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Sensitivity to and Degradation of Pentachlorophenol by *Phanerochaete* spp.

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This research measured mycelial extension rates of selected strains of *Phanerochaete chrysorhiza*, *Phanerochaete laevis*, *Phanerochaete sanguinea*, *Phanerochaete filamentosa*, *Phanerochaete sordida*, *Inonotus circinatus*, and *Phanerochaete chrysosporium* and the ability of these organisms to tolerate and degrade the wood preservative pentachlorophenol (PCP) in an aqueous medium and in soil. Most of the tested species had mycelial extension rates in the range of ≤0.5 to 1.5 cm day^{-1}, but there were large interspecific differences. A notable exception, *P. sordida*, grew very rapidly, with an average mycelial extension rate of 2.68 cm day^{-1} at 28°C. Rank of species by growth rate was as follows: *P. chrysosporium* > *P. sordida* > *P. laevis* > *P. chrysorhiza* = *P. sanguinea* > *I. circinatus* = *P. filamentosa*. There were also significant intraspecific differences in mycelial extension rates. For example, mycelial extension rates among strains of *P. sordida* ranged from 1.78 to 4.81 cm day^{-1}. *Phanerochaete* spp. were very sensitive to PCP. Growth of several species was prevented by the presence of 5 ppm (5 μg/g) PCP. However, *P. chrysosporium* and *P. sordida* grew at 25 ppm PCP, albeit at greatly decreased mycelial extension rates. In an aqueous medium, mineralization of PCP by *P. sordida* 13 (ca. 12% after 30 days) was significantly greater than that by all other tested *P. sordida* strains and *P. chrysosporium*. After 64 days, the level of PCP had decreased by 96 and 82% in soil inoculated with *P. chrysosporium* and *P. sordida*, respectively. Depletion of PCP by these fungi occurred in a two-stage process. The first stage was characterized by a rapid depletion of PCP that coincided with an accumulation of pentachloroanisole (PCA). At the end of the first stage, ca. 64 and 71% of the PCP was converted to PCA in *P. chrysosporium* and *P. sordida* cultures, respectively. In the second stage, levels of PCP and PCA were reduced by 9.6 and 18%, respectively, in soil inoculated with *P. chrysosporium* and by 3 and 23%, respectively, in soil inoculated with *P. sordida*. PCA was mineralized by both *P. chrysosporium* and *P. sordida* in an aqueous medium.

Research on fungal bleaching of kraft pulp mill effluents demonstrated the ability of a wood-degrading fungus, *Phanerochaete chrysosporium* Burds., to degrade chlorinated organics found in the E1 effluent produced from the kraft bleaching process (8, 15, 19, 25). This work led to investigations that demonstrated that this fungus, which causes a white-rot type of wood decay, is capable of transforming a variety of xenobiotics in aqueous media and in soils. A number of xenobiotics are mineralized by ligninolytic cultures of the fungus. These are Arochlor 1254 (14), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) (5, 6), 3,4,3′,4′-tetrachlorobiphenyl (6), 2,4,5,2′,4′,5′-hexachlorobiphenyl (6), 2,3,7,8-tetrachlorodibenzo[*p*]dioxin, lindane (6), chlorinated anilines (1, 2), pentachlorophenol (PCP) (24, 26), polycyclic aromatic hydrocarbons present in anthracene oil (4), and 2,4,5-trichloro-phenoxyacetic acid (33). The last compound was also found to be mineralized by *P. chrysosporium* in soil amended with ground corn cobs (4:1, corn cobs:soil) (33). DDT was also reported to be mineralized by nonligninolytic cultures of the fungus, i.e., cultures in primary growth phase (21). We reported that *P. chrysosporium* is capable of rapidly depleting PCP in soils (14, 22). The ability of this fungus to transform such a wide variety of xenobiotics has generated interest in developing bioremediation technologies that use *P. chrysosporium* or other white-rot fungi as the microbial constituents.

In investigations of white-rot fungal degradation of hazardous compounds, *P. chrysosporium* BKM-F-1767 has been used almost exclusively as the experimental organism. However, there are an estimated 1,400 species of white-rot fungi from all of the major groups of higher basidiomycetes and xylariaceous ascomycetes (29). There is great diversity among these organisms in their ability to degrade lignin (28). Thus, there is reason to believe that this same diversity will be seen in xenobiotic degradation. Indeed, evidence for differences in the abilities of white-rot fungi to degrade xenobiotics was given for the mineralization of DDT (5) and several polynuclear aromatics (A. Huterman, J. Trojanowski, and D. Loske, German patent DE3,731,816, November 1988). However, more information on the intergeneric, interspecific, and intraspecific variations in xenobiotic-degrading abilities of white-rot fungi is needed to fully realize the potential usefulness of these organisms in bioremediation of soil.

Our primary objective was to determine the degree of interspecific and intraspecific variation among selected *Phanerochaete* spp. in growth rate and in their ability to tolerate and degrade the wood preservative PCP. PCP has been classified as a priority pollutant by the U.S. Environmental Protection Agency, and it is a common soil contaminant at wood preservation facilities where commercial formulations of the compound are used. *P. chrysosporium* degrades PCP in liquid culture (24, 26) and in soil (22, 23). Also, lignin peroxidases and manganese peroxidases isolated from the fungus catalyze the *p*-dechlorination of PCP to tetetrachloro-*p*-benzoquinone (18). Thus, *P. chrysosporium* and other white-rot fungi may be useful in remediation of media contaminated with PCP.

