

BIOCONVERSION OF CONIFER WOOD CHIPS INTO SPECIALTY MUSHROOM PRODUCING FUNGAL GROWTH

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Mushroom-producing white-rot fungi can convert conifer wood wastes into gourmet and medicinal mushrooms. These fungi are unable to colonize on conifer wood due to its extractive content. This study evaluates the extractive content of conifer wood before and after treatment with the extractive-degrading fungi *Aureobasidium* spp., *Ceratocystis* spp., and *Ophiostoma* spp. to remove the resinous extractives. The treatment removed the extractives by 70% to 99.9%. The fungi penetrated into the sapwood of

conifer, utilizing nonstructural extractives, simultaneously reducing the extractives. Scanning electron microscopic examination showed that heavy mycelial growth of *Ophiostoma piliferum* (Cartapip 97) occurred with good sporulation on the surface of loblolly (*Pinus taeda*) and other southern yellow pine chips and in the resin canals and parenchyma cells within 4-5 days. We conclude that, mushroom-producing white-rot basidiomycetes can easily colonize on treated conifer wastes.

INTRODUCTION

Wood waste, including thinned material from stagnated and overstocked small diameter wood, poses a serious threat to forest health by increasing fuel load, which results in forest fires that severely damage the ecosystem. At the same time, the global demand for energy and nutritious food has been increasing as has the shortage of natural resources. Thinned material from stagnated and overstocked small-diameter wood stands can serve as a valuable resource for the production of nutritious gourmet and medicinal mushrooms.

White-rot fungi that produce edible and medicinal specialty mushrooms have been cultivated on various agricultural lignocellulosic wastes or hardwood in Asia for centuries but never on conifer wood. White-rot fungi grow mainly on hardwood but rarely attack conifer wood. Conifer contains a high concentration of wood extractives or pitch deposits, ranging from 3% to 9% of the total dry weight of wood (Koch, 1972). Some extractives are toxic to certain fungi and insects (Raffa and Smalley, 1995), but other extractives have a positive function in that they give color and a pleasing odor to wood.

Wood extractive-degrading fungi are initial wood colonizers causing black discoloration in the sapwood of wood; they assimilate available nutrients, primarily nonstructural wood components. However, such treatment has little or no effect on the holocelluloses or lignin content of the wood (Blanchette et al., 1992; Rocheleau et al. 1998 & 1999). Although the fungi do not degrade the major components of wood, the metabolic action substantially reduces wood extractives (Blanchette et al. 1992; 1996).

The objective of our research was to treat conifer wood waste with extractive-degrading fungi so as to grow lignolytic white-rot mushroom-producing basidiomycetes. A subsequent paper will analyze the production of gourmet and medicinal mushrooms on treated conifer wood chips.

METHODS AND MATERIALS

Fungi

Dikaryotic isolates of the following mushroom-producing white-rot fungi were obtained from the culture collection of the Center for Forest Mycology Research at the Forest Products Laboratory (USDA Forest Service, Madison, WI): *Grifola frondosa* (Dicks:Fr.) S.F.Gray (FP-101988, SC# 10), *Hericiium erinaceus* (Bulliard: Fries) Persoon (PF-140075, SC#13), and *Pleurotus ostreatus* (Jacquin: Fries) Kummer (FP 140084, SC# 23). The wood extractive-degrading fungi *Aureobasidium pullulans* (deBary) Arnand [MDX 18], *Ceratocystis coerulea* (Munch) Bakeshe [C-262], and *C. pilifera* (fries) Moreau [RWD 9472B] were also obtained from this collection. A colorless isolate of *Ophiostoma piliferum* (Cartapip97) was obtained from Clariant Co., Charlotte, NC.

The fungi were maintained on 1.5% (w/v) malt extract (Bacto, Difco, Detroit, MI) and 2% (w/v) agar (Bacto, Difco; MEA). 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, SC). Southern yellow pine, SYP, (*Pinus* spp.) wood chips were obtained from the Bienville National Forest, Mississippi. Ponderosa pine (*P. ponderosa*) chips came from the Coconino National Forest near Flagstaff, Arizona. All wood chips were kept frozen until used.

