UTILIZATION OF TREATED CONIFER WOOD CHIPS BY *PLEUROTUS* (Fr.) P. KARST. SPECIES FOR CULTIVATING MUSHROOMS

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Mushroom-producing white-rot basidiomycetes can grow rapidly and produce heavy mycelial growth on treated conifer wastes with extractive-degrading fungi. This study evaluates the treatment of scaled-up conifer wood chips with *Ophiostoma piliferum* (Cartapip 97). Treated conifer chips were used as substrates for cultivating mushroom-producing basidiomycetes of various *Pleurotus* spp. This treatment reduced the extractives by 30% to 90%. These mushroom-producing lignolytic white-rot basidiomycetes can easily colonize and produce mushrooms on treated conifer wood chips.

INTRODUCTION

Pleurotus mushrooms are considered to be one of the most efficient producers of food protein, producing 30% of its dry weight (Ogundana and Okogbo, 1981) and are excellent sources of dietary fiber (Cheung and Lee, 2000); they also produce vitamins (B1, B2, B12, C, D, folates, and niacin) and mineral elements (Mattila et al., 2001). These are nutritious as well as medicinal mushrooms (Wasser and Weis, 1999) that are in great demand by the gourmet mushroom industry (Chang 1999).

Pleurotus mushrooms have been cultivated on various agricultural lignocellulosic wastes or hardwood but never on conifer wood. White-rot fungi primarily attack hardwood but do not grow on softwood (conifers). Loblolly pine and southern yellow pine usually contain a high concentration of wood extractives or pitch deposits (Koch, 1972). The conifer wood chips were treated with extractive-degrading fungi to reduce the extractives, assimilating available nutrients, primarily nonstructural wood components. Although the fungi do not degrade the major components of wood, the metabolic action substantially reduces wood extractives ((Brush et al. 1994; Rocheleau etal. 1998).

The primary objective of this research was to scale up the conifer treatment with extractive-degrading fungi. A secondary objective was to convert treated loblolly pine and southern yellow pine wood wastes into a value-added resource for the production of gourmetandmedicinalmushrooms.

METHODS AND MATERIALS

Fungi

Dikaryotic isolates of mushroom-producing white-rot fungi *Pleurotus citriopileatus* (Singer) (FP-102361, SC17), *P. cornucopiae* Paulet ex Fries (SC 52), *P. cystidiosus* O.K. Miller (SC19), *P. eryngii* (De Candolle ex Fries) Quelet *sensu lato* (SC 54) *P. euosmus* (Berkley apud Hussey) Saccardo (SC 20), *P. ostreatus* (Jacquin: Fries) Kummer (SC 21 and 23), *P. populinus* Hilber and Miller, (SC 24), *P. pulmonarius* (Fries) Quelet (SC 25 and 36), *P. sapidus* Kalchbr, (SC 27, 28, and 55) *Pleurotus sajor-caju* (Fr.) Sing. (SC 29) were obtained from the Center for Forest Mycology Research at the Forest Products Laboratory (USDA Forest Service, Madison, WI). A colorless lyophilized isolate of *Ophiostoma piliferum* (Cartapip 97) was obtained from Clariant Co., Charlotte, NC. The fungi were maintained on 1.5% (w/v) malt extract and 2% (w/v) agar. Malt extract agar 90-mm-diameter plates were inoculated with a mycelium/agar plug (6 mm diameter) of a young, actively growing margin of the colony at the center of the plate and incubated at 24°C in the dark for 1 to 2 weeks or until mycelial growth had covered the entire surface of the MEA plates.

Conifer Chips Treatment

Loblolly pine *(Pinus taeda)* chips were obtained from Bowater, Inc., Catabwa, South Carolina. Southern yellow pine, SYP, *(Pinus* spp.) wood chips were obtained from the Bienville National Forest, Mississippi. All the wood chips were kept frozen until used.

Four kilograms (dry weight 45-50%) of frozen pine chips of various sizes and distilled water were added to an air permeable polyethylene bag (24X36”) to produce a final moisture content of 60%. The bag with wood chips was inoculated with 2 x 10^9 spores of *O. piliferum* (Cartapip 97) per kilogram wet wood chips. The bags were manually mixed and incubated at 24°C in the dark for 5 days. At the end of the incubation period, all conifer wood chips were autoclaved at 121°C for 45 min.

Resinous Extractives Determination

After treatment, the pine wood chips were oven dried at 5O°C and ground into 30-mesh sawdust with a Wiley mill (Authur H. Thomas Co., Scientific Apparatus, Philadelphia, PA). The oven-dried sawdust (dry weight) was extracted in a Soxhlet extractor with diethyl ether overnight (Brush et al., 1994).