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TABLE 1. Basidiomycete strains used in this study

Species	FPL no.[a]	Strain no.	Host species	Collection location (state)
I. circinatus	FP-133668-sp	1	*Pinus*	Oreg.
P. chrysorhiza	HHB-8871-sp	1	*Pinus taeda*	Miss.
	FP-102002-sp	2	Hardwood	Ill.
	MD-143	3	*Pinus*	Miss.
	HHB-9456	4	*Liquidambar styraciflua*	Fla.
	MD-342	5	*Pinus*	Miss.
	HHB-4106-sp	6	Hardwood	Tenn.
	RLG-5507-sp	7	*Populus gradidentata*	N.Y.
	HHB-8917-sp	8	*Cornus florida*	Miss.
P. laevis	MJL-3752-sp	1	*Fagus* sp.	Mich.
	HHB-1625-sp	2	*Acer* sp.	Md.
	MJL-190-sp	3	*Acer* sp.	N.Y.
	HHB-11091-sp	4	*Quercus arizonica*	Ariz.
P. sanguinea	FP-10538-sp	1	*Pinus strobus*	Mass.
	HHB-9985-sp	2	*Pinus* sp.	Fla.
P. filamentosa	RLG-10719-sp	1	*Quercus arizonica*	Ariz.
P. sordida	HHB-8122-sp	1	*Alnus rugosa*	Mich.
	HHB-7808-sp	3	*Betula lutea*	Mich.
	HHB-9638-sp	4	*Quercus* sp.	Fla.
	HHB-8279-sp	5	*Acer saccharum*	Mich.
	HHB-4651-sp	7	Hardwood	Fla.
	HHB-9701-sp	8	*Quercus niger*	Fla.
	HHB-7423-sp	9	*Acer* sp.	Mont.
	HHB-9529-sp	12	Hardwood	Fla.
	HHB-8922-sp	13	*Pinus taeda*	Miss.
	HHB-7827-sp	14	*Alnus rugosa*	Mich.
	HHB-4000-sp	15	Hardwood	N.C.
	HHB-4651-sp	16	*Quercus* sp.	Fla.
	HHB-9702-sp	17	*Quercus niger*	Fla.

[a] FPL, Forest Products Laboratory.

MATERIALS AND METHODS

Approach. We first determined temperature growth optima and growth rates for selected strains and species of fungi. Faster-growing strains of several species were then examined for their sensitivity to PCP. Strains that demonstrated a tolerance to PCP relative to the other tested strains were examined for their ability to mineralize PCP in liquid culture. Finally, the fungus that demonstrated the highest rate of PCP mineralization was compared with *P. chrysosporium* for its ability to deplete PCP in soil.

Fungi. Strains of *Phanerochaete chrysorhiza* (Torr. in Eaton) Bud. et Gilb., *Phanerochaete laevis* (Burt) Parm., *Phanerochaete sanguinea* (Fr.) Parm., *Phanerochaete filamentosa* (Berk. & Curt) Burds., *Phanerochaete sordida* (Karst.) Erikss. & Ryv., *Inonotus circinatus* (Fr.) Teng, and *P. chrysosporium* Burds. (BKM F-1767, ATCC 24725) were obtained from the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis. (Table 1). Each strain was grown and maintained on a complex medium (YMPG) in slants and kept at 24°C. The YMPG medium was composed of (per liter): 10 g of glucose, 10 g of malt extract, 2 g of Bacto-Peptone (Difco Laboratories, Detroit, Mich.), 2 g of yeast extract, 1 g of asparagine, 2 g of KH_2PO_4, 1 g of $MgSO_4 \cdot 7H_2O$, 1 g of thiamine and 23 g of Bacto-Agar (Difco).

Chemicals. [U-^{14}C]PCP, with a specific activity of 12.3 mCi mmol^{-1} and a purity of >99%, *N*,*N*-dimethylformamide (DMF), and spectrophotometric-grade 2,5-diphenyloxazole (PPO) were obtained from Sigma Chemical Co., St. Louis, Mo. Spectrophotometric-grade 1,4-bis(5-Phenyloxazolyl) benzene (POPOP) was obtained from Research Products International, Mount Prospect, Ill. PCP (purity of >99%), spectrophotometric-grade 2-methoxyethanol, ethanolamine (purity of 99+%), technical-grade $Na_2S_2O_4$ (purity of ~85%), and anhydrous Na_2SO_4, (purity of 99%) were obtained from Aldrich Chemical Co., Milwaukee, Wis. Acetone and hexane were B & J Brand high-purity solvents obtained from Baxter Healthcare Corp., McGaw Park, Ill. Pentachloroanisole (PCA) and [^{14}C]PCA were prepared by the reaction of PCP or [^{14}C]PCP with diazomethane in ether.

