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Bioconversion of wood wastes into gourmet and medicinal mushrooms

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Increased wood wastes, including thinned material Ii-em stagnated and overstocked small-diameter forests, are a menace to forest health, to the sustainability of ecosystems, and to community economic viability. The objective of this study is to recycle wood wastes into value-added products, such as gourmet and medicinal mushrooms, by using the white-rot basidiomycetes, *Pleurotus ostreatus, P. populinus, P. pulmonarius,* and other Pleurotus species. When supplemented with low concentrations of dextrose, these basidiomycetes exhibit an excellent ability to colonize and stimulate fruiting body production on wood wastes.

Inoculated wood wastes in air-permeable bags are incubated at 24°C in the dark for 3 to 5 weeks, When exposed to light cycle (10-h day), humidity, and air, they fruit within 4 to 8 weeks. Lyophilization of cultures stimulates filamentous mycelial growth and fruiting is then initiated within 3 to 7 days.

Introduction

Increased worldwide consumption of wood has led to a growing accumulation of wood wastes in the environment. Unutilized lignocellulosic wastes (wood, agricultural, or paper product wastes) are also accumulating. The lack of landfill space is a long-term problem (Peter, 1994); burning is not acceptable because combustible products contain high CO_2, which adds to environmental pollution by emitting carbon into the atmosphere, contributing to global warming. All this poses a serious threat to the environment, to human beings, to animals, and to 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. Mushroom-producing white-rot basidiomycetes can degrade lignocellulosic wastes and gain nourishment from cell wall structural polymer of lignocelluloses producing edible mushroom and are thus a valuable resource for the production of nutritious gourmet and medicinal mushrooms (Chang and Buswell, 1996; Cheung, 1998; Ishizuki et al., 1997; Sheet al., 1998).

The oyster mushroom producing white-rot basidiomycetes, *Pleurotus ostreatus*, *P. populinus*, and *P. pulmonarius*, *grow* primarily on most hardwoods, wood by-products

such as wood chips, sawdust, or various paper products, and all agricultural wastes. Oyster mushrooms have been picked in the wild for centuries. The flavor of *Pleurotus* mushrooms is typically described as oyster-like, hence the generic name, oyster mushroom). Oyster mushrooms are favored by the gourmet mushroom industry (Stamets, 1993; Cuppet et al, 1998) and have medical uses, e.g., as antibacterial, antitumor (Cochran, 1978), and anticholestrol agents (Chovot, 1997).

Method and Materials

Fungi tested. —Dikaryotic isolates of white-rot, mushroom-producing basidiomycetes, *Pleurotus ostreatus* (Jacquin ex Fries) Kummer (FP- 101 509), *P. populinus,* Hilber & Miller (FP- 102575), and *P. pulmonarius* (Fries) Quelet (FP-10645) were obtained from the Center for Forest Mycology Research (CFMR), Forest Products Laboratory, USDA-FS, Madison Wisconsin. *Pleurotus* species (ASI 2001-2), an undetermined species of winter-fruiting-mushroom, resulting from the hybridization between *Pleurotus florida* Eger (ASI2016) and *P.* sajor-caju (Fr) Sing was obtained from Dept. of Mycology, Agricultural Sciences Institute, Suweon, Korea.

Media. –The mycelium 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) plates (90-mm diameter) plates were inoculated with a myceliurn/agar plug (6-mm-diam.) of a young, actively growing margin of the colony. Prior to its use as an inoculum for grain spawn, a mycelium/agar plug was inoculated at the center of the plate and incubated at 24°C in the dark for one to two weeks or until mycelial growth had covered the surface of the whole plates.

Grain spawn production. —A mixture of 500 g barley, 5 g gypsum (calcium sulfate), and 600 ml water was used for spawn production. Calcium sulfate is to help keep substrate loose and thus easily aerated. Each ingredient was individually weighed in polypropylene autoclavable bags with a microporous filter patch, 20.2 cm by 42 cm (Sunbag, Santomi Sangyo, LTD, Japan). The microporous filter patch allows gas exchange but prevents the passage of contaminant spores. Each bag was manually mixed and then autoclave at 121 °C for 45 min. The autoclaved bags were incubated at room temperature for 2 to 5 days to allow inherent fungal spore germination. They were autoclave again at 121 °C for 20 min. After cooling, each bag was inoculated with actively growing mycelial growth on MEA plates as described previously 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 of the pieces of grain.

Fruiting body production.

