Screening of Fungi for Soil Remediation Potential

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17-1 INTRODUCTION

Successful application of bioremediation for treating xenobiotic-contaminated soils requires the identification and implementation of physiochemical and/or nutritional conditions that favor optimum growth and xenobiotic-degrading activities of indigenous (biostimulation) or inoculated (bioaugmentation) xenobiotic-degrading microbes. Biostimulation involves the selection of microbes based primarily on their xenobiotic-degrading abilities. In addition to selecting organisms with superior pollutant-degrading abilities. the bioaugmentation approach allows for selection of microbes based on additional physiological, biochemical, or ecological characteristics that may confer to them, exceptional bioremediation performance. These include attributes such as superior growth rates, competitive ecological strategies and tolerances to high contaminant concentrations, specific nutritional capabilities, and pH or temperature growth optima that could be exploited to obtain dominant colonization and ultimately remediation of a contaminated soil volume by the inoculated organism.

Extensive laboratory study of a group of wood decay basidiomycetes, collectively called *white-rot fungi,* has demonstrated their ability to degrade a wide variety of *contaminants*. This ability makes these organisms attractive for

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use in the remediation of contaminated soils, particularly soils that are contaminated with complex mixtures of hazardous chemicals. The potential use of these organisms for soil remediation using the bioaugmentation approach has been demonstrated in a number of field studies in which the fungal treatment of pentachlorophenol (PCP)-contaminated soils (Lamar & Dietrich, 1990) and soils contaminated with both PCP and creosote (Lamar et al., 1993, 1994, Davis et al., 1993), was evaluated. Most of the work on pollutant degradation and soil remediation by white-rot fungi has focused on very few species and predominantly on *Phanerochaete chrysosporium.* The few reports concerning the evaluation of alternative fungal species have focused on the abilities and selected organisms to degrade specific xenobiotics in liquid culture or on their tolerances to specific xenobiotics. Of relevance to the present research are reports on relative abilities to degrade PCP by a wide range of fungal species from different taxonomic and ecological groups (Zabel et al., 1985; Seigle-Murandi et al., 1991, 1992; Steiman et al., 1994; Benoit-Guyod et al., 1994) and on relative abilities to degrade polycyclic aromatic hydrocarbon (PAH) components of creosote by several white-rot fungi (Field et al., 1992). There also have been several reports on the tolerance of white-rot fungi to PCP (Alleman et al., 1993, 1992; Lamar et al., 1990b). Although these studies have demonstrated significant differences among organisms in their abilities to degrade PCP or other xenobiotics and in their sensitivities to the PCP, the relevance of the results of these studies to bioremediation performance has not been addressed.

Relationships between biochemical or physiological parameters to pollutant transformation or mineralization have been evaluated. Production of lignin (Lip) and manganese (MnP) peroxidases and decolorization of the dye Poly R by several species of white-rot fungi were found to correlate well with PCP removal in liquid culture by these organisms (Lin et al., 1991). In our own work, 29 strains comprising six species of white-rot fungi were evaluated for bioremediation performance (Lamar et al., 1990b). The fungal strains were evaluated in several stages that involved determinations of growth rates, sensitivity to PCP, ability to mineralize PCP in liquid culture and finally ability to deplete PCP in soil. After each stage the most promising strains were selected for continued evaluation. Using this method the potential usefulness of *P. sordida* for remediation of PCP-contaminated soils was identified and this potential has since been confirmed in the field (Lamar & Dietrich, 1990; Lamar et al., 1993, 1994; Davis et al., 1993).

None of the aforementioned studies have elucidated physiological, biochemical, or ecological factors that would be useful for identification and selection of fungi with superior soil remediation performance. The purpose of the present investigation was to determine if physiological and/or biochemical factors such as growth rate, tolerance to and ability to degrade PCP or creosote have use for predicting the potential bioremediation performance of fungi. Because we have focused the initial development of a fungal-based soil remediation technology on PCP- and/or creosote-contaminated soils, the groups of fungi that were evaluated in this study were organisms isolated or known to grow on PCP- or creosote-treated wood.

17-2 MATERIALS AND METHODS

17-2.1 Approach

The optimum temperature and growth rates for a select group of basidiomycetous and deuteromycetous fungi were determined. Each strain was examined for its sensitivity to PCP, ability to mineralize PCP in liquid culture, and to deplete PCP in soil. The fungi also were tested for their tolerance to creosote, and their ability to degrade PAHs in creosote-treated wood.

17-2.2 Fungi

Fungal strains used in this study were obtained from the Center for Forest Mycology Research (Forest Products Laboratory, Madison, WI), American Type Culture Collection (12301 Parklawn Drive, Rockville, MD 20852), C.J.K. Wang (SUNY College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210-2788). and *A. cuboidea* from E. Schmidt (College of Natural Resources, Dep. of Forest Products, Kaufert Laboratory, 2004 Folwell Avenue, St. Paul, MN 55108; Table 17-1). Although three of the strains received from U.K. Wang were identical to those obtained from ATCC, isolates from both

Table 17-1 Basidiomycete and deuteromycete strains investigated.

Species	Culture no.	Type of rot W = white B = brown S = soft	Isolated from treated wood
	Basidiomycetes[†]		
Antrodia carbonica	MD-280	B	creosote
A. xantha	ME-508	B	creosote
Bjerkandera adusta	FP-135160-Sp (ATCC 62023)	W	unidentified
Gloeophyllum trabeum	Mad-5096-15-R	B	penta[‡]
Hyphoderma praetemissum	MB-145	W	penta
Irpex lacteus	Mad-517 (ATCC 11245)	W	mine timber
Phanerochaete chrysosporium[#]	BKM-F-1767 (ATCC 24725)	W	
P. sordida[#]	HHB-8922-Sp	W	
Phlebia brevispora	MD-192 (ATCC 46918)	W	treated wood
P. subserialis	RLG-10693-Sp (ATCC 62007)	W	unidentified
Posita placenta	MD-506	B	creosote
Sistotrema brinkmannii	ME-632	B	creosote
Sistotrema sp.	P-193-Sp	B	unidentified
Trametes versicolor	MD-277	W	copper sulfide/ sodium chromate

Table 17-l. Continued.