Determination of temperature growth optima and growth rates. The temperature optimum for growth of each strain was determined by measuring hyphal extension rate on 2% malt agar. For each strain, inoculum plates were prepared by aseptically transferring pieces of fungal mycelium from YMPG slants to 2% malt agar plates. The fungi were kept at 24°C until colony growth completely covered the plates. Plates of 2% malt agar for determination of temperature growth optima were inoculated with 2-mm plugs taken from inoculum plates and incubated at 14, 16, 20, 22, 24, 26, 28,

30, 32, 36, and 40°C. Five plates were prepared for each strain at each temperature. The mycelial extension rate was recorded as the average daily increase in colony diameter (centimeters) measured in two perpendicular directions. For determination of the maximum temperatures at which the fungi could survive, cultures that showed no growth after 4 days were removed from incubators and kept at 24°C. After 3 days, cultures that still showed no growth were presumed to have been killed. Cultures that grew after removal were presumed to have only been inhibited. For species where two or more strains were examined, data for rate of mycelial extension were analyzed by analysis of variance. If treatment means were shown to be significantly different by analysis of variance, a Fisher Protected Least Significant Difference multiple-comparison test was performed to determine which treatments were different from others.

Sensitivity to PCP of selected fungal strains. The sensitivity to PCP of selected strains was determined by measuring hyphal extension on 2% malt agar that contained PCP at concentrations of 0, 5, 10, 15, 20, and 25 μg of PCP g^{-1} malt agar (ppm). Five replicates were prepared for each fungus at each PCP concentration. The PCP was dissolved in DMF to aid its solution in the agar. The volume of DMF was constant for the control and all concentrations of PCP. The plates were inoculated with 2-mm plugs as described above and incubated at the optimum growth temperature that was previously determined for each strain. Mycelial extension rates were assessed daily.

Mineralization of PCP in liquid culture. The ability of several *P. sordida* strains to mineralize PCP in liquid culture was determined. *P. chrysosporium* was included in this study for comparative purposes. Volatilization of PCP or its transformation products was also measured. Fungi were incubated in stationary-phase cultures containing 10 ml of nitrogen-limited culture medium in 125-ml Erlenmeyer flasks. Culture medium contained (per liter): 10 g of glucose, 1 mg of thiamine HCl, 221 mg of ammonium tartrate, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7\ H_2O$, 1 g of $Ca(H_2PO_4)_2$, and 10 ml of mineral solution (20) in 2,2-dimethylsuccinic acid buffer (1.46 g $liter^{-1}$) adjusted to pH 4.5. Inoculum for *P. chrysosporium* consisted of 1 ml of filtered (glass wool) conidial suspensions (A_{650} = 0.5; 2.5 × 10^6 spores ml^{-1}); conidia were taken from 1-week-old slants. Inocula for *P. sordida* strains consisted of 1-ml portions of mycelial suspensions. Mycelial suspensions were prepared by adding 2 ml of deionized distilled water to each of three 1-week-old slants per strain, scraping the slants with a transfer loop to remove mycelium, and then pooling the mycelium-water slurries from the three slants. Control flasks consisted of noninoculated cultures. Three replicates were prepared for each fungus and the controls. At 3 days after inoculation, each culture received ca. 1.93 × 10^3 Bq of [^{14}C]PCP in 0.25 ml of DMF. Cultures of *P. chrysosporium* and *P. sordida* were held at 39 and 30°C, respectively.

Culture flasks were fitted with inlet-outlet ports. Inlet ports were protected from contamination by sterile, silanized glass-wool traps. Outlet ports were connected to manifold assembly systems for trapping evolved CO_2 and volatile organics. Manifold assembly systems consisted of three fused bubblers. Each bubbler was fitted with a removable tube. Volatile organics were trapped in the first tube in 10 ml of 2-methoxyethanol. Evolved CO_2 was trapped in the second and third tubes in 10 ml of CO_2-trapping scintillation fluid. The scintillation fluid was composed of toluene cocktail, methanol, and ethanolamine (5:4:1; vol/vol/vol). The toluene cocktail contained 4 g of PPO $liter^{-1}$ and 0.1 g of POPOP $liter^{-1}$ in toluene. Headspaces of all cultures were evacuated with air every 2 or 3 days. The first evacuation was conducted 2 days after introduction of [^{14}C]PCP. After each evacuation, the amount of trapped $^{14}CO_2$ was determined by transferring the 10 ml of scintillation cocktail to a 20-ml scintillation vial for counting. ^{14}C-labeled volatiles were determined by measuring the ^{14}C contents of two 2-ml volumes of 2-methoxyethanol per tube. The volumes were transferred to 10 ml of Ecolume scintillation cocktail (ICN Biomedicals, Inc., Irvine, Calif.). Counting was performed on a Packard Tri-Carb 4530 liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.).

Depletion of PCP in soil. *P. chrysosporium* and *P. sordida* 13 were grown on 2% malt agar slants at 39 and 30°C, respectively, for 1 week and then stored at 4°C. Soil inocula consisted of aspen (*Populus tremuloides* Michx.) pulpwood chips (ca. 1.5 by 0.5 by 0.25 cm^3) infested with the related fungus. Chips were sterilized by autoclaving in aluminum foil-covered 500-ml Erlenmeyer flasks at 121°C and 103.5 kPa for 1 h. The moisture content of the chips was adjusted to 60% by the addition of sterile deionized distilled water. Sterile chips were inoculated by exposing them to pieces of mycelium-covered malt agar. Inoculated chips were incubated at 39°C for *P. chrysosporium* and 30°C for *P. sordida* for 1 week.

