Toxicity of Pentachlorophenol to Six Species of White Rot Fungi as a Function of Chemical Dose

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The growth of six species of white rot fungi was a function of pentachlorophenol (PCP) dose, expressed as mass of PCP per mass of mycelia, at PCP doses \leq 35 \mu g of mycelium^{-1}, and not concentration. At higher doses, Inonotus dryophilus, Perenniporia medulla-panis, and Ganoderma oregonense removed less PCP than three other species of white rot fungi. Phanerochaete chrysosporium grown under nitrogen-deficient conditions was inactivated at PCP doses that under nitrogen-sufficient conditions resulted in only 2-day lag periods in growth. Trametes versicolor was the fastest-growing species that remained viable at higher PCP doses. Both Trametes versicolor and Phellinus radius were able to degrade PCP at higher PCP doses.

White rot fungi degrade a wide variety of environmental pollutants, including many chlorinated aromatic compounds such as DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], polychlorinated biphenyls, and pentachlorophenol (PCP) (1, 3–5, 7, 11, 13). This has led to research on using these fungi to degrade organic pollutants in contaminated wastewaters and soils (8, 10–12, 14, 16). Previous studies have shown that these chemicals can be toxic to fungi at higher concentrations (6, 11, 15). However, preliminary experiments in our laboratory indicated that chemical toxicity was better described by the chemical doses, expressed as the ratio of the mass of chemical to the mass of the mycelium (1), than by the solution concentrations routinely reported in other studies (6, 11, 13, 15). In this note, we demonstrate that chemical toxicity should be expressed in terms of dose by examining the toxic effects of PCP on both developing and mature fungal mats in stationary liquid cultures, under both nitrogen-sufficient and -deficient conditions.

Six species of white rot fungi were selected for testing based on their known lignin-degrading capabilities. Phanerochaete chrysosporium ME-446 and Trametes versicolor 697 were obtained from the U.S. Forest Products Laboratory, Madison, Wis. The other four species (Inonotus dryophilus 16297, Ganoderma oregonense 16381, Perenniporia medulla-panis JEA 832, and Phellinus radius JEA 1047) were obtained from the University of Arizona Mycological Collection, Department of Plant Pathology, University of Arizona, Tucson. Cultures were maintained at room temperature by aseptically cutting and transferring a piece of agar (2% malt extract) from a mature slant with a dissecting needle to fresh media every 30 to 60 days.

The media used in these experiments contained (per liter of distilled water): 20 g of glucose, 2.5 g of sodium citrate, 5.0 g of K2SO4, 2.0 g of NH4NO3, 0.068 g of CaCl2, 0.2 g of MgSO4, and 1 mg of thiamine (9). Fungal mats used in experiments were developed on plates consisting of media and agar (15 g liter^{-1}). Plated fungi were used to inoculate liquid cultures after the mycelial colony grew to approximately 30 mm in diameter.

Biomass (mycelium) dry weight samples were filtered onto preweighed glass fiber filters (Whatman GF/C) under a vacuum of 450 mm Hg (60 kPa), rinsed twice with 100 ml of ultrapure water (Milli-Q system; Millipore Corp.), and dried at 105°C for 24 h prior to weighing. Substrate concentration (glucose) was indirectly monitored by measuring the total dissolved organic carbon (DOC) with a Dohrmann DC-80 total organic carbon analyzer. DOC samples analyzed were filtered (GF/C filters), centrifuged (16,000 \times g, 10 min) to remove solids, diluted 1:4 with ultrapure water, acidified below pH 2.0, and purged with high-purity nitrogen to remove inorganic carbon. Samples were stored at 4°C and analyzed within 8 h of harvesting.

PCP was measured by high-performance liquid chromatography (Beckman) in a chromatograph equipped with a C-18 reverse-phase column (4.6 by 250 mm; 5 \mu m-EconoSphere C-18; Alltech Assoc.). PCP was eluted with a 75:25:0.125 mixture of acetonitrile-water-acetic acid, monitored at 238 nm, and concentrations were determined by comparing resulting peak area counts to a standard calibration curve (13). PCP, acetonitrile, and methanol were obtained from Aldrich Chemical Company (Milwaukee, Wis.).

When germinated (<1-day-old) 5-mm plugs of cultures were transferred to test tubes (50 ml, in quadruplicate) containing liquid media (10 ml), all six species failed to grow at PCP concentrations higher than 5 mg liter^{-1} within 2 weeks. Controls (without PCP) showed extensive growth. These results were unexpected, since some reports (11, 13) have shown that white rot fungi are able to grow at PCP concentrations much greater than 5 mg liter^{-1}. However, Mileski (13) reported that spore germination of P. chrysosporium was prevented at PCP concentrations as low as 4 mg liter^{-1}. This suggested that the quantity of biomass was an important factor in determining whether fungi could grow and degrade PCP.

To further examine the dose-response relationship between PCP and fungi, six species of fungi were grown in test tubes without PCP, transferred to new liquid media (50 ml, in quadruplicate) in 250-ml (Erlenmeyer) culture flasks containing PCP, and incubated. Because of differences in growth rates, P. chrysosporium and Phellinus radius cultures were incubated for 6 days and 6 weeks, respectively, while the remaining cultures were all incubated for 2 weeks prior to the addition of PCP. P. chrysosporium was analyzed 6 days...

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and the other cultures were analyzed 2 weeks after the addition of PCP. The initial mass of mycelia varied from 9.4 mg for G. oregonense to 142 mg for Phellinus badius (Table 1). PCP was added at concentrations of 5, 10, 20, and 40 mg of PCP liter\(^{-1}\), resulting in maximum doses ranging from 14 to 212 μg of PCP mg of mycelium\(^{-1}\).

