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# PCB Metabolism by Ectomycorrhizal Fungi

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Since 1976 the use of polychlorinated biphenyls (PCBs) has been banned in the U.S. Prior to this, commercial mixtures (Aroclors) had been used extensively as an industrial lubricant because of their nonflammable, nonreactive properties. These same properties are responsible for their persistence in the environment where they bind to soil particles and resist biodegradation. Decontamination of PCB-laden soil is expensive with excavation followed by either storage or incineration as the primary means of remediation. The use of microorganisms for PCB bioremediation has been gaining popularity in the past few years (Bedard et al. 1986, Furukawa et al. 1978, Unterman et al. 1988)). Bacteria and/or fungi isolated from environmental samples have been used to degrade PCBs under laboratory conditions (Bedard et al. 1984, Eaton 1985, Field et al. 1993, Sahasrabudhe and Modi 1987, Sinton et al. 1986), but in field trials they have not been as effective (Unterman et al. 1988). The most common explanation for the poor performance of PCB-degrading organisms introduced at contaminated sites is that they do not compete well with the existing populations. Plant-ectomycorrhizal systems may overcome this problem. Introduction and cultivation of a known host plant at a contaminated site has the potential of providing a survival advantage for ectomycorrhizal fungi that normally colonize the roots of the introduced plant.

Ectomycorrhizal fungi exist naturally in the soil and normally grow in association with the roots of a host plant in a mutualistic symbiotic relationship (Harley 1989). Preliminary in vitro examination of this group of fungi for their ability to enzymatically degrade xenobiotics is very promising (Bae and Barton 1989, Zhu et al. 1990). In vivo studies have shown that some of these fungi have the ability to degrade chlorinated, aromatic compounds, such as 2,4-D and atrazine (Donnelly et al. 1993, 1994). The aspect of ectomycorrhizal metabolism was investigated further in the current study by determining the ability of 21 different fungi to metabolize 19 different PCB congeners with varying chlorine content and substitution patterns.

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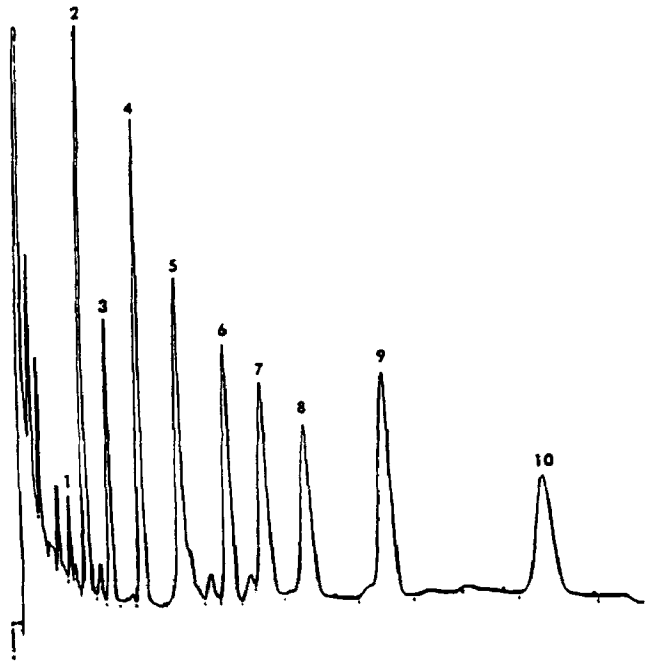
## MATERIALS AND METHODS

Ectomycorrhizal cultures were obtained from three different sources shown in Table I. Stock cultures of each fungus were maintained on solid medium as described in Donnelly et al. (1993). Liquid cultures were started by inoculating 50 ml of medium in 250 ml erlenmeyer flasks with three 3-mm plugs removed from each stock culture (Donnelly et al. 1993). These cultures were incubated at 22°C on a rotary shaker set at 150 rpm for one month. Using aseptic techniques the medium was removed from the fungi by filtration, and mycelium retained on the filter was resuspended and homogenized with a Waring blender in 50 ml fresh medium containing no carbon source. Two milliliters of each fungal preparation were transferred to a sterile 20 ml reaction vessel and 10 µl of a PCB mixture was added. The PCB stock mixture contained either 10 or 11 different PCB congeners in acetone. The final PCB concentration of each congener was 5 µM. Controls were fungi which had been killed by the additions of perchloric acid at a final concentration of 0.7%. Living cells and controls were run in duplicate, and all samples were incubated at 22°C shaking horizontally. After 5 days of incubation, the living cells were killed by the addition of perchloric acid. The PCB extraction method involved the addition of 1.2 g of anhydrous sodium sulfate and 200 µl of 10% Triton X-100. After 3 hours incubation at 55°C, 8.0 ml of hexane was added, mixed, and allowed to incubate overnight shaking. A GC analysis was then performed on the extracted samples. The PCB mixtures used, extraction methods, and GC analysis of extracts were all conducted according to the methods of Bedard et al. (1986).