A Marshan sandy loam (fine-silty, mixed, mesic, Mollic-Hapludalf) was collected from the A horizon, air dried, sieved, and stored in plastic bags at 4°C. The soil had the following chemical characteristics: cation exchange capacity, 50.7 meq 100 g^{-1} (determined by using the ammonium acetate method); base saturation, 66.4%; pH 6.4; organic carbon, 3.55%; Kjeldahl nitrogen, 0.46%; Ca, 4,900.0 mg kg^{-1}; Mg, 1,650.0 mg kg^{-1}; K, 90.0 mg kg^{-1}; P, 17.0 mg kg^{-1}; B, 1.3 mg kg^{-1}; Mn, 9.0 mg kg^{-1}; Zn, 12.2 mg kg^{-1}; and S, 11.3 mg kg^{-1}. The soil was sterilized by fumigating with Bromo Gas (Great Lakes Chemical Corp., West Lafayette, Ind.; 98% methyl bromide-2% chloropicrin). The PCP-amended soil was prepared by adding PCP in 0.25 M NaOH to sterile soil to obtain a final concentration of 80 μg g^{-1}. The pH of the soil was not affected by the addition of the PCP-NaOH solution. Soil for the preparation of blanks was not amended with PCP. Soil water potential of PCP-amended and nonamended soils was adjusted to −50 kPa with sterile deionized distilled water.

To assess the ability of *P. chrysosporium* and *P. sordida* to deplete PCP from soil, cultures were prepared in 125-ml Erlenmeyer flasks fitted with inlet-outlet ports as described above. Each flask contained approximately 24 g (dry weight) of soil. Inoculated cultures were prepared for each fungus by aseptically placing five inoculum chips into PCP-amended soil in each flask. Blank cultures prepared for each fungus were identical to inoculated cultures except that the soil was not amended with PCP. Control cultures consisted of PCP-amended soil that was not inoculated. Flasks were held at 30°C. The headspace of each flask was evacuated with humidified air every 3 or 4 days.

For analysis of PCP and transformation products, two cultures per culture type were harvested at days 0, 2, 4, 9, 21, 35, and 63. The soil in each flask was mixed thoroughly, and a sample was taken for the gravimetric determination of moisture content. Two 5-g subsamples from each flask were then placed in culture tubes (25 by 150 mm) with Teflon-lined screw caps. Approximately 100 mg of $Na_2S_2O_4$ was added to each tube. Soil samples were then extracted for 1 h on a rotating tumbler shaker with two 20-ml volumes of a mixture of hexane-acetone (1:1) acidified to pH 2 with concentrated

H_2SO_4. The extracts were pooled in a clean tube and dried by passing them though a column of Na_2SO_4. The Na_2SO_4 was prepared by muffling for 4 h at 400°C. Culture flasks, drying tubes, and culture tubes were muffled for 1 h at 450°C before use. Culture tubes containing the extracts were placed in a sand bath held at approximately 60°C and evaporated to approximately 5 ml with the aid of a gentle stream of nitrogen. The 5-ml volume was transferred with hexane rinse to a 10-ml volumetric flask, and the extract volume was adjusted to 10 ml with hexane. Extracts were stored at −20°C under nitrogen in amber vials with Teflon-lined screw caps.

Extracts were analyzed by gas chromatography-electron capture detection for PCP and PCA. 2,4,6-Tribromophenol was used as the internal standard. Gas chromatographic analyses of extracts were performed on a Hewlett-Packard model 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector, a model 7673A autosampler, a model 3396A reporting integrator, and a split-splitless capillary column injection port. Operating temperatures were 220°C for the injector and 300°C for the detector. The carrier gas was helium, and the make-up gas was N_2. The column was a DB-5 fused silica capillary column (30 m by 0.321 mm), with a film thickness of 0.25 μm (J & W Scientific, Folsom, Calif.). The temperature program was as follows: initial 60°C, hold for 1 min, split off for 0.5 min, ramp A, 10° min^{-1} for 9 min (60 to 150°C), ramp B, 2° min^{-1} for 20 min (150 to 190°C), and hold at 190°C for 5 min.

Mineralization of PCP and PCA by *P. chrysosporium* and *P. sordida*. PCA was identified as a major extractable product of PCP transformation in soil by *P. chrysosporium* and *P. sordida* 13. Therefore, we compared the ability of these fungi in liquid culture to mineralize PCA with their ability to mineralize PCP. Fungal and control cultures were prepared as described above. For each compound, three replicates were prepared for each fungus and control. Cultures of *P. chrysosporium* and *P. sordida* were held at 39 and 30°C, respectively. Cultures received 1.92×10^3 Bq of either [^{14}C]PCP or [^{14}C]PCA in 0.25 ml of DMF at 3 days after inoculation. Beginning 1 day after the addition of the ^{14}C-labeled compounds, the cultures were flushed with air every 3 to 4 days for 15 days. The volatilized and mineralized ^{14}C-labeled compounds were collected and quantified as described above.