	Microfungi Subdivision Deuteromycotina, Hyphomycetes		
Alternaria alternata[§]	P-3	S	creosote
Arthrographis cuboidea[¶]			creosote
Cladosporium resinae[‡]	ATCC 66686		penta
C. resinae[§]	P-645		unidentified
Leptodontium elatius[‡]	ATCC 66694 (P-247)	S	creosote
L. elatius[§]	P-247 (ATCC 66694)	S	creosote
Paecilomyces variotii[‡]	ATCC 66705	S	creosote
Phialocephala dimorphospora[‡]	ATCC 66712 (P-109)	S	creosote
P. dimorphospora[‡]	ATCC 66713	S	creosote
P. dimorphospora[‡]	ATCC 66714	S	creosote
P. dimorphospora[§]	P-109 (ATCC 66712)	S	creosote
Phialophora heteromorpha[§]	P-33	S	creosote
P. heteromorpha[§]	P-196 (ATCC 66831)	S	creosote
P. heteromorpha[‡]	ATCC 66831 (P-196)	S	creosote
Rhinocladiella atrovirens[‡]	ATCC 66758		penta
R. atrovirens[‡]	ATCC 66759		penta
R. atrovirens[§]	P-154		penta
Scytalidium circinatum[‡]	ATCC 66463	S	CCA[‡‡]
S. circinatum[‡]	ATCC 66464	S	creosote
S. lignicola[§]	P-53	S	creosote
Taxon 121[§]	P-121 (ATCC 66761)	S	creosote

[†] Cultures obtained from the Center for Forest Mycolqy Research.
[‡] Cultures obtained from the American Type Culture Collection.
[§] Cultures obtained from C.J.K Wang.
[¶] Culture obtained from Elmer Schmidt.
[#] *P. chrysosporium* and *P. sordida* were included in this study for comparison purposes because they have demonstrated potential for use in remediation of PCP–and creosote-contaminated soils (Lamar et al., 1990, Lamar & Evans, 1993; Davis et al., 1993).
[††] Penta refers to a technical-grade formulation of chlorophenols in which PCP is the major component.
[‡‡] CCA = copper-chromium-arsenate

sources were studied. The strains were maintained on 2% malt extract agar (MBA) containing L^{-1}: 20 g malt extract and 15 g Bacto agar (Difco Laboratories, Detroit, MI) and stored at -20°C. Master plates were prepared by aseptically transferring pieces of fungal mycelium from stock slants to 2% MBA or potato dextrose agar (PDA) plates that were then incubated at 24°C.

17-23 Chemicals

[^{14}C]-labeled PCP (5.8 mCi M^{-1}, purity >98%) and [^{14}C]-labeled phenanthrene (13.1 mCi $mmol^{-1}$, purity >99%) were purchased from Sigma Chemical Company (St Louis, MO). [^{14}C]-labeled benzo[a]pyrene (9.65 mCi $mmol^{-1}$ purity >98%) was purchased from California Bionuclear Corporation (Los Angeles, CA). Unlabeled PCP (purity >99%), POP (2,5-diphenyloxazole), and

sodium dithionite were obtained from Aldrich Chemical Company (Milwaukee, WI). Water was purified with a Mini-Q Water System (Millipore Corp., Bedford, MA). Poly-Fluor liquid scintillation cocktail was purchased from Packard (Meriden, CT). Soil moist (JRM Chemical, Cleveland, OH) consisted of 99.7% cross-linked polyacrylamide and 0.3% inert ingredients. The coal tar creosote was obtained from Koppers Industries (Pittsburgh, PA). The POPOP (p-*bis*-[2-(5-Phenyloxazolylo]-benzene) was obtained from Research Products International Corporation (Elk Grove Village, IL). All solvents were HPLC grade. All other chemicals were the highest available grade.

17-2.4 Dry Weight Determinations

The moisture contents of soil and wood were determined gravimetrically after drying at about 80°C for 18 h. Hyphal mats from liquid cultures were collected by vacuum. filtration on preweighed filters (G6 glass fiber filters, Fischer Scientific Co., Pittsburgh,.PA), rinsed with distilled water, allowed to air dry for 3 d, and then weighed.

17-25 Determination of Temperature Growth Optima and Growth Rates

The optimum temperature for growth of each strain was determined by measuring hyphal extension rates. For this study, 2% MEA plates were inoculated with 6-mm plugs taken from the actively growing margin of fungal colonies from master plates, and incubated at 6, 12, 16, 20, 22, 24, 28, 30, 32, 36, 40, and 44°C. Three plates were prepared for each strain at each temperature, and kept in open Zip-Loc bags. The hyphal extension rate was recorded as the average daily increase in colony diameter (mm) measured in two perpendicular directions. If the cultures demonstrated no growth after 7 d, they were removed from the incubators and kept at 24°C. After 3 d, cultures that did not grow were presumed to have been killed. Those that grew were assumed to have been inhibited by the initial incubation temperature.

17-2.6 Sensitivity to Pentachlorophenol or Creosote

The sensitivity of the fungi to PCP or creosote was determined by measuring the rate of hyphal extension on yeast malt peptone glucose (YMPG) agar that contained different concentrations of PCP or creosote. The concentrations for PCP were 0, 5, 10, and 15 mg L^{-1} and for creosote 0, 100, 250, 500, and 1000 mg L^{-1}. The YMPG agar consisted of (per 750 mL): 7.5 g of glucose, 7.5 g of malt extract, 1.5 g of bacto-peptone, 1.5 g of yeast extract, 0.75 g of asparagine, 1.5 g KH_2PO_4 0.75 g $MgSO_4$i7 H_2O, 0.75 g of thiamine, 17.25 g of Bacto-agar, and 725 mL of distilled H_2O. Both the PCP and creosote were dissolved in acetone prior to addition to the agar. The volume of acetone added to the agar was constant for the controls and all concentrations of PCP and creosote. The plates were inoculated with 6-mm fungal plugs as previously described, kept in Zip-Lot bags and incubated at 24°C. Three replicates were prepared for the controls and every fungus at each concentration. Mycelial growth was measured daily, as described previously, for 14 d and hyphal extension rates were determined.

17-2.7 Mineralization of Pentachlorophenol in Liquid Culture

Fungi were incubated in stationary-phase cultures containing 10 mL of N-limited BIII culture medium (Kirk et al., 1978) in 125-mL Erlenmeyer flasks. Culture medium contained (per L): 10 g Of glucose, 1 mg of thiamine HCl, 221 mg of ammonium tartrate, 1 g of KH_2PO_4, 0.5 g of $MgSO_4$•7 H_2O, 1 g of $Ca(H_2PO_4)_2$, and 10 mL of mineral solution in 2.2~dimethylsuccinic acid buffer (1.46 g L^{-1}) adjusted to pH 4.5. Inoculum consisted of one 6-mm plug taken from the active growing region from each fungal master plate. Control flasks were prepared using an agar plug from a sterile plate. Three replicates were prepared for each fungus and the control. On Day 3, each culture received approximately 90 000 dpm of [^{14}C]PCP in 100L of N,N- dimethylformamide. Cultures were incubated at 24°C in complete darkness for 30 d.

Culture flasks were fitted with inlet-outlet ports to permit flushing of culture headspaces. Inlet ports were protected from contamination by a piece of sterile cotton. Outlet ports were connected to volatile traps (Orbo 43, Supelco, Bellefonte, PA) and then to a bubbler tube containing 10 mL of CO_2-trapping scintillation fluid. Scintillation fluid was composed of toluene cocktail, methanol, and ethanolamine (5:4:1; v/v/v). Toluene cocktail contained 4 g of PPD L^{-1} and 0.1 g of POPOP L^{-1} in toluene. Headspaces of all cultures were evacuated with O_2 every 3 to 4 d. The first evacuation was 3 d after the addition of [$C^{14]}$PCP, 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 radioactivity present. Counting was performed on a 1214 Rackbeta liquid scintillation counter (Pharmacia). The dry weight of hyphal mats was determined after harvesting as described above.