One hundred grams (dry weight 45-50%) of frozen SYP chips of various sizes (0.5 to 3.5 cm by 0.2 to 0.25 cm) and distilled water were added to Pyrex storage dishes (Corning No. 3250) to produce a final moisture content of 60%. Each dish was autoclaved and inoculated with actively growing mycelia from 1/2 MEA plate with the extractive-degrading fungi *Aureobasidium pullulans*, *Ceratocystis coerulea*, and *C. pilifera*. A colorless isolate of *Ophiostoma piliferum* (Cartapip™ 97) was inoculated with 2×10^8 spores (1x) and 4×10^8 spores (2x) per storage dish. After thorough mixing, the chips in storage dishes were incubated at 24°C in the dark for 30 days and autoclaved to kill the extractive-degrading fungi. To evaluate the growth of mushroom-producing fungi, the treated SYP chips were inoculated with actively growing mycelia from 1/2 MEA plate with *Grifola frondosa*, *Hericiium erinaceus*, or *Pleurotus ostreatus*. After thorough mixing, they were incubated at 24°C in the dark for 50 days.

Resinous extractives determination

The treated pine wood chips were oven dried at 50°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).

Scanning electron microscopy

Treated loblolly pine chips were cut radially using razor blades, mounted on aluminum stubs using silver paste, and gold coated using a Denton Desk-1 (Denton Vacuum, Inc., Cherry Hill, NJ) sputter coater. Samples were examined and photographed using Polaroid film in a JEOL JSM-840 (JEOL Ltd., Tokyo, Japan) scanning electron microscope at 15 kV.

RESULTS AND DISCUSSION

Three mushroom-producing basidiomycetes, *Grifola frondosa*, *Hericiium erinaceus*, and *Pleurotus ostreatus*, were inoculated on untreated conifer wood chips (loblolly pine, ponderosa pine, or southern yellow pine, SYP, chips); they did not grow or occasionally grew very poorly on some areas of the surface of the fresh conifer chips. Martinez-Inigo et al. (1999) found that Scots pine extractives were toxic to various wood-inhabiting fungi. Resin acids accounted for 88% of total extractives. Fungal growth was enhanced by removing the extractives. Similarly, certain resin acids in pine cone extractives were found to be toxic to wood-inhabiting fungi (mold, sapstain, and wood-rotting fungi) (Micales et al. 1994).

When conifer chips were treated with the extractive-degrading fungi *Aureobasidium pullulans*, *Ceratocystis coerulescens*, and *Ophiostoma piliferum*, and lyophilized *O. piliferum* (Cartapip 97), rapid and heavy filamentous mycelial growth occurred on the entire surfaces of SYP chips. The fungi removed 70% to 99.9% of the extractives (Table 1). In addition to heavy mycelial growth, *Grifola frondosa*, *Hericium erinaceus*, and *Pleurotus ostreatus* produced abnormal fruiting bodies, aerial spines, or aerial mycelia (Fig. 1).

Table 1. Removal of wood extractives from southern yellow pine chips by extractive-degrading fungi

Treatment	Remaining extractives (%)	Removed extractives (%)
No treatment (control)	9.02 ± 0.04	0
<i>Aureobasidium pullulans</i> MDX-18	2.73	69.7
<i>Ceratocystis coerulescens</i> C-256	2.69	70.2
<i>C. pilifera</i> RWD9427	0.05	94.5
<i>Ophiostoma piliferum</i> (Cartapip 97)		
1x inoculation	0.009	99.9
2x inoculation	0.009	99.9

The aerial spines of *Hericium erinaceus* were 24 to 35 mm high and extended 28 to 33 mm beyond the surface of the chips in deep dishes. By contrast, the aerial spines of *G. frondosa* remained relatively flat although they were elongated (20 to 28 mm). *Pleurotus ostreatus* produced flat and shorter aerial spines (8 to 15 mm) and abnormal fruiting bodies (20 to 50 mm by 5 to 25 mm) with many small hyphal proliferations.

Aerial spines and abnormal fruiting bodies were produced on treated SYP chips in storage dishes without special treatment, e.g., light/dark cycles, added humidity, or decreased temperature. They may have been caused by the accumulation of carbon dioxide, which can occur in deep dishes. Aerial spines and abnormal fruiting bodies were initially very pale yellow to white; as the cultures aged, spines and fruiting bodies became yellowish brown.