RESULTS

Temperature growth optima and growth rates. All strains exhibited growth over the range of 14 to 28°C and as such would be classified as mesophiles (Table 2). Strains of *P. filamentosa* and *I. circinatus* had the slowest growth rates and were severely inhibited or killed at temperatures ≥30°C. Strains of all other species were able to grow at temperatures ≤32°C. Most strains of *P. sordida*, *P. sanguinea*, and *P. chrysorhiza* were able to grow at 36°C. Strains of *P. sanguinea* and *P. chrysorhiza* that were able to grow at ≥36°C had been collected from southern states. Of all the strains tested, only four *P. sordida* strains were able to grow at 40°C. All four strains were from Florida, suggesting that strains collected from southern states possess higher maximum growth temperatures (Table 2). Geographic origin did not appear to affect the optimum temperature for growth or the rate of mycelial extension (Table 2).

The temperature optimum for most strains fell in the range of 22 to 32°C (Table 2). Two strains of *P. sordida* had optima above 32°C. One of these, *P. sordida* 8, had the widest temperature optimum range and grew well up to 40°C. This strain also grew at 44°C, although the rate of growth was significantly less than that at 40°C.

TABLE 2. Optimum and maximum temperatures for growth and growth rates at optimum temperature for selected strains of white-rot fungi

Isolate	Optimum temp[a] (°C)	Maximum temp[b] (°C)	Growth rate[c] (cm day^{-1})
P. sordida			
1	26–30	36	2.09 cd
3	24–32	36	1.78 e
4	22–32	36	2.41 c
5	24–30	36	2.77 b
7	26–30	40	2.74 b
8	30–40	44	4.81 a
9	28–30	36	2.76 b
12	26–30	36	2.47 c
13	24–36	40	2.68 b
14	28	36	2.26 cd
15	28–30	36	2.27 cd
16	28–30	40	2.31 c
17	30	40	2.88 b
P. sanguinea			
1	28	32	0.99 a
2	26–30	36	1.01 a
P. chrysorhiza			
1	28	36	1.11 b
2	24–30	32	0.97 d
3	26–30	36	1.01 c
4	26–30	36	0.84 e
5	26–30	36	1.02 c
6	28	36	0.96 d
7	26–30	32	0.95 d
8	28–30	36	1.19 a
P. laevis			
1	24–30	32	1.64 a
2	24–26	32	1.62 a
3	26	32	1.51 b
4	26–28	36	1.43 c
P. filamentosa			
1	22–28	30	0.31
I. circinatus			
1	24	28	0.47

[a] Ranges determined by Fisher Protected Least Significant Difference multiple-range test.

[b] Maximum temperature is the highest temperature at which a fungus survived.

[c] Mean growth rate at temperature optimum. Means followed by the same letter are not significantly different as determined by Fisher Protected Least Significant Difference multiple range test.

A statistical analysis of growth rate differences among the species was not possible because of the different numbers of strains examined for each species. However, there appeared to be large interspecific differences in growth rates. Mean growth rates ranged from 0.31 cm day^{-1} for *P. filamentosa* to 2.68 cm day^{-1} for *P. sordida*. Rank in terms of growth rates was as follows: *P. sordida* > *P. laevis* > *P. chrysorhiza* = *P. sanguinea* > *I. circinatus* = *P. filamentosa* (Table 3). There were also significant intraspecific differences in growth rates, particularly among strains of *P. sordida* and *P. chrysorhiza* (Table 2).

Sensitivity of selected strains of *Phanerochaete* spp. to PCP. The tested strains varied greatly in their sensitivity to PCP

TABLE 3. Mean maximum growth rates of selected species of white-rot basidiomycetes on 2% malt agar

Species	Maximum growth rate (cm day^{-1})
P. sordida	2.68
P. sanguinea	1.01
P. chrysorhiza	0.99
P. laevis	1.57
P. filamentosa	0.31
I. circinatus	0.46

(Table 4). As a group, the *P. sordida* strains were less sensitive to PCP than were strains of the other *Phanerochaete* spp. except for *P. chrysosporium*, which demonstrated the most rapid growth rate at all concentrations of PCP except at 25 ppm. *P. sordida* 7, 8, 9, and 13 and *P. chrysosporium* demonstrated a significant tolerance to PCP compared with the other *Phanerochaete* spp. and *P. sordida* 5. No growth of *P. laevis* 1, *P. sanguinea* 2, and *P. filamentosa* 1 was observed when PCP was present in the medium. Growth rates in control cultures (no PCP added to the medium) for all strains were slower than those observed in the temperature optima studies. The presence of DMF in the medium to solubilize the PCP may have had an inhibitory effect on the growth of the fungi.

Mineralization of PCP in liquid culture by several *P. sordida* strains and *P. chrysosporium*. Mineralization of PCP by *P. sordida* 13 was significantly greater than that by all other *P. sordida* strains and *P. chrysosporium* (Table 5). For all strains except *P. sordida* 13, the percentage of ^{14}C trapped as volatiles was 4 to 10 times greater than the amount mineralized. In cultures of *P. sordida* 13, mineralization exceeded volatilization. However, volatilization from cultures of *P. sordida* 13 was not significantly different than that from cultures of the other strains. Although *P. chrysosporium* and *P. sordida* 8 were the least-sensitive fungi to PCP, they did not demonstrate a superior ability to mineralize PCP. The small amount of PCP that was added to the cultures may not have been enough to inhibit growth or activity of any of these strains. Mineralization and volatilization from control cultures was insignificant.