17-2.8 Depletion of Pentachlorophenol in Soil

Marshan sandy loam (fine-loamy over sandy or sandy-skeletal, mixed, mesic Typic Haplaquoll) was air dried, sieved (<2 mm), and stored in plastic bags at 4°C. The soil was sterilized by fumigating with Bromo Gas (Great Lakes Chemical Corp., West Lafayette, IN; 98% methyl bromide-2% chloropicrin). Soil physical and chemical properties were reported by Lamar et al. (1990a). Nonlabeled PCP was dissolved in acetone and added to the sterile soil to obtain a final concentration of 100 mg kg^{-1}. The moisture content was adjusted to 39% using sterile distilled water.

To determine the ability of the fungi to degrade PCP in the soil, approximately 5 g of PCP-amended soil (wet weight, 39% moisture content) was placed in sterile 20-mL vials fitted with polyurethane foam stoppers. Cultures were inoculated by aseptically placing seven 6-mm plugs from fungal master plates on the soil surface. Two sets of control cultures were prepared containing PCP-amended soil with either seven sterile agar plugs or none. The vials were incubated for 30 d at 24°C. Three replicates were performed for each culture.

For analysis of PCP, the agar plugs were removed and the 5 g of soil transferred to 25 × 150 mm culture tubes (Corning, Corning, NY) with Teflon-lined screw caps. In order to determine percentage of moisture of the soil, every fifth sample had approximately 1 g of soil removed for gravimetric determination of

moisture content. Approximately 100 mg of $Na_2S_2O_4$ were added to each tube. The samples were then extracted twice, under N, with 20-mL of hexane-acetone (1:1) acidified to pH 2 with H_2SO_4, for 1 h on a rotating tumbler shaker at about 33 revolutions min^{-1}. The extracts were pooled in a clean tube, dried by passing them through a column of anhydrous Na_2SO_4 and collected in a culture tube (20 × 150 mm). The Na_2SO_4 was prepared by muffling for 4 h at 350°C and all glassware was muffled for 1 h at 450°C. Culture tubes containing the extracts were placed in a Zymark (Hopkinton, MA) Turbo Vap ZW700 evaporator held at 35°C and evaporated, under N, to approximately 5 mL. The 5 mL volume was filtered through a 0.5µm MILLEX-SR filter (Millipore Products, Bedford, MA) into a 10-mL volumetric flask and the final volume adjusted with hexane. To determine the effectiveness of the extraction procedure, selected soil samples were spiked with approximately 40 000 dpm of [^{14}C]-labeled PCP prior to extracting. Following extraction, the amount of ^{14}C associated with 0.5-mL aliquots of the extracts was determined as described above, and the percentage of recovery calculated. Recoveries averaged 87%. Extracts were stored at -20°C in amber vials with Teflon-lined screw caps.

Extracts were analyzed for PCP by gas chromatography using electron capture detection, as described previously (Lamar et al., 1993). 2,4,6-Tribromophenol was used as the internal standard and Sylon BSA (Supelco) was used as the derivatizing reagent. Gas chromatographic analyses of extracts were performed on a Hewlett Packard Model 5890A or 5890II gas chromatograph equipped with a ^{63}Ni electron capture detector, a Model 7673A autosampler, and split-splitless capillary column injection ports. Operating temperatures for both instruments were injector 220°C and detector 300°C; carrier gas, He; and makeup gas, N_2. The columns were a DB-5 fused-silica capillary column (30 m × 0.321 mm) with a film thickness 0.25 µm (J&W Scientific, Folsom, CA). Injections were splitless with split off for 1 min. The temperature program was initial temperature 60°C; hold for 1 min; ramp A, 10°C min^{-1} for 9 mm (60-150°C); ramp B, 2°C min^{-1} for 20 min (150-190°C); and hold at 190°C for 5 min. Data are reported as percentage decrease of the PCP concentration after 30 d.

17-2.9 Degradation of Creosote in Creosote-Treated Wood

Nonsterile creosote-treated wood (railroad ties, approximately 30 yr of age) was cut into cubes (2.5-3.5 cm^3). Approximately 10.4 g of wood cubes were placed in a sterile 256-mL jelly jar with a lid modified to allow adequate aeration and minimize moisture loss by gluing a piece of microporous polypropylene film (Cell-Guard, Hoechst Celanese Corp., Somerville, NJ) over a 32 mm diam. hole, on the underside of the lid. Jars and lids were sterilized by autoclaving at 121°C for 20 min. Because of the hydrophobic nature of the creosote-treated wood, it was impossible to adjust the moisture content to a level that would be optimum for fungal growth (e.g., 60%). Therefore 0.1 g of Soil Moist was added to the Wood in each jar to absorb the water that was not adsorbed by the wood and to release the water as needed. For each isolate fungal cultures were prepared by aseptically adding agar pieces, infested with the proper fungus, from one-half of a master plate prepared with PDA, to the cubed wood. Control cultures were prepared by adding one-half of the agar from a sterile PDA plate to the wood cubes.

Three replicates were prepared for each fungal isolate and the control. The cultures were incubated at 24°C in complete darkness for 30 d.

Prior to extraction, the agar pieces and soil moist were scraped from the woo d cubes and the wood cubes were ground in a commercial coffee grinder until the wood was light and fluffy (about 1 - 2 min). Initial PAH concentrations were determined using nontreated wood cubes. A sample was taken from each culture to determined moisture content of the ground wood, gravimetrically. Approximately 1 g (wet weight) was mixed with 6 g of anhydrous sodium sulfate in a culture tube 25 X 150 mm). Each sample was extracted by sonicating in 20 mL of acetone-methylene chloride (1:1, v/v) for 2 min at power setting 5 using a Heat System (Farmingdale, NY) XL2020 sonicator with a microtip probe. The wood was allowed to settle to the bottom of the tubes for about 1 to 2 h and the extraction solvents were vacuum filtered through a G6 glass fiber filter (Fischer Scientific Co., Pittsburgh, PA) into a disposable culture tube (20 × 150 mm). The ground wood was then extracted a second time using the same solvent system, After the second extraction, the extraction solvent-wood mixture was poured onto the filter surface. The wood trapped on the filters was rinsed with 5 mL methylene chloride and then 5 mL acetone. The two extracts were combined and evaporated to dryness under N at 40°C using a Turbo Vap. The residue was solubilized in 10 mL of acetonitrile in disposable culture tubes (20 × 150 mm) sealed with parafilm and incubated for 1 h at 24°C. The tubes were gently vortexed and decanted into 12-mL amber screw cap vials with Teflon-lined caps and stored at 24°C.