(a) Wood wastes. – Seven hundred grams of frozen aspen,*Populus tremuloides,* chips of various sizes (0.5 to 3.5 by 0.2 to 0.25 cm) and 650 mL distilled water were placed in an autoclavable bag. Each bag was mixed manually, loosely tied, and autoclave at 121 °C for 45 min. The bags were reautoclaved after 3 to 5 days at 121 C for 20 min. After cooling, 45 mL of 40% glucose was added to each bag. The bags were then inoculated with grain spawn at a level of ca. 10%, on a basis of wet weight. They were manually mixed, loosely tied, and incubated at 24°C in the dark for 3 to 5 weeks or until the mycelium had completely colonized the substrate.

(b) Paper. – Rolls of commercial-grade toilet paper were saturated with distilled water (approximately 500 to 600 mL distilled water depending upon roll of paper), placed in an autoclavable bag, and then autoclave at 121 °C for 45 min. They were reautoclaved after 3 to 5 days at 121 °C for 20 min. After cooling, the bags were inoculated with grain spawn by filling the hole in the center of roll of the paper. The bags were loosely tied and incubated at 24oC in the dark for 3 to 5 weeks or until the mycelium had completely colonized the substrate.

The bags were cut open and the colonized substrate was exposed to the air in a sink. The temperature was maintained at 22-28°C under light cycles (approximately 8-10 h days) of fluorescent ceiling light (General electric, 2-15 watt, Standard, cool white) and a constant vapor-like spray of water. The fruiting bodies were harvested at 5-15 cm (or 20 cm) in diameter of caps. They were harvested for up to 11 flushes.

Lyophilization. – Cultures of Pleurotus *ostreatus*, *P. populinus*, and *P. pulminaris* were lyophilized. Four to five mycelium/agar plugs of a stationary growth colony on growth medium (Croan, in preparation) were transferred into cotton-plugged 2-mL sterile constricted ampoules and lyophilizer as previously described (Croan, in preparation).

Results and Discussion

Inasmuch as there was an excess of aspen wood chips, they were recycled into edible gourmet mushrooms using oyster mushroom-producing white rot basidiomycetes. The basidiomycetes colonized the wood chips rapidly in the air-permeable bags. The contents in the bags were exposed to the air and subjected to a constant vapor-like spray of water in a sink to initiate fruiting. Fruiting bodies of oyster mushrooms were usually

produced within 3 to 5 weeks. Originally hollow with a curved-like inner surface, the caps expanded into a broadly concave shape and eventually became flat. The caps of the fruiting bodies were harvested at 5-15 cm in diameter; their color was initially grayish blue on the inner surface and became lighter gray to pale yellowish brown to light tan with age. The fruiting bodies produced by *Pleurotus ostreatus* are white to light grayish tan in color. They are summer fruiting mushrooms with wavy and tan color margins and white gills (photo 1). They can be flushed up to 5-10 times with 50 to 250 g harvested at each flush. *Pleurotus populinus* fruited easily and rapidly. The fungi produced gorgeous light grayish blue mushrooms with tan edges cap clusters with robust, long, and thick stems (7-10 cm long). They are summer mushrooms and can be flushed up to 7-11 times with 76 g to 300 g harvested at each flush. Unlike other oyster mushrooms, these fruiting bodies are not flat but concave (Photo 2). *Pleurotus pulmonarius* fruited in attractive clusters with robust, and thick stems (5-8 cm long) of summer mushrooms. This species could be flushed up to 5-10 times with 55 g to 300 g harvested at each flush (Photo 3). *Pleurotus sp.*, a winter mushroom, grew faster on grain and wood chips than other oyster mushroom-producing fungi but they only produced small, dark blue bouquets of cluster of mushrooms once after 10 weeks under the conditions described in methods and materials section above (Photo 4).

The bioconversion of substrate into fresh fruiting body production is biologically very efficient. According to the biological efficiency (BE) formula, 1 lb of fresh mushrooms grown from 1 lb. of dry substrate or 4 lb. of moist substrate is 100% BE (Stamets, 1993). Most fresh mushrooms contain approximately 90% water. We obtained 300 to 500% BE by oyster mushrooms under the conditions described above.