Depletion of PCP in soil by *P. chrysosporium* and *P. sordida* 13. Inoculation of PCP-contaminated Marshan soil with either *P. sordida* 13 or *P. chrysosporium* resulted in a rapid decrease in the level of PCP (Fig. 1). The rate and extent of PCP depletion were slightly greater in soils inoculated with *P. chrysosporium*. After 64 days, the level of extractable PCP was reduced by 96% in soil inoculated with *P. chrysosporium* and by 82% in soil inoculated with *P. sordida*. Depletion of PCP by these fungi appeared to occur in a two-stage process. In the first stage, the concentration of PCP in the soil decreased rapidly. In soil inoculated with *P. chrysosporium*, this stage lasted 9 days, during which time the level of extractable PCP was decreased by 86%. In soil inoculated with *P. sordida*, this stage was approximately twice as long (21 days) and coincided with a 79% decrease in PCP. The rapid depletion of PCP in both cultures coincided with an accumulation of PCA (Fig. 1). At the end of the first stage, ca. 64 and 71% of the PCP was converted to PCA in *P. chrysosporium* and *P. sordida* cultures, respectively.

TABLE 4. Effect of PCP on maximum growth rate of strains of *P. sordida*, *P. laevis*, *P. chrysorhiza*, *P. sanguinea*, and *P. filamentosa*[a]

Strain	Maximum mycelia extension rate (cm day^{-1}) at PCP level (ppm) of:					
	0	5	10	15	20	25
P. chrysosporium	3.30	2.45	1.70	1.03	0.62	0.43
P. sordida 5	2.13	0.84	0.32	0.10	0	0
P. sordida 7	2.12	1.62	0.93	0.53	0.32	0.12
P. sordida 8	3.49	2.12	1.33	0.82	0.57	0.46
P. sordida 9	2.12	1.72	0.98	0.59	0.39	0
P. sordida 13	2.13	1.90	1.23	0.74	0.44	0.28
P. laevis 1	0.67	0	0	0	0	0
P. chrysorhiza 1	0.78	0.28	0.11	0	0	0
P. chrysorhiza 8	0.60	0.15	0.09	0	0	0
P. sanguinea 2	0.73	0	0	0	0	0
P. filamentosa 1	0.18	0	0	0	0	0

[a] Fungi were grown at optimum growth temperature on 2% malt agar containing various amounts of PCP.

TABLE 5. Percentage of total [^{14}C]PCP mineralized and volatilized in liquid cultures of *P. chrysosporium* and several strains of *P. sordida*[a]

Strain	Mineralization (%)	Volatilization (%)	Total ^{14}C evolved (%)
P. chrysosporium	1.97 (0.22)a	13.82 (1.20)a	15.79
P. sordida 7	2.67 (1.09)a	12.91 (2.79)a	15.58
P. sordida 8	1.92 (0.44)a	8.88 (2.21)a	10.80
P. sordida 9	1.22 (0.54)a	11.92 (2.35)a	13.14
P. sordida 13	11.64 (2.54)b	8.48 (0.52)a	20.12
Control	0.17 (0.03)c	0.06 (0.03)b	0.23

[a] Figures in parentheses represent the standard deviation of three observations. Means followed by the same letter are not significantly different.

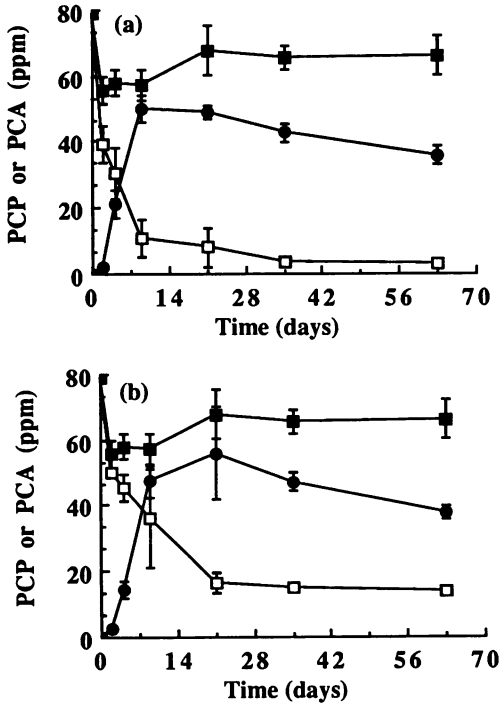

FIG. 1. Effect of inoculation with *P. chrysosporium* (a) or with *P. sordida* 13 (b) on the concentration of PCP and PCA in Marshan soil. Symbols: □, concentration of PCP in fungus-inoculated soil; ■, concentration of PCP in noninoculated soil; ●, concentration of PCA in fungus-inoculated soil.