To assess the extraction efficiency, randomly selected ground wood samples were spiked with about 100 000 dpms each of [^{14}C]phenanthrene or [^{14}C]benzo[a]pyrene dissolved in acetone. Following the extraction, 0.5 mL aliquots of the concentrated extraction solvents were counted and the percentage of recoveries calculated. Phenanthrene recoveries averaged 85% and benzo[a]pyrene recoveries averaged 69%.

The extracts were analyzed for 14 different PAH components (Table 17-8) by high-performance liquid chromatography (HPLC) using a Hewlett Packard 1090L Series II equipped with a diode-array detector, as described in Lamar et al. (1994). Separations were achieved on a Vydac 201TP Cl8 analytical column (0.46 × 25 cm; Separation Group, Hesparia, CA), preceded by a Vydac 211GCC54T Cl8 guard column. Analyte peaks were identified based on their elution times and ultraviolet absorption spectra when compared with pure PAH standards. For quantitation, interferences due to poorly-resolved components in the complex mixtures were minimized by monitoring the different analytes for absorbance at wavelengths that gave the greatest selectivity for each analyte. Thus, acenapthene was monitored at 228 nm, fluorene, and phenanthrene at 2.58 nm, chrysene at 265 mn, fluoranthene and benzo[*a*]anthracene at 280 nm, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and dibenzo[*a,h*]anthracene at 300 nm, pyrene at 312 nm, and benzo[*a*]pyrene, benzo[*g,h,i*]perylene and indeno[1,2,3-*cd*]pyrene at 382 nm.

17-2.10 Statistical Analysis

Analysis of variance (ANOVA, $\alpha = 0.05$) was used to test for equality of means for PCP mineralization efficiency, percentage of PCP depletion from soil

and PAH concentration in wood. If by ANOVA, treatment means were shown to be significantly different, Fisher's protected LSD (a = 0.05) was performed to identify treatment differences.

17-3 RESULTS

17-3.1 Temperature Growth Optima, Temperature Growth Range, and Growth Rates

All tested strains grew between 12 and 32°C except for *Sistotrema* sp., which grew to a maximum temperature of 24°C (Table 17-2). Only two fungi, the basidiomycete *Phlebia subserialis* and the dueteromycete *Cladosporium resinae,* grew at all temperatures evaluated. There were no differences between the taxonomic groups in temperature growth ranges; however, in general, the basidiomycetes had greater hyphal extension rates than the deutemmycetes. Mean growth rates for the basidiomycetes ranged from 5.5 mm d^{-1} for *A. carbonica* to 47.3 mm d^{-1} for *P. subserialis. Phlebia subserialis* and *P. brevispora* possessed the greatest hyphal extension rates (Table 17-2). Rank in terms of hyphal extension rates for the basidiomycetes *was* as follows: *P. sabserialis* > *P. brevispora* > *I. lacteus* > *B. adusta* > *T. versicolor* > *S. brinkmannii* > *P. placenta* > *G. trabeum* > *A. xantha* > *Sistotrema* sp. = *H. praetermissum* > *A. carbonica.* The optimum temperature for growth of basidiomycete strains fell in the range of 22 to 40°C.

With a few exceptions the dueteromycetes had very low (>5 mm d^{-1}) hyphal extension rates (Table 17-2). *Arthrographus cuboidea* had the greatest hyphal extension rate (21.2 mm d^{-1}). Ranking of these organisms from highest to lowest extension rates *was as* follows: *A. cuboidea* > *P. variottii* > *A. alternata* > *C. resinae* (P-545) > *S. lignicola* > *S. circinatum* (ATCC 66464) > *C. resinae* (ATCC 66686) > *S. circinatum (ATCC 66463)* > *P. dimorphospora* (ATCC 66712) > *P. dimorphospora* (ATCC 66713) *P. dimorphospora* (P-109) > *P. dimorphospora* (ATCC 66714) > *L. elatius* (ATCC 66694) *L. elatius* (P-247) *P. heteromorpha* (ATCC 66831) > *P. heteromorpha* (P-196) > Taxon 121 > *P. heteromorpha* (P-33) > *R. atrovirens* (P-154) > *R. atrovirens* (ATCC 66758) > *R. atrovirens* (ATCC 66759). Temperature growth optima for the dueteromycetes ranged from 22 to 36°C. Different isolates of the same species generally exhibited the same optimum growth temperature, temperature range for growth, and hyphal extension rates (Table 17-2):

17-3.2 Sensitivity of the Strains to Pentachlorophenol

The tested isolates varied greatly in their sensitivity to PCP (Table 17-3). Basidiomycetes were more sensitive to PCP than deuteromycetes, with only one-half of the former able to grow in the presence of 5 mg L^{-1}PCP. Basidiomycete genera that were relatively less sensitive to PCP were *Irpex Phanerochaete, Phlebia,* and *Sistotrema.* The other tested strains were unable to grow when PCP was present. Of all the tested fungi, *P. sordida* demonstrated the most rapid growth rate at all concentrations of PCP. All duetemmycete strains were able to grow up to 10 mg L^{-1}, with the exception of Taxon 121 (Table 17-3).

Table 17-2. Temperature range for growth, optimum temperature, and maximum growth rate at the optimum temperature for selected fungi.

	Optimum temperature	Growth range	Growth rate[†]
	°C	°C	mm d^{-1}
	Basidiomycota		
A. carbonica	24	12-36	5.5
A. xantha	28	6-36	7.3
B. adusta	28	6-36	21.7
G. trabeum	28-36	12-40	8.8
H. praetermissum	24	6-36	6.2
I. lacteus	36	6-40	24.2
P. chrysosporium[‡]	40	12-50	35-42
P. sordida[§]	24-36	14-40	26.8
P. brevispora	30	6-40	36.9
P. subserialis	28	6-44	47.3
P. placenta	24-28	12-36	9.4
S. brinkmannii	24	6-32	11.5
Sistotrema sp.	22	6-24	6.2
T. versicolor	23-32	6-40	19.8
	Mitosporic Ascomycota		
A. alternata (P-3)	24	6-36	10.4
A. cubaidea	28	6-36	21.2
C. resinae (ATCC 66666)	22-36	6-44	4.7
C. resinae (P-545)	36	6/-44	6.8
L. elatius (ATCC 66694)	22-24	6-32	2.2
L. elatius (P-247)	22	6-32	2.2
P. variotti (ATCC 66705)	32-36	12-44	15.6
P. dimorphospara (ATCC 66712)	24	6-36	3.5
P. dimorphospora (ATCC 66713)	24	6-36	3.4
P. dimorphospora (ATCC 66714)	22-24	6-36	3.0
P. dimorphospora (P-109)	24	6-36	3.4
P. heteromorpha (ATCC 66631)	30-32	16-40	2.2
P. heteromorpha (P-33)	28	12-40	1.8
P. heteromorpha (P-196)	322	12-40	2.1
R. atrovirens (ATCC 66753)	24	12-36	1.3
R. atrovirens (ATCC 66759)	24	12-36	1.2
R. atrovirens (P-154)	22-24	12-26	1.4
S. circinatum (ATCC 66463)	24	6-36	4.2
S. circinatum (ATCC 66464)	24	6-36	4.9
S. lignicola (P-53)	30	6-40	5.3
Taxon 121 (P-121)	30	16-40	

[†] Most rapid growth rate observed.
[‡] Burdsall and Eslyn (1974).
[§] Lamar et al. (1990).