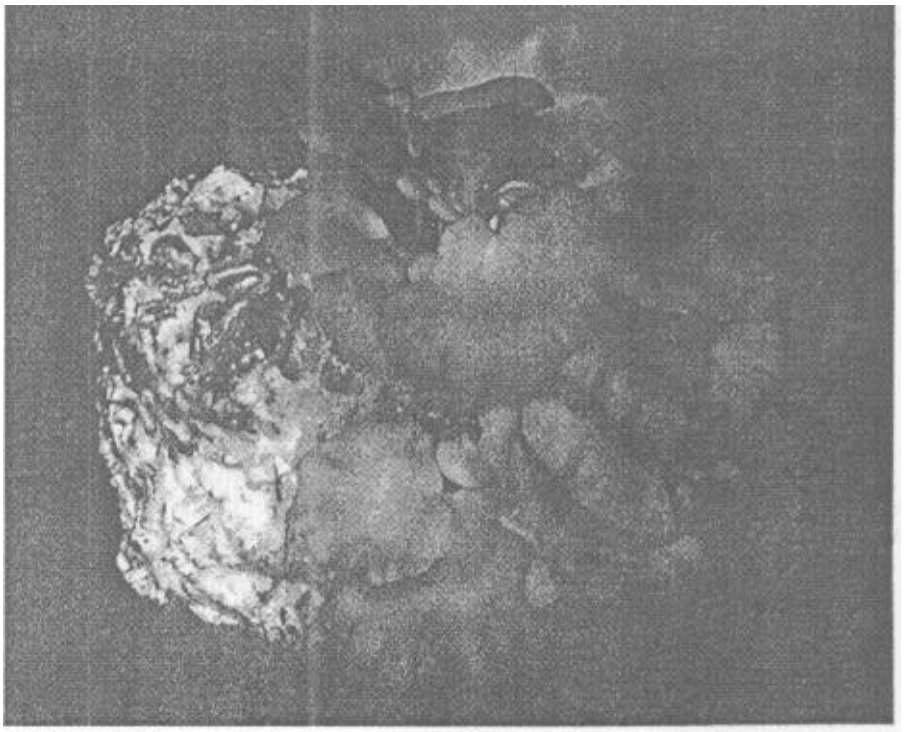


Photo 1. Oyster mushroom variety *Pleuotus ostreatus* fruiting on wood chips.