TABLE 6. Percentage of mineralization and volatilization of PCP and PCA in liquid cultures of *P. chrysosporium* and *P. sordida* 13 and in control (noninoculated) cultures

Culture	Compound	Mineralization (%)	Volatilization (%)
P. chrysosporium	PCP	8.91	7.95
	PCA	7.95	9.54
P. sordida	PCP	16.13	8.25
	PCA	13.07	10.02
Control	PCP	1.05	0.08
	PCA	0.40	15.92

The second stage in cultures of both fungi was characterized by a slow but steady decline in the levels of PCP and PCA. During this stage, levels of PCP and PCA were reduced by 9.6 and 18%, respectively, in soil inoculated with *P. chrysosporium* and 3 and 23%, respectively, in soil nonoculated with *P. sordida*. The amount of extractable PCP in noninoculated soil did not change significantly throughout the experiment. No PCA was recovered in extracts from control culture soils.

Comparative mineralization and volatilization of PCP and PCA by *P. chrysosporium* and *P. sordida*. *P. sordida* mineralized significantly greater amounts of both PCP and PCA than did *P. chrysosporium* (Table 6). Both fungi mineralized slightly greater amounts of PCP than PCA. Mineralization from control cultures was insignificant. Mineralization of PCP by *P. chrysosporium* was ca. five times greater than in the previous study (Table 5). We have found mineralization of PCP by this fungus to be quite variable, ranging from 1 to 10%.

There was a significant amount of volatilization from all cultures, except control cultures amended with PCP (Table 6). The amount of volatiles collected was independent of fungal species. However, volatilization was significantly greater from cultures amended with PCA than from cultures amended with PCP. Volatilization was particularly high in control cultures amended with PCA (Table 6). Most of the volatiles collected from these cultures (12% of the total 16%) were collected the first time the cultures were flushed. This indicates that most volatilization from these cultures was probably from nontransformed PCA. In contrast, the amount of materials volatilized in fungal cultures was fairly consistent over the course of the experiment, indicating that the presence of the fungi in some way decreased volatilization of PCA.

DISCUSSION

The results of the studies reported here demonstrate significant differences among members of a group of taxonomically closely related white-rot basidiomycetous fungi in mycelial extension rate, sensitivity to PCP, and ability to mineralize PCP in an aqueous medium. These differences suggest that fungal strains with desirable characteristics for use in xenobiotic degradation, e.g., rapid growth rate, relatively high tolerance to high contaminant levels, and superior contaminant-degrading ability, could be identified and used for specific applications.

One often-cited disadvantage of bioremediation is the longer treatment time relative to alternative treatment technologies. Thus, microorganisms for use in bioremediation must possess rapid growth rates, and the environmental conditions for the optimum growth and activity of these organisms must be identified to ensure expeditious production of microbial biomass. Our results demonstrate the presence of great variability in growth rates among *Phanerochaete* spp. grown on 2% malt agar. Experimental evidence suggests that determination of growth rates on malt agar may be useful for discriminating between fast- and slow-growing soil fungi. Relative rates of mycelial extension of wood-degrading basidiomycetes on agar correlated well with the relative growth rates of these same fungi in soil (13). However, under optimal conditions, fungal growth rate in soil was, in general, several times greater than that on 2% malt agar (3, 13, 34).

We found both interspecific and intraspecific differences in mycelial extension rates. Interspecific differences in rates of mycelial extension were demonstrated previously for wood-degrading basidiomycetes (3, 13, 29). Reported rates on 2% malt agar at the optimum temperature for growth of the studied organisms ranged from ≤0.5 to 1.5 cm day^{-1} (29). Most of the fungi we examined fell within this range. A notable exception, *P. sordida*, grew very rapidly, with an average mycelial extension rate of 2.68 cm day^{-1} at an average optimum growth temperature of 28°C. This rate compares well with the mycelial extension rate of *P. chrysosporium*, which ranged from 1.8 to 2.4 cm day^{-1} at 28°C (7). Mycelial extension rates among strains of *P. sordida* varied from 1.78 to 4.81 cm day^{-1}. Intraspecific differences in mycelial growth rate were also observed among ectomycorrhizal fungi (9), which are taxonomically related to the fungi studied here. Intraspecific variation in rates of mycelial extension indicate that characterization of the growth of a particular fungal species should be based on the performance of a number of strains.

The ability of a microorganism to tolerate high contaminant concentrations would be a distinct advantage for bioremediation of highly contaminated media. Reported tolerances of fungi to PCP vary greatly (10, 31). The maximum concentration of PCP allowing fungal growth varied from 12 ppm for *Chaetomium globosum* Kunze:Fr. to 180 ppm for *Cephaloascus fragrans* Hanawa (10). In the same study, adapted strains of *C. fragrans* grew at 280 ppm PCP. Mean inhibitory concentrations of PCP for fungi representing 14 genera, reported as the lowest concentration that completely inhibited growth, ranged from 15 to 133 ppm (31). *Phanerochaete* spp. are comparatively very sensitive to PCP. Growth of several species was prevented by the presence of only 5 ppm PCP. However, *P. chrysosporium* and *P. sordida* were much less sensitive than the other *Phanerochaete* spp. and were able to grow at 25 ppm PCP, albeit at greatly decreased growth rates. In another study, *P. chrysosporium* and *P. sordida* 13 were able to grow and deplete PCP in a field soil with initial concentrations of from 250 to 400 mg kg^{-1} PCP (22). Cultures of *P. chrysosporium* in a liquid medium could not be established by inoculation with conidiospores at PCP concentrations greater than 4 mg $liter^{-1}$ (4 ppm) (26). The lethal effects of PCP were avoided by allowing the fungus to establish a mycelial mat before the addition of PCP (26). The ability of *P. chrysosporium* and *P. sordida* to grow on agar and in soil at PCP concentrations greater than 4 ppm was probably a result of the presence of mycelia in the inocula. Thus, inocula that contain mycelia should be used for applications of white-rot fungi to contaminated media.