Table 17-3. Effect of PCP on growth rates, at 24°C, on the studied fungal strains.

	Hyphal extension rate (mm d^{-1}) at PCP concentration (µg ml^{-1})			
	0	5	10	15
	Basidiomycota			
P. sordida	29.1	12.8	7.3	7.3
P. subserialis	23.6	4.6	0.0	0.0
I. lacteus	21.9	8.8	0.0	0.0
B. adusta	20.4	0.0	0.0	0.0
P. chrysosporium	19.0	7.6	0.0	0.0
T. versicolor	18.5	0.0	0.0	0.0
S. brinkmannii	10.7	5.9	4.4	3.1
P. placenta	9.4	0.0	0.0	0.0
P. brevispora	8.8	7.2	0.0	0.0
Sistotrema sp.	8.1	2.5	1.8	1.3
G. trabeum	6.5	0.0	0.0	0.0
A. xantha	6.5	0.0	0.0	0.0
H. praetermissum	6.3	0.0	0.0	0.0
A. carbonica	2.7	0.0	0.0	0.0
	Mitosporic Ascomycota			
A. cuboidea	23.0	3.4	1.7	1.4
P. variotii (ATCC 66706)	14.2	2.7	0.9	0.0
A. alternata (P-3)	10.4	5.0	2.3	1.9
C. resinae (ATCC 66686)	7.8	2.7	1.5	0.0
S. lignicola (P-53)	6.0	3.8	3.6	3.2
C. resinae (P-545)	5.7	2.4	0.9	0.6
S. circinatum (ATCC 66464)	5.4	1.1	0.7	0.0
S. circinatum (ATCC 66463)	4.1	1.3	0.8	†
P. dimorphospora (ATCC 66712)	2.8	1.1	0.5	†
P. dimorphospora (ATCC 66713)	2.7	1.0	0.5	0.0
P. dimorphospora (ATCC 66714)	2.2	1.1	0.6	0.3
P. dimorphospora (P-109)	2.2	1.0	0.6	0.3
L. elatius (P-247)	1.8	1.0	0.6	0.4
L. elatius (ATCC 66694)	1.6	0.6	0.2	0.0
P. heteromorpha (ATCC 66831)	1.5	0.8	†	0.0
P. heteromorpha (P-33)	1.3	0.7	0.5	0.5
R. atrovirens (P-154)	1.2	0.7	0.6	0.6
P. heteromorpha (P-196)	1.2	0.7	0.4	0.0
Taxon 121 (P-121)	1.2	0.6	0.0	0.0
R. atrovirens (ATCC 66759)	1.0	0.9	0.5	0.4
R. atrovirens (ATCC 66758	0.9	0.7	0.4	0.4

†Indicates that the fungus began growing late in the second week with minimal growth, therefore a growth rate was not calculated.

Table 17-3. Effect of PCP on growth rates, at 24°C on the studied fungal strains.

	Hyphal extension rate (mm d^{-1}) at PCP concentration (μg ml^{-1})			
	0	5	10	15
	Basidiomycota			
P. sordida	29.1	12.8	7.3	7.3
P. subserialis	23.6	4.6	0.0	0.0
I. lacteus	21.9	8.8	0.0	0.0
B. adusta	20.4	0.0	0.0	0.0
P. chrysosporium	19.0	7.6	0.0	0.0
T. versicolor	18.5	0.0	0.0	0.0
S. brinkmannii	10.7	5.9	4.4	3.1
P. placenta	9.4	0.0	0.0	0.0
P. brevispora	8.8	7.2	0.0	0.0
Sistotrema sp.	8.1	2.5	1.8	1.3
G. trabeum	6.5	0.0	0.0	0.0
A. xantha	6.5	0.0	0.0	0.0
H. praetermissum	6.3	0.0	0.0	0.0
A. carbonica	2.7	0.0	0.0	0.0
	Mitosporic Ascomycota			
A. cuboidea	23.0	3.4	1.7	1.4
P. variotii (ATCC 66705)	14.2	2.7	0.9	0.0
A. alternata (P-3)	10.4	5.0	2.3	1.9
C. resinae (ATCC 66686)	7.8	2.7	1.5	0.0
S. lignicola (P-53)	6.0	3.8	3.6	3.2
C. resinae (P-545)	5.7	2.4	0.9	0.5
S. circinatum (ATCC 66464)	5.4	1.1	0.7	0.0
S. circinatum (ATCC 66463)	4.1	1.3	0.8	†
P. dimorphospora WCC 66712)	2.8	1.1	0.5	†
P. dimorphospora (ATCC 66713)	2.7	1.0	0.5	0.0
P. dimorphospora (ATCC 66714)	2.2	1.1	0.6	0.3
P. dimorphospora (P-109)	2.2	1.0	0.6	0.3
L. elatius (P-247)	1.8	1.0	0.6	0.4
L. elatius (ATCC 66694)	1.6	0.6	0.2	0.0
P. heteromorpha (ATCC 66831)	1.5	0.8	†	0.0
P. heteromorpha (P-33)	1.3	0.7	0.5	0.5
R. atrouirens (P-154)	1.2	0.7	0.6	0.6
P. heteromorpha (P-196)	1.2	0.7	0.4	0.0
Taxon 121 (P-121)	1.2	0.6	0.0	0.0
R. atrovirens (ATCC 66759)	1.0	0.9	0.5	0.4
R. atrovirens (ATCC 66758)	0.9	0.7	0.4	0.4

†Indicates that the fungus began growing late in the second week with minimal growth, therefore a growth rate was not calculated.

17-3.3 Mineralization of Pentachlorphenol in Liquid Culture

Percentage of mineralization of PCP by tested basidiomycetes was quite variable and ranged from about 1% for *Sistotrema* sp. to about 45% for *B. adusta* and *T. versicolor* (Table 17-4). The percentage of PCP mineralized by the latter two organisms were far superior to any of the other tested strains. A group of organisms comprised of *H. praetermissum, I. lacteus, P chrysosporium, P. sordida,* and *P. subserialis* also mineralized a significant percentage of PCP (about 20 to 29%). The other tested isolates mineralized $\leq$ 2% PCP. Although the deuteromycetes produced mycelial mats in the BIII medium, none of the tested isolates mineralized >2% PCP.

