the colonized substrate to the air. The temperature was maintained at 22°C-28°C under a standardized light cycle (approximately 8-10 h light, 14-16 h dark) using a fluorescent ceiling light (General Electric, 2-15 W, standard, cool white). Humidity and moisture were maintained with a constant vaporlike spray of water. Fruiting bodies were harvested when caps reached 5-20 cm in diameter. Fruiting bodies were harvested for up to 10 flushes.

Lyophilization

Cultures of *P. ostreatus*, *P. populinus,* and *P. pulminaris* were lyophilized. Four to five mycelia/agar plugs of a stationary growth colony on growth medium were transferred into cotton-plugged 2-ml sterile constricted ampoules. Approximately 0.5-0.6 ml of a mixture of 10% (w/v) skimmed milk (Difco) with 10% (w/v) D (+)-trehalose (Sigma, St. Louis, MO) as the lyoprotectant was added to the ampoules. The ampoules were then placed in the refrigerator (4°C) overnight to permit cold hardening. Using liquid nitrogen as the coolant, the ampoules were slowly frozen in a microcomputer-programmed freezer (Cryomed Model 1010 programmable cooler, Stremikon, Mt. Clemens, MI) to about -45°C at -1°C/min, and then frozen to -90°C at -10°C/min. The frozen samples were lyophilized in an ethanol-dry ice bath. The ampoules were then sealed under vacuum and stored in the refrigerator (Croan, 2000).

Determination of Lignin

Total lignin content in the spent substrates was extracted with 72% H_2SO_4. Acid-insoluble lignin (Klason lignin) was measured gravimetrically (Effland, 1977) and acid-soluble lignin was determined by spectrophotometry based on absorption of ultraviolet radiation (Tappi standard method T 222 om-88; Tappi, 1988).

RESULTS AND DISCUSSION

Pleurotus is a globally distributed wood-inhabiting ligninolytic white-rot wood decay genus, belonging in the Pleurotaceae, within the Agaricales s.l. This classification is supported by the most current molecular systematic studies (Thorn et al., 2000). The *Pleurotus* species are tree pathogenic and saprotrophic on hardwoods, particularly beechwood. These fungi are also commonly found in forests and woodland, where they grow on fallen branches, dead tree stumps, and on felled logs, forming basidioma that are fan-shaped, whitish, spore-bearing gills on the underside of the cap.

Fruiting bodies of *Pleurotus* species were usually produced within 3-5 weeks. The caps of the fruiting bodies were initially hollow with a curved inner surface; with time, they usually became broadly concave and finally flat. The caps were harvested at 5-15 cm (up to 20 cm) in diameter. They were initially grayish blue on the inner surface and became lighter gray to pale yellowish brown to light tan with age.

Pleurotus ostreatus (FP- 101509) produced mushrooms with wavy and tan-colored margins and white gills (Fig. 2). This mushroom could be flushed up to 5-10 times under optimum condi-

FIGURE 2. Fruiting of *Pleurotus ostreatus* (FP-101509) on wood chips.

tions, harvesting 45-250 g/flush. The fruiting bodies produced by *P. ostreatus* HHB-9790 appeared to be lighter in color (whitish tan with light yellowish tan edges) than those of *P. ostreatus* FP-101509. In addition, the caps of *P. ostreatus* HHB-9790 were smaller (3-12 cm) than other caps *of* the same species. *Pleurotus populinus* fruited easily and rapidly. These Basidiomycetes produced attractive light grayish-blue mushrooms with tan edges and cap clusters with robust, long (7-10 cm), and thick stems (Fig. 3). Like *P. ostreatus*, *P. populinus* is a summer mushroom: it could be flushed up to 7-9 times, harvesting 76 g-300 g/flush. Unlike the fruiting bodies of other *Pleurotus* species, *P. populinus* fruiting bodies remained concave (Fig. 3). *Pleurotus pulmonarius*, another summer mushroom, fruited in clusters with robust, long (5-8 cm), and thick stems (Fig. 4). This species could be flushed up to 5-10 times, harvesting 55 g-300 g/flush. *Pleurotus* sp., a winter mushroom, grew faster on grain and wood chips than did the other oyster mushroom-producing fungi, but it produced small, dark blue bouquets of mushroom clusters only once after 8 weeks under the test conditions (Fig. 5).

The bioconversion of substrates into mushrooms is biologically efficient. According to the formula for biological efficiency (BE), the production of 454 g of fresh mushrooms from 454 g of dry substrate or 1816 g of moist substrate has a BE of 100% (Stamets, 1993). We obtained 300-500% BE for the production of oyster mushrooms under the conditions described. Because most fresh mushrooms contain approximately 90% water, it is possible for BE to be above 100%.