The amounts of PCP mineralized by *P. chrysosporium* and strains of *P. sordida* grown in liquid culture were low (e.g., 1-11% PCP mineralized to CO_2), and in fact these amounts were lower than a previously reported value of 23% mineralization by *P. chrysosporium* after 30 days (26). The amount

of PCP mineralized by *P. chrysosporium* grown in liquid culture was reported to depend on the concentration of extracellular enzymes and the amount of living cell biomass, with increasing amounts of mineralization obtained when amounts of either parameter were increased (24). Thus, variation in reported values is probably the result of differences in amounts of fungal biomass and extracellular enzyme concentration produced in response to differences in culture preparation and maintenance.

The culture conditions that we used to assess fungal mineralization of PCP were initially developed to obtain optimal mineralization of lignin model compounds by *P. chrysosporium* (20). Mineralization of PCP is greatest when *P. chrysosporium* cultures are ligninolytic (26), and it can be increased by raising the concentrations of ligninolytic enzymes in the culture medium (24). These factors, and the oxidation of PCP to *p*-chloranil (tetrachloro-*p*-benzoquinone) by lignin peroxidases and manganese peroxidases, suggest that the lignin-degrading system is involved in PCP mineralization. *P. sordida* 13 was clearly superior to *P. chrysosporium* and the other tested *P. sordida* strains in its ability to mineralize PCP under conditions optimal for lignin degradation by *P. chrysosporium*. It is interesting that *P. sordida* 13 was the only strain isolated from a coniferous host (Table 1). White-rot fungi are more frequently associated with angiospermous hosts (16, 29). This preference is thought to be the result of the allegedly more refractory nature of coniferous lignin as compared with angiospermous lignin (29). Therefore, white-rot fungi that have the ability to metabolize coniferous lignin may have a more powerful lignin-degrading system and thus superior PCP-degrading abilities.

Despite an apparently superior ability to mineralize PCP in liquid culture, *P. sordida* 13 did not deplete PCP from a sterile soil as rapidly or to as great an extent as did *P. chrysosporium*. However, rates and extents of PCP depletion by the two fungi in a contaminated field soil were similar (22). Also, the loss of PCP via mineralization in soils inoculated with *P. chrysosporium* was negligible (i.e., $\leq 2\%$) (23). Since loss via mineralization is not a major fate of PCP depletion in soils inoculated with white-rot fungi, a superior ability to mineralize PCP in liquid culture does not appear to be useful for screening fungi for remediating PCP-contaminated soils.

We reported previously that inoculation of Marshan soil with *P. chrysosporium* resulted in conversion of ca. 60% of the PCP to extractable transformation products (23). This correlates well with the 64 to 71% loss of PCP in the same soil caused by methylation of PCP by *P. chrysosporium* and *P. sordida* in the present study. The similarity between the percentage of extractable transformation products found in the previous study and the percentage of PCP converted to PCA in the present work suggests that PCA was the main extractable product produced as a result of fungal activity in the previous study.

The amount of extractable as opposed to soil-bound PCP transformation products was greatly influenced by soil type (23). It follows that soil type also has a great influence on the amount of PCP that is lost as a result of fungal methylation to PCA. We found that in a field soil contaminated with 250 to 400 ppm PCP, only 8 to 13% of the PCP decrease could be attributed to methylation to PCA (22). Fungi are known to methylate chlorinated phenolic compounds to the corresponding methylated derivatives (11, 12, 17). Studies on the relative toxicities of PCP and PCA to selected microbes suggest that microbial methylation may function as a detoxification mechanism. For example, PCA was less toxic than PCP to 17 bacterial strains with the exception of *Streptomyces* spp. (32), to 16 strains of blue-stain and wood-rotting fungi (31), and to *Trichoderma* spp., *Penicillium* spp., and *Cephaloascus* spp. (11). Methylation of chlorinated phenolic compounds increases their lipophilicity and thus their tendency to bioaccumulate (27, 35). However, depending on the method of administration, PCA was two to five times less toxic than PCP to mice (30). We found that *P. chrysosporium* and *P. sordida* are able to metabolize PCA in liquid culture and possibly in soil. After an initial accumulation of PCA in soil inoculated with either *P. chrysosporium* or *P. sordida*, the concentration of PCA decreased significantly. This decrease might have been the result of either an increasing resistance to extraction with time as a result of the extreme lipophilicity of PCA or to the degradative activity of the fungi. The magnitude of the decrease would suggest that fungal activity was involved.

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