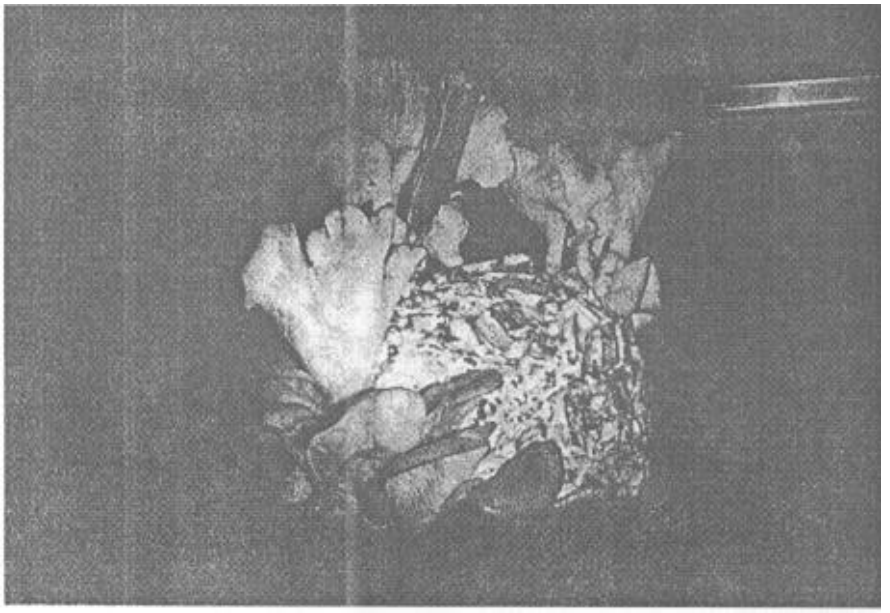


Photo 2. Oyster mushroom variety *Pleutous populinus* fruiting on wood chps

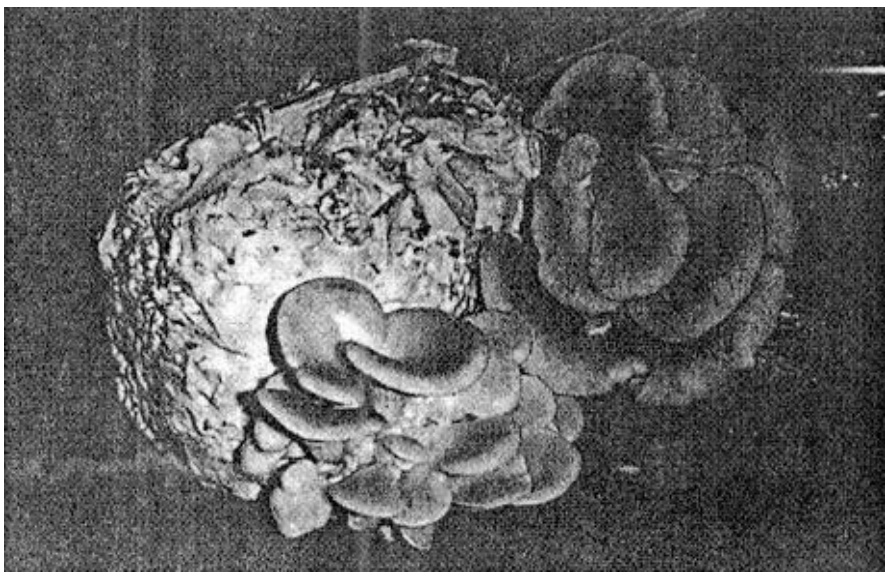


Photo 3. Oyster variety of *Pleurotus pulmonarius* fruiting on wood chips

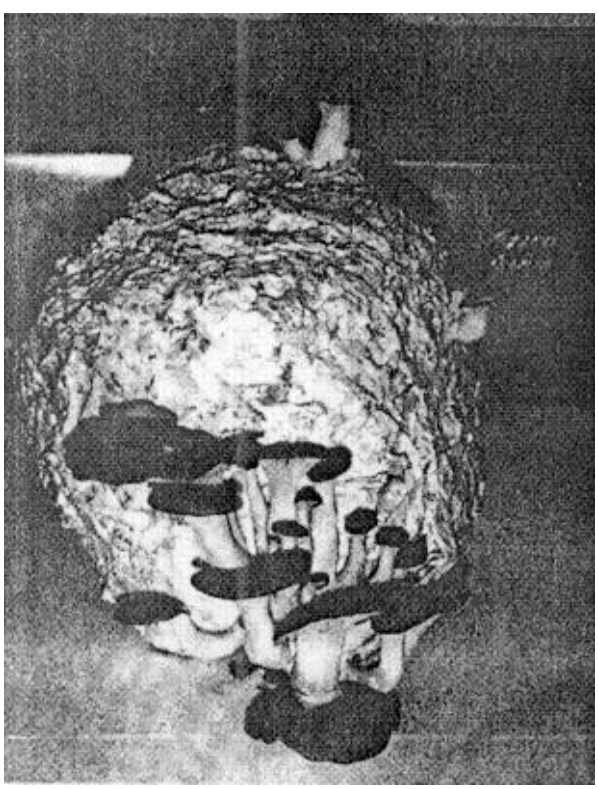


Photo 4. Oyster variety of *Pleurotus sp.* fruiting on wood chips

After the third or fourth flushes, the substrates were occasionally contaminated with one or two colonies *of Penicillium* spp. or *Trichoderma* spp. The contaminants did not spread but dissipated.

Using a roll of commercial toilet paper as substrate, the fruiting bodies of *P. ostreatus* produced 110 g in the first flush and 48 g in the second flush (photo 5). *Pleurotus pulmonarius* produced the fruiting bodies 86 g in the first flush and 45 g in the second flush by (photo 6). A roll of commercial grade of toilet paper is an ideal substrate for growing mushrooms because it is like a ground wood without any inhibitory substances. The hole provides an easy way to inoculate the grain spawn. The drawback is that mushrooms can only be harvested twice.