Grain Spawn and Fruiting Body Production

Grain spawn and mushroom production were prepared as outlined (Croan 2000). Pretreated pine chips of various sizes with or without 20% wheat bran, 1% gypsum, and 3% glucose were placed in autoclavable bags with a microporous filter patch. Distilled water was added to increase moisture content of the mixture to 60%, resulting in a final weight of 1,000 g. The bags were then inoculated with grain spawn of various *Pleurotus* species at approximately 20% wet weight. The bags were manually mixed, sealed with thermal impulse sealer, and incubated at 24ºC in the dark far 1 to 2 weeks or until the mycelium had completely colonized the substrate.

The bags were placed in a refrigerator for 1 to 2 days, and then were cut open, exposing the colonized substrate to the air, and placed in an incubator. The temperature was maintained at 20ºC to 22°C under a standardized light cycle (approximately 10 h light, 14 h dark) using a fluorescent ceiling light (40 W, cool white). Humidity and moisture were maintained at 70-95% with a constant vapor-like spray of distilled water for an hour every six hours, using a Herri-michifier. Fruiting bodies were harvested when the caps reached 5 to 10 cm in diameter. Fruiting bodies were harvested 3 to 7 flushes.

Determination of Lignin

The 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

Mushroom-producing white-rot fungi are unable to colonize conifer wood because of its extractive content. After conifer chip treatment with extractive-degrading fungi, rapid and heavy filamentous growth occurred. *Ophiostoma piliferum* (Cartapip 97) was selected for large-scale treatment of conifer chips because it is easily obtained in large quantities and simple to use. This treatment removed 30% to 90 % of the extractives within 4 to 5 days and induced excellent mycelial growth and mushroom production. All these *Pleurotus* spp showed dense, filamentous heavy mycelial growth on the entire surface of treated conifer chips; in fact, after 6 days these fungi grew faster on treated conifer chips than on hardwood (red oak sawdust, *Quercus* spp). The caps of *Pleurotus* mushrooms are generally oyster- to fan-shaped; their color ranges from creamy white, tan, yellow, grayish blue and brown to almost black (Figs. 1-8). The white stems (20-150 mm) are usually positioned at the cap margin (20-180 mm). The caps were relatively flat and fan-shaped with wavy margins with relatively shorter stems on treated conifer chips, resulting in a lower level of biological efficiency than when the conifer chips were supplemented with 20% bran (Table 1). When the red oak sawdust, *Quercus* spp., was compared with treated conifer chips, the biological efficiency of mushrooms produced by *P. pulmonarius* (SC 36) was 155% and 159% respectively (Table 1).