Amount of fungal biomass (i.e., mycelial mat weight) did not relate directly with percentage of mineralization: regression of mycelial mat weight on percentage of mineralization gave an R^2 = 0.386, P = 0.0101. For example, some isolates like *P. brevispora* produced relatively large mats (0.038 g mat-‘) but mineralized only small amounts of PCP (Table 174). Other organisms like *H. praetermissum, I. lacteus,* and *P. chrysosporium,* were able to mineralize significant amounts of PCP with intermediate-sized mats (0.021-0.24 g mat^{-1}). Because of the lack of a direct relationship between percentage of mineralization and mycelial mat weight, the fungi were compared on the basis of mineralization efficiency (i.e., % PCP mineralization/weight mycelial mat). Although their mineralization efficiencies were not significantly greater than several white-rot fungi, the mycelium of the brown-tot fungi G. *trabeum* and *P. placenta* possessed the

Table 17-4. Pentachlorophenol mineralized (%) in BIII medium by basidiomycetes.[†]

	Mineralization	Mycelial mat weights
	%	mg
T. versicolor	45.1 ± 8.0	43 ± 11
B. adusta	44.8 ± 3.3	45 ± 6
P. subserialis	29.4 ± 1.1	45 ± 7
P. chrysosporium	28.7 ± 6.2	24 ± 4
I. lacteus	25.1 ± 2.3	21 ± 2
H. praetermissum	25.0 ± 1.2	22 ± 1
P. sordida (old)	20.3 ± 3.9	55 ± 5
P. sordida (new)	12.4 ± 0.5	38 ± 2
G. trabeum	11.5 ± 0.3	9 ± 1
P. placenta	10.1 ± 0.4	8 ± 1
A. carbonica	8.0 ± 1.0	4 ± 2
P. brevispora	3.6 ± 1.2	38 ± 11
Sistotrema sp.	1.2 ± 0.2	10 ± 2
S. brinkmannii	1.1 ± 0.7	25 ± 1
Control	0.7 ± 0.3	
A. xantha	0.5 ± 0.3	4 ± 1

[†] Although the deuteromycetes grew in the BIII medium, they did not mineralize > 2% of the PCP, therefore these results were not included in the table.

Table 17-5. Mineralization efficiency for PCP of the basidiomycetes (% comulative mineralization per gram of mycelial weight produced after 30 d).

	Mineralization efficiency
G. trabeum	13.5 ± 1.7a[†]
P. placenta	12.9 ± 2.7a
P. chrysosporium	12.5 ± 4.5a
I. lacteus	11.8 ± 1.8a
H. praetermissum	11.2 ± 0.7a
T. versicolor	10.6 ± 1.3ab
B. adusta	10.1 ± 1.4ab
P. subserialis	6.6 ± 0.8bc
P. sordida (old)	3.7 ± 1.0cd
P. sordida (new)	3.3 ± 0.2cd
A. carbonica	1.8 ± 6.9d
A. xantha	1.3 ± 0.3d
Sistotrema sp.	1.1 ± 0.0d
P. brevispora	0.9 ± 0.2d
S. brinkmannii	0.42 ± 0.0d

[†] Values followed by the same letter are not significantly different as determined by Fisher's Protected least significant difference multiple comparison procedure ($\alpha = 0.05$).

greatest capacity for mineralizing PCP under the experimental conditions (Table 17-5); however, with the exception of those two organisms, white-rot fungi possessed greater mineralization efficiencies than brown-rot fungi.

17-3.4 Depletion of Pentachlorophenol in Soil

The abilities of both basidiomycetous and dueteromycetous fungi to decrease the concentration of PCP in soil varied greatly (Table 17-6). The most effective basidiomycete was *P. sordida* Interestingly this isolate decreased the PCP concentration by about 7% more than the same *P. sordida* isolate that had been transferred on 2% malt agar, repeatedly, during a 7 yr period. Other basidiomycetes that caused PCP decreases that were significantly greater than those observed in control cultures were the white-rot fungi *P. chtysosporium, I. lacteus, T. versicolor,* and *B. adusta* and the brown-rot fungi *G. trabeum* and *A. carbonica* (Table 17-6).

The most effective PCP-degrading dueteromyetes were *C. resinae,* two isolates of *Phialocephala dimorphospora,* and one isolate of *Phialophora heteromorpha* (Table 17-6). Although these organisms caused significant percentage decreases in the PCP concentration (about 33-44%), their decreases were not as great as those caused by the most effective basidiomycetes. In several cases, the percentage decrease of PCP caused by different isolates of the same species varied greatly. For example, the percentage decreases effected by isolates of *Phialophora heteromorpha* were 32.5, 25.8, and 9.5%, respectively, for isolates P-196, P-33, and ATCC66831 (Table 17-6). The percentage PCP decreases caused by two isolates of *R. atrovirens* were less then was observed in control cultures.

Table 17-6. Percentage of decrease in the PCP concentration in soil culture.

Fungal isolate	Decrease in PCP concentration
Basidiomycota	
	%
P. sordida (new isolate)	71.9 ± 24.9a
P. chrysosporium	66.1 ± 14.9ab
P. sordida (old isolate)	65.0 ± 27.4ab
I. lacteus	49.7 ± 1.3bc
T. versicolor	39.1 ± 2.8cde
B. adusta	32.8 ± 8.8cde
G. trabeum	28.2 ± 203def
A. carbonica	24.9 ± 8.2def
H. praetermissum	20.3 ± 6.7defg
A. xantha	19.3 ± 13.4defg
P. subserialis	13.8 ± 17.4efgh
P. placenta	11.8 ± 2.6fgh
S. brinkmannii	10.8 ± 4.4fgh
Sistotrema sp.	9.5 ± 2.3fgh
P. brevispora	4.0 ± 8.9gh
Control (no agar)	2.5 ± 7.6gh
Control (agar)	0.3 ± 6.6gh
Microsporic Ascomycota	
C. resinae (ATCC 66686)	43.8 ± 8.8a
P. dimorphospora (ATCC 66714)	35.3 ± 12.2a
P. dimorphospora (P-109)	32.5 ± 5.1a
P. heteromorpha (P-196)	32.5 ± 6.0a
L. elatius (P-247)	30.7 ± 2.1ab
l? dimorphospora (ATCC 66713)	28.9 ± 2.7ab
P. dimorphospora (ATCC 66712)	26.7 ± 6.6abc
C. resinae (P-545)	26.6 ± 7.6abc
P. heteromorpha (P-33)	25.6 ± 13.1abc
A. cuboidea	25.8 ± 16.4abe
A. alternata (P-3)	25.6 ± 4.0abc
L. elatius (ATCC 66694)	22.4 ± 15.0abcd
P. variotii (ATCC 66705)	17.6 ± 7.7bcde
S. circinatum (ATCC 66463)	13.9 ± 7.0cdef
S. *circinatum* (ATCC 66464)	13.8 ± 7.1cdef
Taxon 121 (P-121)	11.6 ± 9.2def
P. heteromorpha (ATCC 66831)	9.5 ± 4.2def
R. atrovirens (P-154)	3.6 ± 6.4ef
Control (agar)	8.2 ± 7.4ef
Control (no agar)	5.4 ± 6.9ef
R. atrovirens (ATCC 66758)	3.1 ± 4.5f
R. atrovirens (ATCC 66759)	1.7 ± 7.1f

†Values followed by the same letter are not significantly different as determined by Fisher's Protected least significant difference multiple comparison procedure (α = 0.05)

17-3.5 Sensitivity of Strains to Creosote

The majority of the basidiomycetes grew up to 500 ppm creosote before growth was completely inhibited (Table 17-7). Several isolates including *A. xantha, B. adusta, I. lacteus, P. placenta,* and *S. brinkmanii* were able to grow in the presence of 1000 g L^{-1}creosote. *Hyphoderma praetermissum* was the most sensitive isolate and it failed to grow in the presence of 100 μg L^{-1}. The most creosote-tolerant deuteromycete was *C. resinae.* The hyphal extension rate of this organism was only slightly decreased in the presence of 1000 g L^{-1} creosote. There was some intraspecific variation in creosote tolerance among deuteromycete isolates. For example, *Leptodontium* elatius strain ATCC 66694 did not tolerate 100 mg L^{-1}, whereas strain P-247 was able to grow in the presence of 1000 μg L^{-1} creosote.

