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Biological Degradation of Resin Acids in Wood Chips by Wood-Inhabiting Fungi

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Resin acids in many pulp mill effluents are primary sources of toxicity to fish. Inconsistent biological detoxification of chlorinated and nonchlorinated resin acids in secondary treatment of pulp mill effluents is a continuing source of concern. An alternative approach to effluent detoxification is to remove or modify the toxic compounds present in wood chips prior to pulping. Results from experiments in which lodgepole pine sapwood chips were inoculated with several fungal candidates indicate that the total resin acid content can be reduced by up to 67% after fungal growth. Such a treatment could be an efficient and environmentally acceptable way for deresinating wood chips and so decreasing the toxicity of pulp mill effluents.

The pulp and paper industry is currently under pressure to reduce or eliminate the release of toxic chemicals into the environment (15). Naturally occurring resin acids, a group of diterpenoid carboxylic acids present mainly in softwoods, are liberated from wood during pulping. They are toxic to fish and microorganisms. The 96-h 50% lethal concentrations of most resin acids are between 0.5 and 1.5 ppm for rainbow trout, and 20 to 70% of the toxicity of whole effluents has been linked to resin acids (6, 12–14). Inconsistent biological detoxification of chlorinated and nonchlorinated resin acids in secondary treatment of pulp mill effluents is a continuing source of concern. Resin acids have been found in receiving waters and sediments downstream from pulp mills.

An alternative approach to effluent detoxification is to remove or modify the toxic compounds present in wood chips prior to pulping. Such a wood chip pretreatment to reduce lipids, and so pitch (2, 5), is presently under mill trial. The same biological pretreatment could substantially decrease the resin acid content of wood. In this paper, we present results obtained after inoculation of sapwood chips from lodgepole pine with four different fungal strains.

MATERIALS AND METHODS

Two sap-staining fungi causing blue stain, *Ophiostoma piceae* and *Ophiostoma ainoae*, isolated from softwood species, were obtained from Forintek Canada Corp. (Ottawa, Canada). The commercial product Cartapip, an albino strain of *Ophiostoma piliferum*, another sap-staining fungus, was a gift from Repligen Sandoz Research Corporation, Lexington, Mass. A white wood-inhabiting fungus, belonging to the genus *Lecythophora*, was isolated from a healthy aspen. This hyphomycetous fungus was obtained from the Canadian Forest Service (Edmonton, Canada). The strains were grown on 2% malt extract agar plates. To avoid degeneration on successive transfer on agar medium, cores of 3 mm in diameter were taken from colony edges and kept in 10% sterile glycerol at −70°C. For all the fungal strains, the inoculum was prepared by transferring a core to a minimal liquid medium containing starch as a carbon source (1). After 4 days of growth at 23°C on a rotary shaker, each culture was homogenized and centrifuged and the fungal pellet was rinsed before being resuspended in sterile water. The fungal biomass was determined by filtering a known volume of washed mycelium through a pretared glass microfiber filter.

Fresh lodgepole pine sapwood blocks (30 by 10 by 5 mm) were sterilized by γ radiation, and their moisture contents were determined gravimetrically after they had been dried at 105°C overnight. Wood blocks (1.5 g [wet weight]) were transferred to sterile petri dishes containing a plastic mesh on the top of several layers of humidified Whatman paper and were inoculated with 50 μg of fungal mycelium per g of wood and then incubated at 23°C. After various incubation periods, infected and noninfected wood samples were removed, ground into powder while being cooled with liquid N_2, and then extracted with acetone by using a Soxhlet extraction apparatus. The resin acids were separated and quantified by gas chromatography after fractionation with solid-phase extraction (4, 9). To compare how efficiently the different organisms removed resin acids, we measured fungal growth by determining the ergosterol contents of infected wood chips (8). All the experiments and analyses were done in duplicate.

RESULTS AND DISCUSSION

Softwoods contain high concentrations of resin acids such as dehydroabietic acid (DHA), abietic acid, pimaric acid, and isopimaric acid, etc. (Table 1; Fig. 1). However, the concentrations vary within a tree and between tree species. Similarly, the types and concentrations of resin acids in effluents vary substantially, depending on the wood species, pulping and bleaching processes, and biological treatments (13, 18). It is also well-known that the toxicities of individual resin acids differ. In order to compare the relative toxicities of resin acids in different woods and effluents, and to evaluate the effectiveness of different detoxifying treatments, we defined the following index of relative toxicity (T_{rel}): $T_{rel} = F_{tox} \cdot C$, where F_{tox} is a toxicity factor equal to the inverse of the 50% lethal concentration and C is the concentration of an individual resin acid in wood (milligrams per kilogram) or water (milligrams per liter).