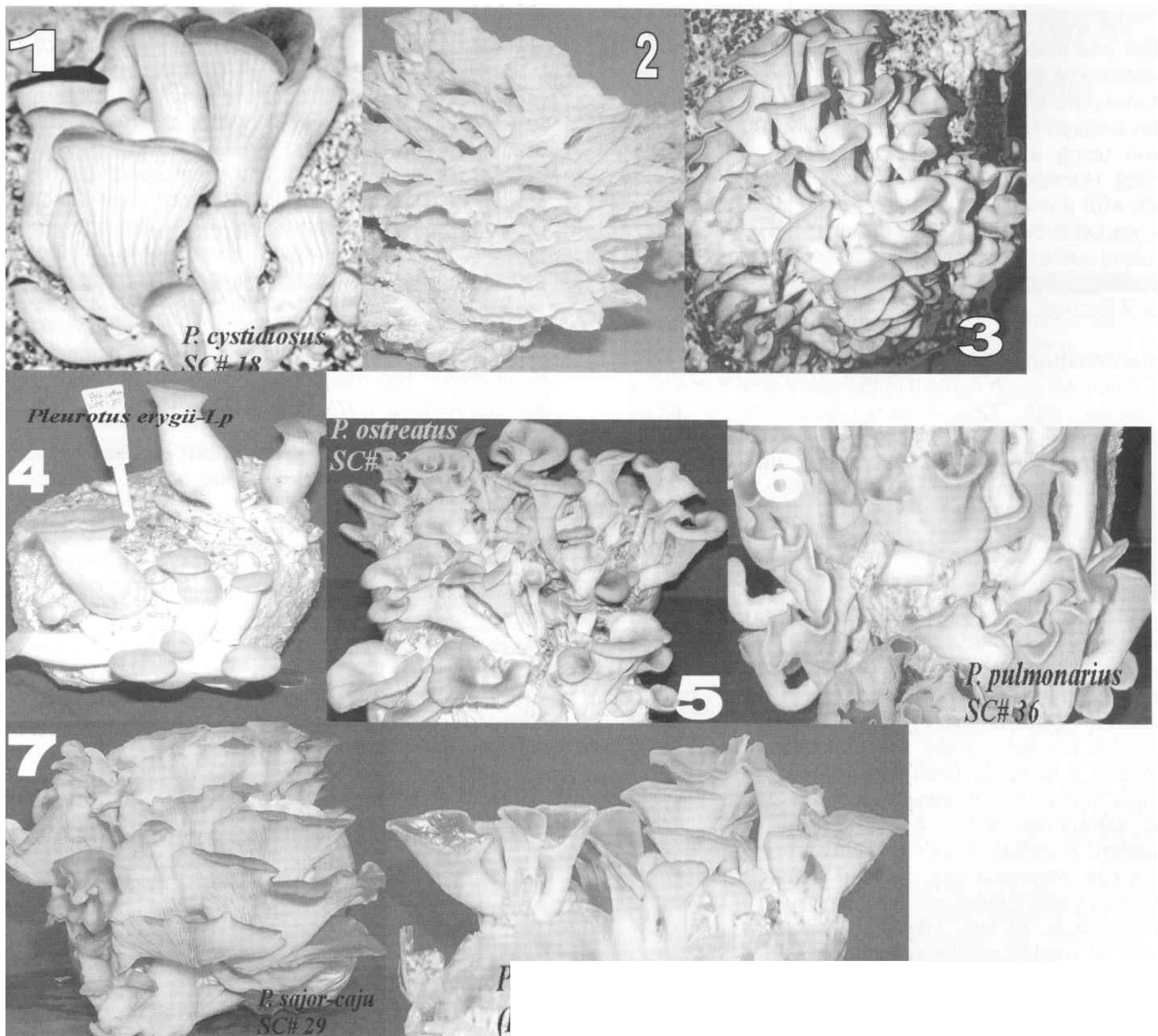
In our study, the treated conifer chip substrates were supplemented with additional glucose to protect holocelluloses in treated conifer chips. When additional glucose was added to *Phanerochaete chrysosporium* stationary cultures on thermomechanical pulp, the utilization of cellulose and hemicellulose in lignocellulosic substrates was suppressed and only the lignin fraction was degraded (Yang et al., 1980). Southern yellow pine chips were analyzed as 31.1% Klason lignin and 1.0% acid soluble lignin. After the fruiting bodies of *P. castreatus* (SC 23) were harvested a sixth time, the spent substrates were analyzed as 12.1% to 16.1% Klason lignin and 0.54% to 0.72% acid soluble lignin. Therefore, the lignin had been degraded by 51.9% to 61.1% Klason lignin. In addition, the chips in the spent substrates appeared to be brittle, indicating that the remaining lignin in the conifer chips might be partially degraded, selectively removed, or modified. Ruminants may therefore digest the spent substrates with fungi. Thus, the spent substrate may be used as animal feed (Bisaria et al., 1997), animal bedding, soil conditioner (Stewart et al., 1998), or fertilizer (Chiu et al. 2000). The spent wood substrates with fungus can also be utilized since their lignolytic enzyme systems can remove or modify lignin and may serve as a biopulping agent (Breen et al., 1999) as well as for bioremediation (Chiu et al. 2000).

In conclusion, treated conifer wood chip substrates with extractive-degrading fungi, *O. piliferum,* can be recycled to produce valuable gourmet and medicinal mushrooms. The many uses of spent substrates can achieve the total utilization of conifer waste.

Table 1. Mushroom production on southern yellow pine chips, loblolly pine chips, and red oak sawdust[a]

Species	SC	Substrate	Total biological efficiency[b] (%)
Pleurotus citriopileatus	17	P	94
		PB	113
Pleurotus cornucopiae	52	LPB	188
Pleurotus cystidiosus	18	P	54
		PB	100
Pleurotus euosmus	20	P	109
		PB	137
Pleurotus eryngii	54	LPB	131
Pleurotus ostreatus	21	P	90'
		PB	138
Pleurotus ostreatus	23	P	132
		PB	177
Pleurotus populinus	24	P	82
		PB	83
Pleurotus pulmonarius	25	P	104
		PB	120
Pleurotuspulmonarius	36	P	116
		PB	159
		ROB	155
Pleurotus sapidus	28	P	20
		PB	42
Pleurotus sapidus	55	LPB	54
Pleurotus sajor–caju	29	P	18
		PB	39

[a]LP is treated loblolly pine chips; P, treated southern yellow pine chips; RO, red oak sawdust; and B, supplemented with 20% wheat grain; total biological efficiency, the total of 3 flushes.
[b]Percentage of biological efficiency = (fresh mushroom harvested / dry substrate) x 100.



Fruiting bodies of *P. cystidiosus,* SC 19, PB (1), golden mushrooms; *P. citrinopileatus,* SC 17, P (2), *P. cornucopiae,* SC 52, P (3), king oyster mushrooms *P. eryngii,* SC 54, LPB (4), blue oyster *P. ostreatus,* SC 23, P (5), Phoenix oyster mushrooms *P. pulmonarius,* SC 36, PB (6), *P. sajor-caju,* SC 29, PB (7) and *P. sapidus;* SC 28, PB (8).

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