## RESULTS AND DISCUSSION

Fourteen of the 21 ectomycorrhizal fungi tested degraded PCBs (Table 1). Both the number of PCB congeners metabolized and the extent of metabolism varied among the fungi (Table 2). The number of PCB congeners metabolized ranged from zero to seven (Table 2). The lower chlorinated congeners were more readily degraded than the higher chlorinated congeners (Table 2). Our work had a % recovery which ranged from 40% to 100% depending on the species and the congener. The average % recovery was 78%. The average standard error was 6%. Radiigera atrogleba and Gautieria crispa metabolized the highest number of congeners, seven and five respectively. Radiigera atrogleba also showed the most pronounced ability to metabolize the congeners which it acted upon; two congeners (2,3 and 2,2') were almost entirely metabolized, and one (2,5,2') in the 60 to 79% range (Fig. 1). No single congener was metabolized by all of the fungi. Analysis of the data showed no correlation between taxonomically related species and metabolism of structurally similar PCB congeners. It was observed that several of the fungi that metabolized PCBs in this study had previously been shown to degrade the chlorinated, aromatic herbicides 2,4-D and atrazine (Donnelly et al. 1993, 1994).

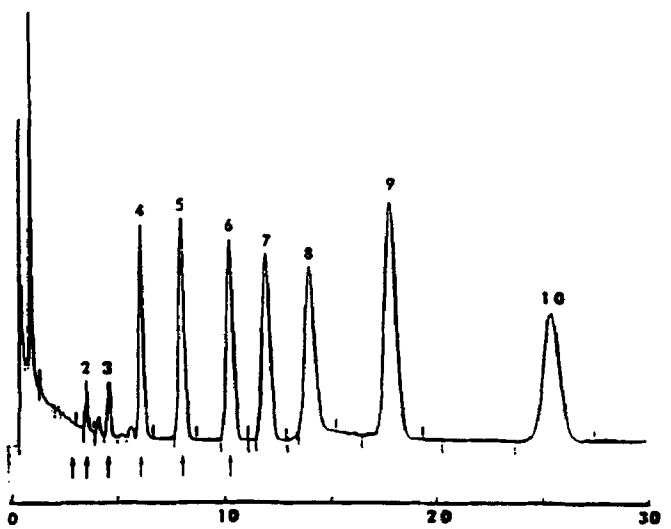
Control - Dead Cells




PCB Mix 2B

1 2,2'
2 2,3
3 2,5,2'
4 2,5,4'
5 2,4,2',4'
6 2,4,6,2',4'
7 2,5,3',4'
8 2,4,5,2',5'
9 2,3,4,2',5'
10 2,4,5,2',4',5'

Living Cells




RETENTION TIME (min)

**Figure 1** GC chromatograms of PCBs incubated with dead (top) or living (bottom) cells of Radiigera atrogleba. Arrows mark congeners which were metabolized by >20%.