Table 1 shows the wide range of concentrations and relative toxicity indexes for the eight resin acids detected in lodgepole pine wood. DHA and palustric acid are the dominant resin acids by concentration, but palustric acid's relative toxicity index is twice as high as DHA's. Isopimaric and abietic acids also have significantly high toxicity indexes; DHA, palustric acid, abietic acid, and isopimaric acid contributed almost 87% of the total relative toxicity.

Recently, we and a few other researchers showed that wood-inhabiting fungi could substantially decrease the level of resin acids when the organisms grew in wood (3, 5, 10). However, none of these studies correlate fungal growth with resin acid disappearance or show that fungi can use resin acids as a carbon source. In the present work, there was a good correlation between the total amount of fungal growth and the decrease in total resin acid content for all four fungi (Fig. 2). Up to 60% of the total resin acid content was degraded after 2 weeks of treatment, and longer treatment times gave no fur-

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TABLE 1. Relative toxicities of resin acids in lodgepole pine sapwood[a]

Resin acid	Amt (mg/kg of OD wood)	LC_{50} (mg/liter)	F_{tox} (liters/mg)	T_{rel}
DHA	1,036	1.1[b]	0.91	943
Palustric acid	891	0.5[b]	2.00	1,782
Abietic acid	440	0.7[b]	1.43	629
Isopimaric acid	364	0.4[b]	2.50	910
Pimaric acid	213	0.8[b]	1.25	266
Neoabietic acid	156	0.6[c]	1.67	261
Sandaracopimaric acid	51	0.4[b]	2.50	142
Levopimaric acid	tr	0.7[d]	1.43	

[a] OD, oven-dried; LC_{50}, 50% lethal concentration.
[b] Determined by Leach and Thakore (13).
[c] Determined by Chung et al. (6).
[d] Determined by Leach and Chung (12).

ther significant resin acid reduction. The *Lecythophora* sp. and *O. ainoae* were more efficient than the commercial product, which was comparable to the staining fungus *O. piceae* in removing total resin acids (Fig. 3). Figure 4 compares how effectively individual resin acids were removed by the four fungi. There were important differences between the two dominant resin acids, palustric acid and DHA. Palustric acid, which had the highest T_{rel} in the wood, was reduced by 96% by *Lecythophora* sp. and *O. ainoae*, by 92% by Cartapip, and by 89% by *O. piceae*. DHA, the dominant resin acid by concentration in the wood before treatment, decreased by between 35 and 52%. Contents of neoabietic, abietic, and pimaric acids were also reduced by 30 to 70%. The *Lecythophora* sp. and *O. ainoae* decreased isopimaric acid by 40 to 50%, while for Cartapip and *O. piceae* we observed a slight increase in the concentration of this compound. The increase may have been due to the isomerization of other resin acids. Similar behavior has been reported to occur in seasoning wood (16, 19). With the exception of their activity on isopimaric acid, the four fungal

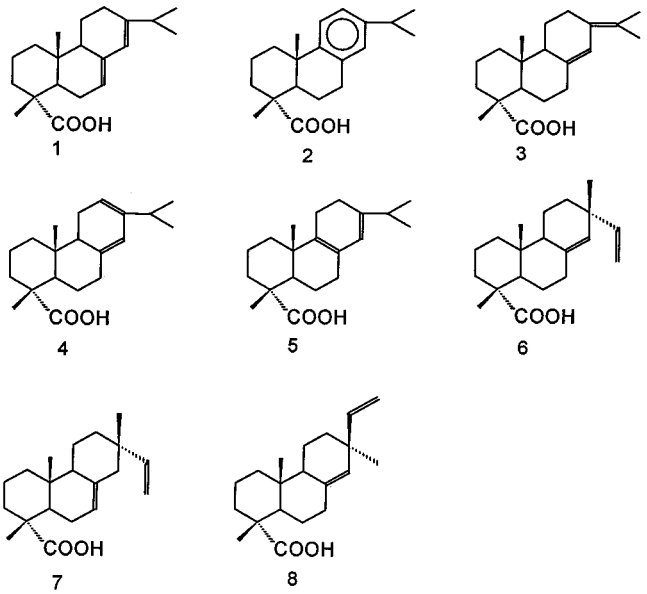

FIG. 1. Chemical structures of the most common resin acids in softwood species. No. 1, abietic acid; no. 2, dehydroabietic acid; no. 3, neoabietic acid; no. 4, levopimaric acid; no. 5, palustric acid; no. 6, sandaracopimaric acid; no. 7, isopimaric acid; no. 8, pimaric acid.