After the third or fourth flush, the substrates were occasionally contaminated with one or two colonies of *Penicillium* or *Trichoderma* species. The contaminants did not spread but dissipated. An antifungal substance was identified from *P.*

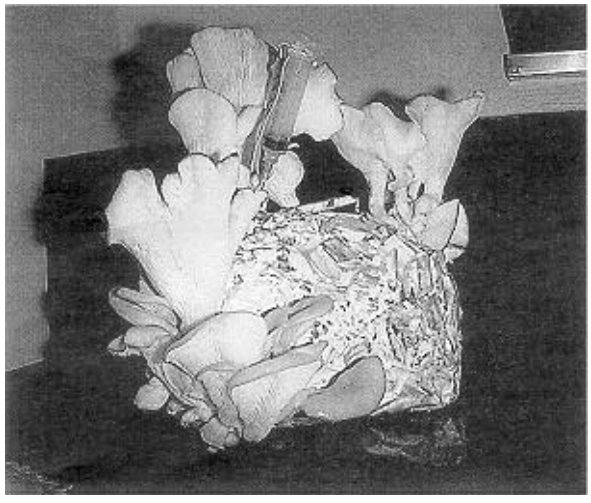

FIGURE 3. Fruiting of *Pleurotus populinus* on wood chips.

FIGURE 4. Fruiting of *Pleurotus pulmonarius on* wood chips.

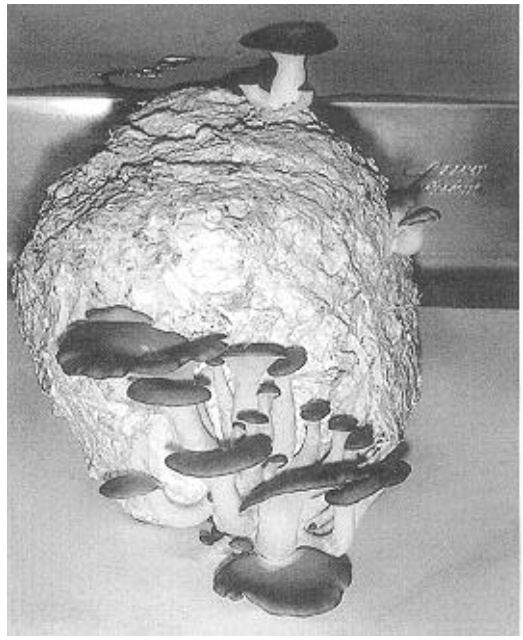

FIGURE 5. Fruiting of *Pleurotus* sp. on wood chips.

pulmonarius (Wasser and Weis, 1999). All *Pleurotus* species tested apparently produced antifungal activity that might help control contaminants.

When commercial-grade toilet paper was used as substrate, *P. ostreatus* (Fig. 6) produced 110 g of fruiting bodies in the first flush and 48 g in the second. *Pleurotus pulmonarius* (Fig. 7) produced 86 g of fruiting bodies in the first flush and 45 g in the second. A roll of commercial-grade toilet paper is an ideal substrate for growing mushrooms because it is like ground wood without any inhibitory substances. The hole in the roll provides an easy way to inoculate the grain spawn. A drawback is that mushrooms can be harvested only twice.

Lyophilized cultures of *P. ostreatus, P. populinus,* and *P. pulmonarius* exhibited heavy filamentous mycelial growth. The oyster mushroom-producing fungi exhibited faster, more dense, and filamentous heavy mycelial growth in the MEA plates immediately after lyophilization, than did the unlyophilized cultures. This may have been due to the survival of the more vital parts of mycelia through the lyophilization process. The fungi also often formed mycelial aggregates and/or primodia or abnormal fruiting bodies (stem without any spore-bearing basidioma) on MEA plates (Fig. 8). These mycelial aggregates and/or primodia or abnormal fruiting bodies formed along the inside periphery of the Petri dish and/or around site of inoculum that usually preceded their development into small fruiting bodies under optimum conditions of temperature, humidity, and light cycles.