17-3.6 Degradation of Polycyclic Aromatic Hydrocarbons in Creosote-Treated wood

Concentration of PAH analyses in nonsterile, creosote-treated wood cubes were significantly less in fungal inoculated and control cultures compared with those found in nontreated wood samples (Table 17-8). Inoculation with either basidiomycetes or deuteromycetes did not always result in concentrations that were significantly less than those found in wood amended with sterile agar (controls). Residual concentrations of acenapthene, fluorene, and phenanthrene in fungal inoculated wood cubes were never significantly less than those found in control treated cubes (Table 17-8). Decreases in the PAH analyte concentrations in control wood cubes may have been due to the presence of dueteromycete and/or bacterial strains naturally associated with the creosote-treated wood. Two dueteromycete fungi, *Curvularia* sp. and *Alternaria alternata* were identified growing on wood cubes and agar from the control treatment. Residual concentrations of anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*]anthracene, benzo[*g,h,i*]perylene, indeno[1,2,3-*c,d*]pyrene were significantly less in some fungal-treated blocks than in control blocks (Table 17--8). In particular, treatment with the basidiomycyes *G. trabeum* and *P. brevispora* and the dueteromycetes *C. resinae* (ATCC 66686) and *S. circinatum* (ATCC 66463), resulted in residual concentration of theses compounds that were significantly less than those found in wood from control cultures (Table 17-8).

17-4 DISCUSSION

Identification of the most effective fungus for a particular set of contaminatn-soil conditions and provision of environmental conditions in the field, that give optimum growth and xenobiotic-degrading activities of the selected fungus, are the keys to successful application of fungal bioaugmentation. Ideally fungi would be selected on the basis of biochemical, physiological, and ecological attributes that would confer to them superior performance under a particular set of contaminant-media (e.g., soil) conditions. Currently treatability studies, using

Table 17-7. Effect of creosote on growth rates, at 24°C, on various strains of fungi.

Fungal isolate	Mycelia extension rate mm d^{-1}) at creosote concentration (µg ml^{-1})				
	0	100	250	500	1000
		Basidiomycota			
P. subserialis	23.6	1.9	1.2	1.8	0.0
I. lacteus	21.9	4.4	5.6	3.7	1.0
B. adusta	20.4	16.0	5.8	4.7	3.8
P. chrysosporium	19.0	4.2	0.0	0.0	0.0
T. versicolor	18.5	6.7	2.9	2.2	0.0
P. sordida	15.6	4.8	†	†	0.0
S. brinkmannii	10.7	2.7	3.1	2.0	1.3
P. placenta	9.4	3.8	1.8	1.2	1.0
P.. brevispora	8.8	2.6	1.4	0.9	0.0
Sistotrema sp.	8.1	1.2	1.5	1.0	0.0
A. xantha	6.5	4.1	2.9	3.0	1.8
G. trabeum	6.5	1.5	0.9	†	0.0
H. praetermissum	6.3	0.0	0.0	0.0	0.0
A. carbonica	2.7	0.7	0.6	0.5	†
		Mitosporic Ascomycota			
A. cuboidea	8.0	7.2	6.5	5.5	3.1
A. alternata (P-3	7.3	3.5	†	0.0	0.0
C. resinae (ATCC 66686)	6.3	5.0	4.7	4.2	4.1
P. variotii (ATCC 66705)	5.8	6.3	3.2	4.2	2.3
C. resinae (P-545)	4.6	4.3	4.0	4.0	4.0
S. lignicola (P-53)	4.1	3.7	2.6	1.8	1.8
S. circinatum (ATCC 66464)	2.8	†	0.0	0.0	0.0
P. dimorphospora (P-109)	2.4	1.6	1.3	1.5	†
P. dimorphospora (ATCC 66712)	2.3	1.4	†	†	0.0
S. circinatum (ATCC 66463)	2.2	0.0	0.0	0.0	0.0
P. dimorphospora (ATCC 66113)	2.1	1.6	†	†	0.0
P. dimorphospora (ATCC 66114)	1.8	0.9	0.8	0.9	†
L. elatius (ATCC 66694)	1.4	†	0.0	0.0	0.0
L. elatius (P-247)	1.4	1.0	1.1	1.1	0.9
P. heteromorpha (P-33)	1.1	1.0	0.8	0.5	0.4
P. heteromorpha (P-196)	1.0	1.3	0.9	0.5	†
R. atrovirens (P-154)	1.0	0.8	0.6	0.5	0.5
Taxon 121 (P-121)	1.0	1.0	1.0	0.9	0.7
R. atrovirens (ATCC 66758)	0.8	0.7	0.0	0.0	0.0
R. atrovirens (ATCC 66759)	0.8	0.7	†	0.0	0.0

†Indicates that the fungus began growing late in the second week with minimal growth, therefore a growth rate was not calculated.

Table 17-8. Concentrations (mg kg^{-1}) of extracted PAHs in creosote-treated wood blocks before (initial)† and after 6 wks of treatment with selected fungi and controls† (noninoculated).