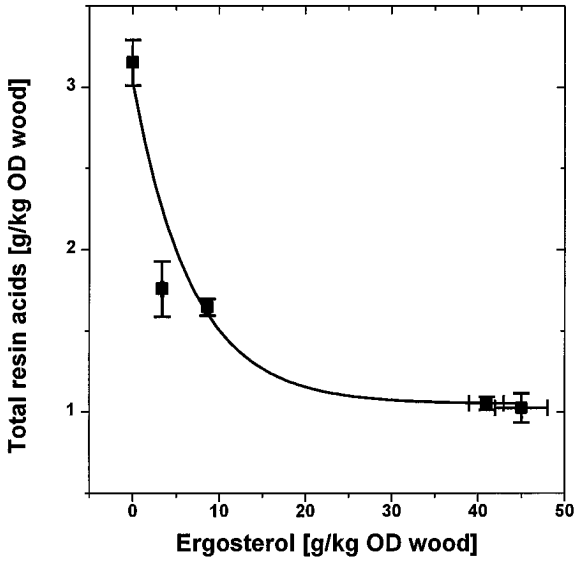

FIG. 2. Relationship between total resin acid content and degree of growth of the *Lecythophora* sp. measured at days 0, 4, 8, 14, and 36 after inoculation. Each datum point represents a different day, and error bars indicate standard deviations. OD, oven-dried.

strains showed similar trends on the different resin acids. It is interesting that *Ophiostoma* species are among the first fungi to appear on freshly cut wood and their hyphae are highly concentrated in the nutrient-rich ray parenchyma cells, but that they also invade resin canals and tacheids. *O. piliferum* is one of the most common species on conifers in the southern United States, while *O. piceae* is the predominant species in Canada (17). Usually, these fungal species do not damage wood structurally as decay fungi do; however, they seem to be very efficient in retrieving the nonstructural components of wood such as wood extractives (5, 7, 10). We chose strains for their abilities to rapidly invade wood and to remove wood extractives. Surprisingly, the *Lecythophora* sp. isolated from the aspen, a wood species with no detectable resin acids, was as effective as fungal species growing in coniferous trees, which contain high levels of resin acids. It is important that none of the species

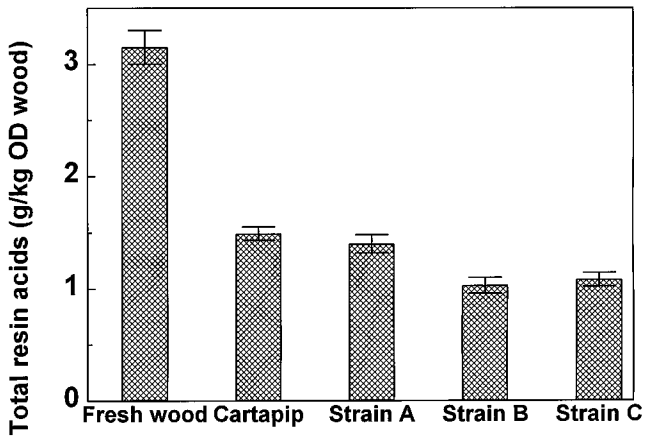

FIG. 3. Total acetone-extracted resin acids from lodgepole pine sapwood without biotreatment (Fresh wood) and with biotreatment by four different fungi: *O. piliferum* (Cartapip), *O. piceae* (Strain A), the *Lecythophora* sp. (Strain B), and *O. ainoae* (Strain C). Error bars indicate standard deviations. OD, oven-dried.