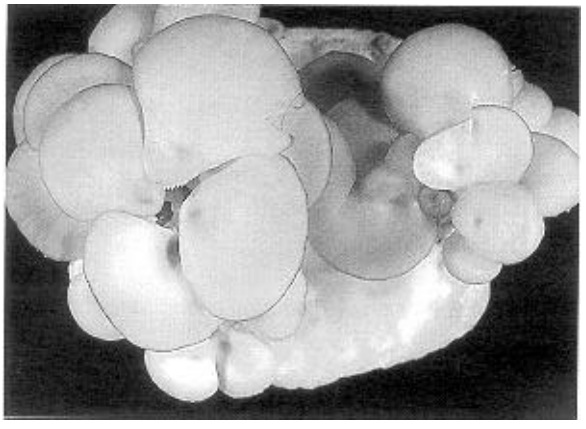

FIGURE 6. Fruiting of *Pleurotus ostreatus* on roll of paper.

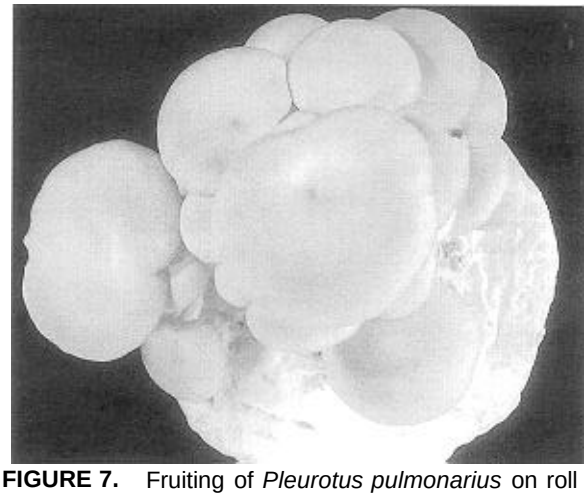

FIGURE 7. Fruiting of *Pleurotus pulmonarius* on roll of paper.

The *Pleurotus* species also colonized wood waste faster and exhibited heavy filamentous mycelial growth immediately after lyophilization. Fruiting was accelerated within 3-5 days after the polypropylene bags were cut open and the colonized substrate exposed to air. These results may be due to the survival of vital parts of mycelia during the process of lyophilization.

Mushroom-producing ligninolytic white-rot fungi can convert wood waste into nutritious edible mushrooms, which are in great demand. The protein content (dry weight basis) of *Pleurotus ostreatus* is 30% compared to 18% for *Lentinus edodes* (Berk.) Sing., 13% for wheat, and 25% for milk. *Pleurotus* species are considered to be one of the most efficient producers of food protein (Ogundana and Okogbo, 1981).

Pleurotus species grow fast; they degrade a variety of lignin as well as hemicellulose and cel-

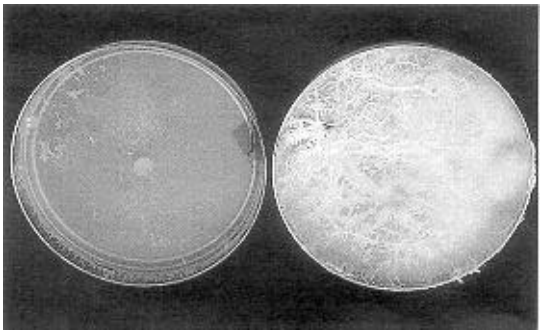

FIGURE 8. Heavy filamentous growth (abnormal fruiting) after lyophilization.

TABLE 1
Loss of Klason and Acid-Soluble Lignin in Spent Substrates

Pleurotus sp.	Collection number	Klason lignin[a] (%)	Acid-soluble lignin[b] (%)
P. ostreatus	FP-101509	37.82	35.41
P. ostreatus	HHB-9790	36.27	25.89
P. populinus	FP-102575	37.66	44.46
P. pulmonarius	FP-10645	37.20	35.41

[a]Loss based on percentage of Klason lignin (19.53%) in wood chips.
[b]Loss based on percentage of acid-soluble lignin (3.53%) in wood chips.

lulose in lignocellulosic waste. In our study, the wood waste was supplemented with 1.5% glucose to protect holocelluloses. After the fruiting bodies were harvested, the basidiomycetes degraded Klason lignin by 36-38% and acid-soluble lignin by 26-45% in the spent substrates (Table 1).

Because ruminants can digest the spent substrate with fungi, the spent substrate can be used for animal feed (Bisaria et al., 1997). It can also be used for animal bedding, soil conditioner, and fertilizer (Stewart et al., 1998), as well as for "biopulping" and "bioremediation" (Kirk et al., 1992a,b; Akhtar et al., 1993; Lamar et al., 1994; Eggen and Majcherczyk, 1998; Semple et al., 1998). Thus, the many uses of spent substrate allow total utilization of wood waste.

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