**P. chrysosporium** and Perenniporia medulla-panis did not grow after exposure to PCP at doses of ≤17 and 40 μg of PCP mg of mycelium\(^{-1}\), respectively, indicating these doses were lethal to the cultures (Table 1). The other four species grew at these PCP concentrations, but there was little relationship between growth and PCP concentration between different species (Fig. 1A). However, when the PCP doses are calculated by using the mass of mycelia initially present, the growth of five of the six species of fungi in the presence of PCP was similar. Only T. versicolor exhibited substantially higher growth rates than other fungi. Growth was also monitored by measuring the concentration of DOC after PCP addition and followed the same trend observed for production of mycelia (1, 2).

The mass of PCP removed by the fungi was also a function of PCP dose (Fig. 2A and Table 1). PCP adsorption experiments with inactivated mycelia accounted for between 2 and 3% of the PCP disappearance (data not shown). Furthermore, when the amount of PCP removed is normalized for the mycelial biomass in each test, the amount of PCP removed by different species is similar for all six species of white rot fungi, at PCP doses below 35 μg of PCP mg of mycelium\(^{-1}\) (Fig. 2B). At higher doses, two species (I. dryophillus and Perenniporia medulla-panis) removed less PCP than the other four species. Neglecting data for these two species at higher doses, PCP removed was in proportion to dose (\(r^2 = 0.99; n = 19\)) at doses below 100 μg of PCP mg of mycelium\(^{-1}\) (Fig. 2B). However, only two species (T. versicolor and Phellinus badius) remained viable and able to degrade PCP at higher doses used in these experiments.

We further examined the effect of sublethal PCP doses (2 to 6 μg of PCP mg of mycelium\(^{-1}\)) on the long-term viability of cultures for three species of fungi, P. chrysosporium, I. dryophillus, and T. versicolor, under both nitrogen-deficient and -sufficient conditions (0.178 and 2.0 g of NH\(_4\)NO\(_3\) liter\(^{-1}\), respectively [13]). Cultures grown in nitrogen-sufficient media all achieved similar biomass production and DOC utilization levels compared to controls (without PCP) within 2 to 6 days after PCP addition. However, cultures of P. chrysosporium in nitrogen-deficient media were completely inactivated (produced no additional biomass) at a dose (5.0 μg of PCP mg of mycelium\(^{-1}\)) found not to be lethal in nitrogen-sufficient media. In addition, mycelia transferred from the nitrogen-deficient P. chrysosporium culture to fresh media did not grow. Nitrogen-deficient cultures of both I. dryophillus and T. versicolor, however, remained viable and comparable in biomass production to controls within 5 to 10 days. Therefore, although nitrogen-sufficient cultures of P. chrysosporium are known to degrade PCP less efficiently than nitrogen-deficient cultures (13), our studies indicate that nitrogen-sufficient cultures remain viable at higher PCP doses.

From these results, we hypothesize that previous reports of the ability of P. chrysosporium to degrade PCP at concentrations as high as 500 mg liter\(^{-1}\) (13) may primarily be due to the high growth rate of this species, i.e., to the high

### Table 1. Lethal and residual PCP concentrations for six species of fungi

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Initial biomass (mg)</th>
<th>Chemical dose (μg of PCP mg of biomass(^{-1}))</th>
<th>Residual PCP concn (mg liter(^{-1})) at initial PCP concn (mg liter(^{-1})) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>Lethal</td>
</tr>
<tr>
<td><em>P. chrysosporium</em> ME-446</td>
<td>58</td>
<td>35</td>
<td>≤17</td>
</tr>
<tr>
<td><em>I. dryophillus</em> 16297</td>
<td>24</td>
<td>85</td>
<td>ND*</td>
</tr>
<tr>
<td><em>T. versicolor</em> 697</td>
<td>49</td>
<td>41</td>
<td>ND</td>
</tr>
<tr>
<td><em>G. oregonense</em> 16381</td>
<td>9.4</td>
<td>212</td>
<td>ND (≤50)b</td>
</tr>
<tr>
<td><em>Perenniporia medulla-panis</em> JEA 832</td>
<td>26</td>
<td>79</td>
<td>≤40</td>
</tr>
<tr>
<td><em>Phellinus badius</em> JEA 1047</td>
<td>142</td>
<td>14</td>
<td>ND</td>
</tr>
</tbody>
</table>

a ND, not detected.
b Based on DOC utilization. Other numbers based on biomass produced.

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**Fig. 1.** Growth of different species of white rot fungi as a function of PCP added. (A) Mycelia produced as a function of PCP concentration; (B) mycelia produced as a function of dose. (C) P. chrysosporium, (D) I. dryophillus, (E) T. versicolor, (F) G. oregonense, (G) Perenniporia medulla-panis, (H) Phellinus badius.
concentrations of biomass used in those experiments. When the response of several species of white rot fungi was compared on a dose basis, we found the ability of *P. chrysosporium* to remove PCP was comparable to that of several other species. However, since culture viability of *P. chrysosporium* was lost at higher PCP doses, other species may be more useful in biodegradation studies. Of the six species examined, *T. versicolor* was the fastest-growing species able to remain viable at higher PCP concentrations (Fig. 2B), produced the most biomass after addition of PCP (Fig. 1B), and remained viable when grown with both nutrient-deficient and -sufficient media. Other research in our laboratory has shown that this fungus is also able to more effectively dehalogenate PCP (1). Therefore, of the six species examined, we conclude that *T. versicolor* might be the best choice to inoculate soils and bioreactors used for PCP degradation in bioremediation and wastewater treatment studies. These results also suggest the need to report chemical doses in biodegradation studies using white rot fungi.

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REFERENCES