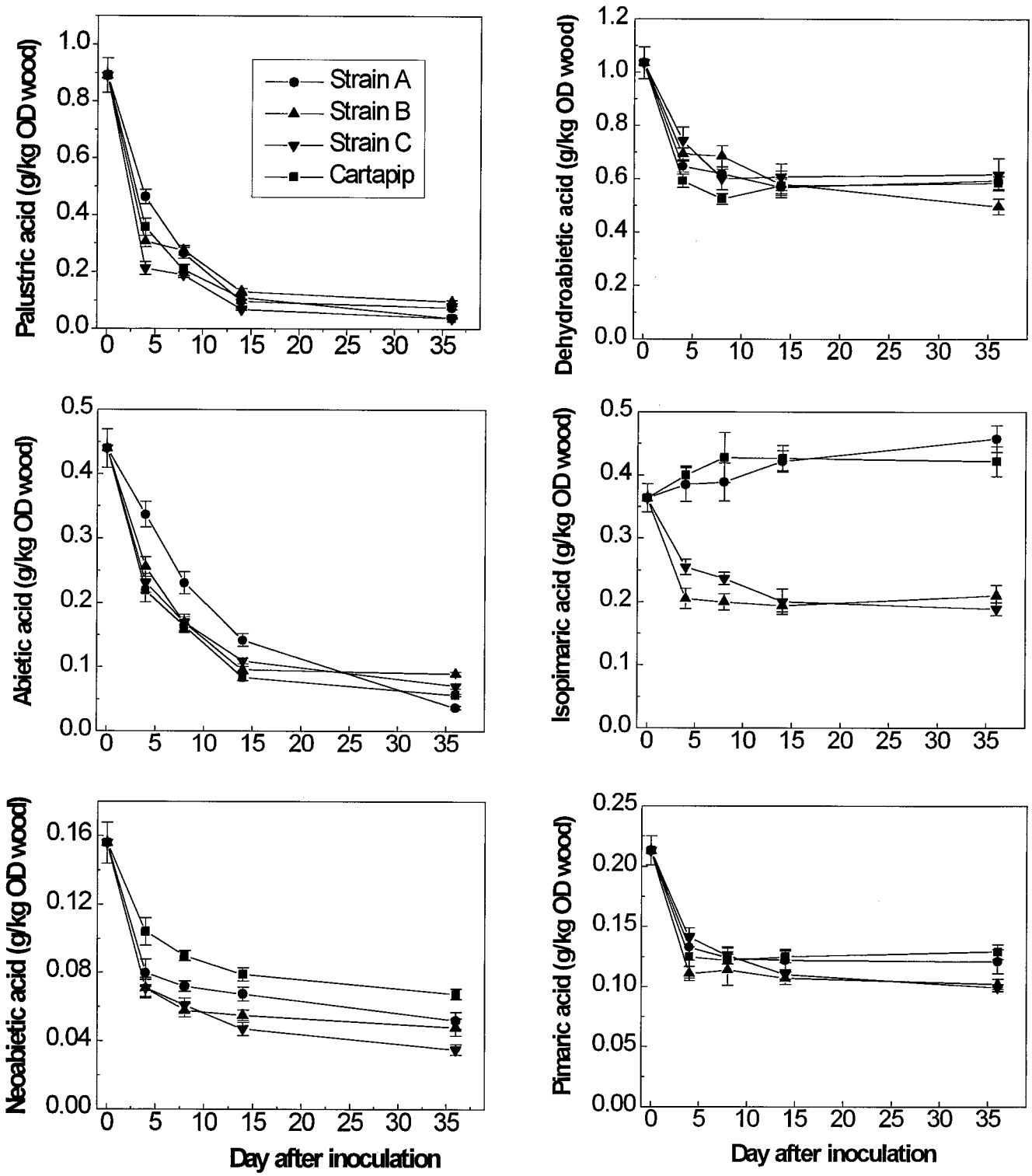

FIG. 4. Concentrations of individual resin acids in lodgepole pine sapwood during treatment with four different fungi: *O. piceae* (Strain A), the *Lecythophora* sp. (Strain B), *O. ainoae* (Strain C), and *O. piliferum* (Cartapip). Error bars indicate standard deviations. OD, oven-dried.

were able to grow in the heartwood of lodgepole pine, where the resin acid content can be as high as 5% (9). At this point, we cannot conclude whether the resin acids were completely degraded or only modified into less toxic metabolites, as was reported for the fungus *Mortierella isabellina* (11). Because of difficulties in isolating, purifying, and radioactively labelling individual resin acids, very little information is available on the degradation of individual resin acids or on their effect on fungal growth.

Changes in relative toxicity indexes due to treatment for the

eight resin acids can be summarized as follows. Palustric acid, with the dominant toxicity index, was strongly reduced by all fungal strains. Similarly, abietic acid was greatly reduced by all strains. Pimaric and neoabietic acids had low indexes before and after treatment, and their indexes were close to those of posttreatment palustric acid. Before treatment, DHA and isopimaric acid were the next most important contributors to the relative toxicity index after palustric acid. There were significant differences between the removal patterns for the different fungal strains. Cartapip and *O. piceae* showed increases in isopimaric acid but were moderately effective in decreasing DHA. The *Lecythophora* sp. was very active in decreasing both DHA and isopimaric acid. *O. ainoae* was active in decreasing isopimaric acid and moderately effective in decreasing DHA.

Although there were substantial decreases in total resin acids after fungal treatment, DHA and isopimaric acid were left as the dominant factors in the overall remaining relative toxicity. The results suggest that biological pretreatments could be an efficient way of deresinating wood chips.

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