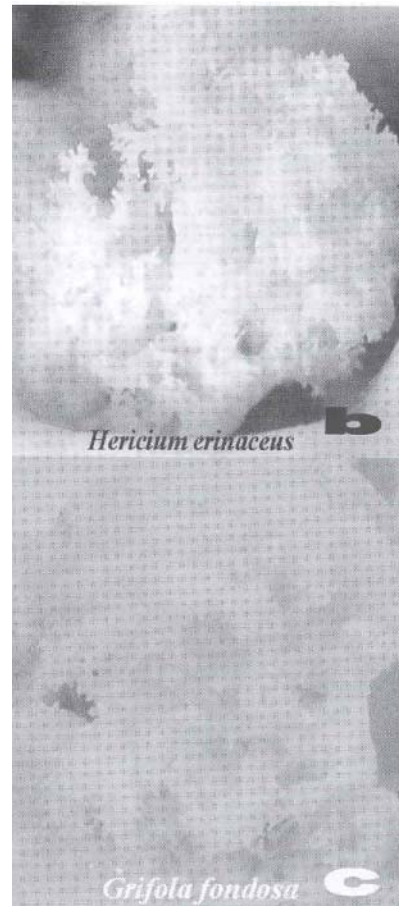
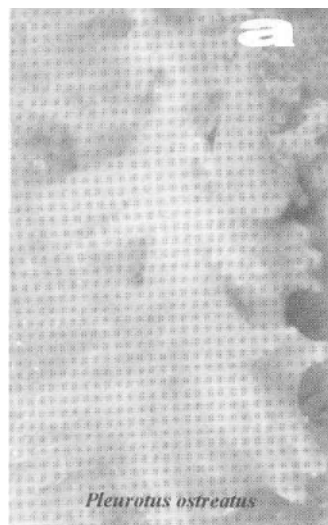


Fig. 1. Abnormal fruiting body and aerial spine formation of *Grifola frondosa* (a), *Hericiu erinaceus* (b), and *Pleurotus ostreatus* (c).

The wood extractives are mostly low molecular weight compounds that are easily extracted by solvents such as acetone, alcohol, diethylether, dichloromethane, benzene, or water. The percentage of extractives varies, depending upon the solvents used for extraction, the batch of wood chips received, the season, and the amount of rainfall when they were harvested (Terry Conners, Mississippi Forest Products Laboratory, personal communication). The percentage of extractives amounted to 3.05% to 9.02%, depending on the batch of chips used. SEM examination showed that *O. piliferum*, Cartapip 97, were colonized on the entire surface of the SYP or ponderosa pine chips within 2 days, producing filamentous heavy mycelial growth (Fig. 2) with sporulation (Fig. 3). The treatment removed 25.9% of the extractives within 2 days. The mycelium of *O. piliferum* passed from one cell to the next through bordered pits and tracheids (Fig. 4). Heavy mycelial growth was observed in radial tracheid cells (Fig. 5) and ray parachyma cells within 5 to 10 days (Fig. 6).

All these basidiomycetes showed dense, filamentous heavy mycelial growth on the entire surface of treated conifer chips. Subsequently, they produced mushrooms on treated conifer wood chips.

Fig. 2-6. The colonization of *Ophistoma pilliferum*:

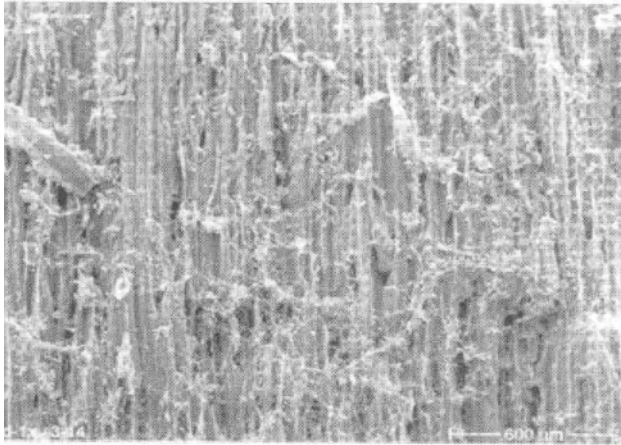


Fig. 2. Filamentous 2-day-old mycelial growth on SYP chips

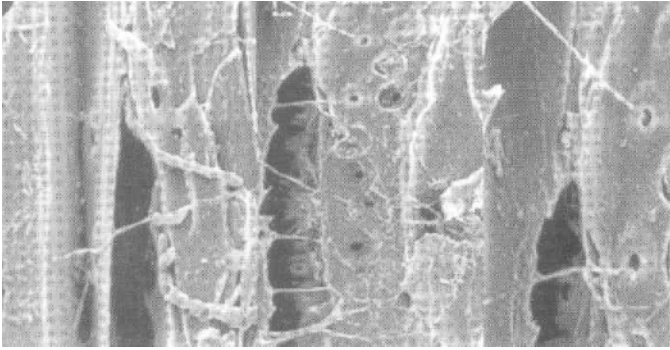


Fig.4. Mycelial growth in bordered pits and tracheids on SYP chips

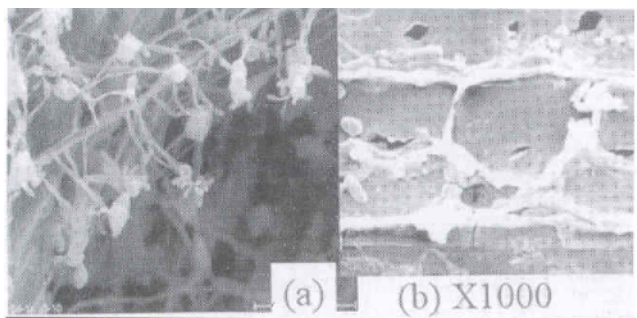


Fig. 3. Sporulation (a) 6-day-old on SYP chips and (b) 10-day-old on ponderosa pine chips

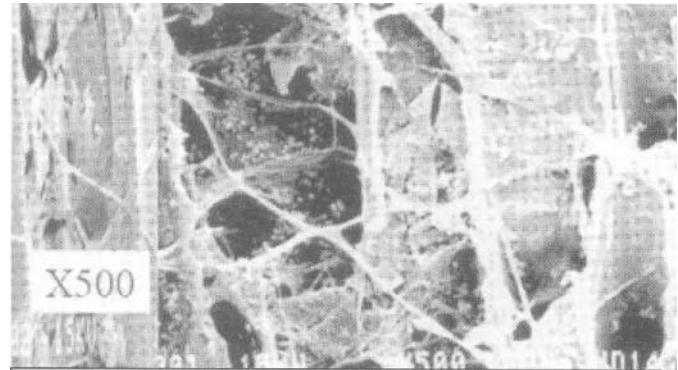


Fig. 5. Mycelial growth in 5 tracheid cells, radial section of ponderosa pine chips.

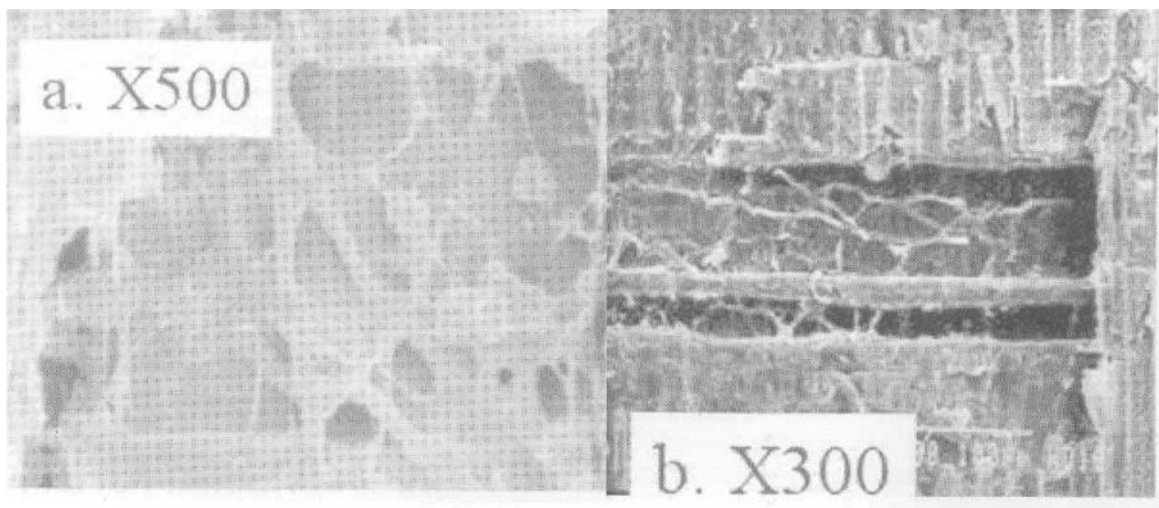


Fig. 5. Mycelial growth in Ray parenchyma cells on (a) ponderosa pine and (b) SYP chips

CONCLUSIONS

Mushroom-producing basidiomycetes grow fast, degrading a variety of lignin as well as hemicelluloses and cellulose in treated conifer lignocellulosic substrates. The mushroom-producing lignolytic white-rot fungi can convert conifer into a valuable resource, producing nutritious edible mushrooms that are in great demand. Unused conifer wood wastes pose a danger to the ecosystem. In conclusion, this study demonstrated that the extractive-degrading fungi can remove 70% to 99.9% of extractives from conifer wood chips. Treated conifer wood chips can foster rapid mycelial growth of white-rot mushroom-producing basidiomycetes, which can be utilized for the production of gourmet and medicinal mushrooms.

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