**Table 1** Name and source of ectomycorrhizal fungi screened for PCB metabolism.

| Fungus | | | | Abbreviation[a] | Source | PCB metabolism |
|---|---|---|---|---|---|---|
| Family | Genus | Species | Strain | | | |
| Corticiaceae | Byssoporia | terrestris | Z-30 | BT | 1 | + |
| Elaphomycetaceae | Cenococcum | geophilum | A-145 | CG | 1 | - |
| Gautieriaceae | Gautieria | caudata | 8481 | GCa | 1 | - |
| Gautieriaceae | Gautieria | crispa | 4936 | GCr | 1 | + |
| Gautieriaceae | Gautieria | othii | 6362 | G0 | 1 | + |
| Geneaceae | Genabea | cerebriformis | 6264 | GCe | 1 | + |
| Cortinariaceae | Hebeloma | crustuliniforme | 1 | HC1 | 2 | + |
| Cortinariaceae | Hebeloma | crustuliniforme | 2 | HC2 | 2 | + |
| Helotiaceae | Hymenoscyphus | ericae | 1318 | HE | 1 | - |
| Hysterangiaceae | Hysterangium | gardneri | 8405 | HG | 1 | + |
| Sporendonemataceae | Oidiodendron | griseum | | 0G | 1 | + |
| Corticiaceae | Piloderma | croceum | S-163 | PC | 1 | + |
| Sclerodermataceae | Pisolithus | tinctorius | S-370 | PT1 | 1 | - |
| Sclerodermataceae | Pisolithus | tinctorius | S-471 | PT2 | 1 | + |
| | Radiigera | atrogleba | 9470 | RA | 1 | + |
| Rhizopogonaceae | Rhizopogon | vinicolor | 6861 | RV1 | 1 | - |
| Rhizopogonaceae | Rhizopogon | vinicolor | 7534 | RV2 | 1 | - |
| Rhizopogonaceae | Rhizopogon | vulgaris | S-251 | RVu | 1 | - |
| Boletaceae | Suillus | granulates | 7589 | SG1 | 1 | + |
| Boletaceae | Suillus | granulates | CK13 | SG2 | 2 | + |
| Elasmomycetaceae | Zelleromyces | B | H1091 | ZE | 1 | + |
| | Phanerochaete | chrysosporium | 1767[b] | PH | 3 | + |

[a]Abbreviation used in Table 2.
[b]Nonmycorrhizal white-rot fungus as positive control.
1 = Jane Smith, USDA Forest Service, Pacific Northwest Forest Research Station, Corvallis, Oregon.
2 = Merline Robideaux, ManTech Services, Environmental Research Laboratory, Corvallis, Oregon.
3 = Andrzej Paszczynski, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho.

**Table 2** The number and relative amounts of PCB congeners degraded by ectomycorrhizal fungi with the greatest potential for PCB-degradation.

| PCB Congener | Fungi | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BT[a] | GCr | GO | HC2 | HG | OG | PC | RA | SG1 | SG2 | ZE | PH[b] |
| 2,3 | +[c] | ++ | + | +++ | +++ | - | + | ++++ | + | +++ | +++ | ++ |
| 2,2' | - | +++ | +++ | NA[d] | ++++ | + | NA | ++++ | NA | NA | NA | ++++ |
| 2,4' | + | ++ | + | - | - | + | - | + | ++ | + | ++ | - |
| 4,4' | - | + | - | - | - | - | - | - | - | - | - | - |
| 2,4,4' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,5,2' | - | + | + | ++ | ++ | - | + | +++ | + | ++ | ++ | + |
| 2,5,4' | - | - | - | + | + | - | + | ++ | + | - | + | - |
| 2,3,2',3' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,3,2',5' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,4,2',4' | - | - | - | - | - | - | - | ++ | - | - | - | - |
| 2,4,3',4' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,5,2',5' | + | - | - | - | - | - | - | - | - | - | - | - |
| 2,5,3',4' | - | - | - | - | - | - | - | - | - | - | - | - |
| 3,4,3',4' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,3,4,2',5' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,4,5,2',3' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,4,5,2',5' | - | - | - | - | - | - | - | - | - | - | - | - |
| 2,4,6,2',4' | - | - | - | - | - | - | - | + | - | - | - | - |
| 2,4,5,2',4',5' | - | - | - | - | - | - | - | - | - | - | - | - |

[a]Abbreviations of fungi are the same as used in Table I.
[b]Phanerochaete chrysosporium, a nonmycorrhizal fungus included for comparative purposes.
[c]Symbol signifying the amount metabolized; - 0 to 19%, + 20 to 30%, ++ 40 to 59%, +++ 60 to 79%, ++++ 80-100%.
[d]NA = not available

This study disclosed for the first time that several ectomycorrhizal fungi have the ability to metabolize PCBs. Under the conditions used in this study this ability equaled or exceeded that of the white-rot fungus Phanerochaete chrysosporium (Table 2), the only fungus which has received a great deal of attention in bioremediation research to date. The significance of our findings with ectomycorrhizal fungi is that this group of fungi exists naturally in the soil environment in combination with host plants. Because of the mutualistic arrangement between host plants and ectomycorrhizal fungi, such systems are environmentally sound. Therefore plant-ectomycorrhizal systems have the potential to survive and degrade soil contaminants when introduced at PCB contaminated sites, whereas other bacteria and saprophytic fungi have failed to display sustained growth and activity (Unterman et al. 1988), presumably because of their inability to compete with indigenous organisms.

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