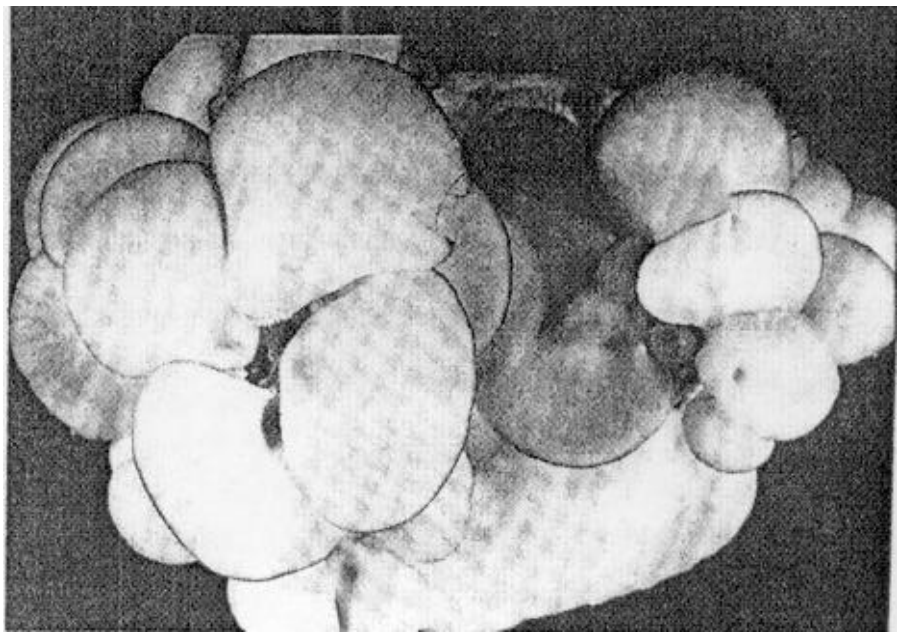
Lyophilizing cultures of *P. ostreatus, P. populinus,* and *P. pulmonarius* stimulated filamentous mycelial growth and fruiting within 3 to 7 days instead of 3 to 5 weeks after polypropylene bags were cut open and the colonized substrate exposed to air. This results may be due to the survival of the more vital parts of mycelia during the process of the lyophilization.

The ligninolytic mushroom-producing, white-rot fungi can convert worthless wood wastes into nutritious edible mushrooms, which are in great demand.

The protein content on the basis of dry weight consists of 30% by *Pleurotus ostreatus,* 18% by *Lentinus edodes,* 18%, wheat 13%, and milk 25%. The oyster mushrooms are considered to be one of the most efficient producers of food protein (Ogundana and Okogbo, 1981). *Pleurotus* species are the most versatile, fast colonizers, and they also degrade a variety of lignocellulosic wastes.

P. ostreatus (Photo 5) & *P. pulmonarius* (Photo 6) fruiting on a roll of paper

P5



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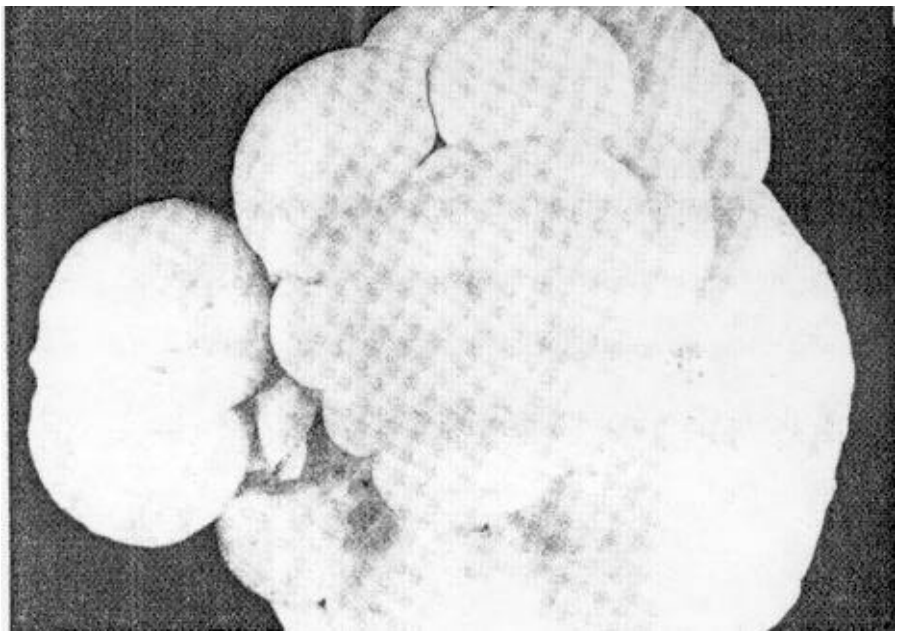

After harvesting the fruiting bodies, the spent substrates with fungi can be as an excellent source of fiber for paper, a process termed "biopulping" (Akhtar et al., 1993; Kirk et al., 1992a). They can be used for bioremediating toxic polyaromatic pollutants as an inoculum (Eggen et al., 1998; Kirk et al., 1992b; Lamar et al., 1994; Semple et al., 1998). Spent substrate can also be used as animal feed (Bisaria, et al., 1997), animal bedding, a soil conditioner, or a fertilizer (Stewart et al., 1998).

Further studies are planned to develop methods of mushroom production using softwood and a various mixtures of softwood and hardwood wastes as substrates for mushroom production.

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