	PAHs‡													
	ACP	FLU	PHE	ANT	FLA	PYR	BAA	CHR	BBF	BKF	BAP	DAA	BGP	IP
Basidiomycota														
B. adusta	22	11	109	61	349	260	121	141	101a	47a	82a	17	26a	33a
G. trabeum	14	12	72	45a	270a	224a	109a	122a	89a	41a	65a	14a	23a	29a
H. praetermissum	24	19	106	65	331	249a	125	138	99a	45a	76a	16	25a	32a
I. lacteus	18	15	89	64	358	278	139	157	115	52	86a	19	30	38
P. chrysosporium	18	16	101	59	327	250a	124	138	95a	44a	76a	15a	23a	30a
P. sordida	21	17	106	64	343	262	129	143	106	48a	83a	17	38	35a
P. brevispora	16	14	82	49a	310a	247a	121a	136a	95a	43a	75a	15a	24a	31a
P. subserialis	26	22	129	74	402	309	149	166	125	57	107	20	36	45
P. placenta	21	16	104	62	336	253	126	143	105	48a	86a	17	29	36a
S. brinkmannii	21	19	104	63	346	267	128	143	103a	47a	85a	16	28a	35a
T. versicolor	23	20	125	67	371	278	134	149	101a	46a	84a	16a	25a	32a
Mitosporic Ascomycota														
A. alternata	21	20	110	57	310a	24a	116a	130a	93a	43a	77a	15a	25a	32a
A. cuboidea	37b	32b	186b	94	463	325	153	168	106	49	91	16a	25a	32a
C. resinae (ATCC 66686)	18	15	86	51a	308a	243a	122a	136a	101a	46a	81e	18	29	36a
C. resinae (P-545)	19	16	104	57	356	278	138	155	113	52	89a	20	31	40
P. uariotii	26	22	117	67	333	248a	121a	135a	96a	43a	72a	15a	23a	30a
S. circinatum (ATCC 66463)	22	18	94	53a	269a	207a	99a	110a	75a	35a	61a	12a	17a	23a
S. circinatum (ATCC 66464)	15	13	80	50a	309a	266a	134	150	121	55	94	21	35	44
S. lignicola	25	18	110	59	373	291	141	158	115	52	87a	20	32	40
Control	23	20	111	77	423	326	159	177	134	62	116	22	38	48
Initial	194b	139b	791b	322b	1497b	1011b	507b	563b	408b	186b	355b	69b	112b	142b

§ Means within columns followed by an a orb are significantly less or greater, respectively, than the control as determined by Fisher's protected least significant difference multiple comparison procedure (α = 0.05). †Abbreviations: Acenapthene (ACP), Fluorene (FLU), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLA), Pyrene (PYR), Benzo[*a*]anthracene (BAA), Chrysene (CHR), Benzo[*b*]fluoranthene (BBF), Benzo[*k*]fluoranthene (BKF), Benzo[*a*]pyrene (BAP), Dibenzo[*a,h*]anthracene (DAA), Benzo[*g,h,i*]perylene (BGP), and Indeno[1,2,3-*c,d*]pyrene (IP).

† Initial values are from non-inoculated creosote-treated wood at the start of the experiment. Control values are from creosote-treated wood which was amended with half the agar from a sterile PDA plate. The control treatment contained two contaminant fungi which were identified as *Curvularia* sp. and *Alternariaalternatat*

representative soil samples, are employed to select the best fungus to use on a particular soil. Because of the laborious and time-consuming nature of these studies, the number of fungi that can be evaluated is limited and results do not yield information on what biochemical, physiological, and/or ecological attributes are responsible for superior bioremediation performance.

Much of the knowledge of the biochemistry and physiology of pollutant degradation by white-rot fungi has been generated from experiments in which the degradation of a pollutant by a fungus grown in defined aqueous media is determined or by evaluating the activity of crude extracts or purified enzymes from aqueous fungal cultures on various pollutants in in vitro experiments. While providing valuable insights into the biochemistry and physiology of pollutant degradation by selected fungi, the applicability of the results of the results of aqueous culture work to complex environments like polluted soils, is questionable. This is because of the complex effects of physiochemical and biological soil factors on both the fungus, its pollutant-degrading system, and the pollutant. For example, in the present work, neither percent mineralization of PCP in liquid culture ($r^2 = 0.276$, $P = 0.0536$) or mineralization efficiency ($r^2 = 0.168$, $P = 0.146$) of basidiomycetes correlated well with their abilities to decrease the concentration of PCP in soil. Thus these attributes were not useful for predicting bioremediation performance of these organisms in the PCP-contaminated Marshan soil. *Phanerochaete chrysosporium* was found to convert PCP in soil to primarily nonvolatile products, both soil bound and extractable, whereas loss via mineralization was negligible (Lamar et al., 1990). If the fate of PCP in soils inoculated with other white-rot fungi is similar to that in soils inoculated with *P. chrysosporium* it is not surprising that the ability of a fungus to mineralize PCP in aqueous media does not necessarily correlate well with its ability to degrade PCP in soil. Measurement of the activities of fungal enzymes in aqueous culture (Lin et al., 1991; Okeke et al., 1994), disappearance of pollutants (Lin et al., 1991; Fletcher et al., 1994) and decolorization of various dyes (Lin et al., 1991; Fletcher et al., 1994 Field et al., 1993) also have been suggested as corollaries of fungal degradative abilities. In particular the activities of Lips, MnPs, and laccases associated with the ligninolytic system of white-rot fungi and with pollutant degradation by these organisms have been measured, however, white-rot fungi have been shown to produce different combinations of ligninolytic enzymes and to produce different sets of isozymes of the same enzyme, when grown in different media. For example, *P. chrysosporium* produced different LiP mRNA transcripts in soil compared with those produced in liquid media (Lamar et al., 1995). Therefore, transformation and mineralization abilities in aqueous media may have little potential for predicting degradative abilities in contaminated soils.

In addition to a demonstrated ability to degrade a contaminant, two factors that do show potential for predicting bioremediation performance, at least for degradation of PCP by white-rot and brown-rot basidiomycetes, are hyphal extension rate and sensitivity to contaminant. The three most effective strains for removal of PCP from the Marshan soil, *P. sordida, P. chrysosporium* and *I. lacteus* (Table 17-6), all had rapid hyphal extensions rates and high tolerances to PCP, relative to the other tested basidiomycetes. Although *T. versicolor* and *B. adusta* possessed rapid growth rates (Table 17-2) and superior abilities to mineralize PCP in liquid culture (Table 17-4). they were both extremely sensitive to

the presence of the chlorophenol (Table 17-3) and were only intermediate in their relative abilities to decrease the concentration of PCP in soil (Table 17-6).

The soil remediation potential of dueteromycetous fungi has not been evaluated previously. As a group, they varied greatly in their ability to transform PCP (Benoit-Guyd et al., 1994) and in general were more tolerant of PCP than were basidiomycetes as shown in the present work (Table 17-3) and by Zabel et al. (1985); however, despite a general greater tolerance to PCP, the dueteromycete strains that decreased the soil PCP concentration by the greatest percentages, C. *resinae, P. dimorphospora,* and *P. heteromorpha,* were not as effective as the most competent basidiomycetes (Table 17-6). Also, in contrast to the basidiomycetes, neither relative sensitivity to PCP or growth rate were related to the ability of deuteromycetes to deplete PCP in soil. *Cladosporium resinae* (ATCC 66686) was not very tolerant of PCP and like most of the dueteromycetes, grew very slowly. Therefore, characteristics that prove to be valuable for predicting the bioremediation performance of an ecological group, such as white-rot fungi, may have little or no value for predicting the performance of fungi from other groups.

For degradation of PAHs in wood, tolerance to contaminant and hyphal extension rate were not useful for predicting the contaminant-degrading ability of a fungus. The most effective fungi for degrading PAHs in wood were the brown-rot basidiomycete, *G. trabeum,* particularly for the lower molecular weight PAHs, and the dueteromycete, *S. circinatum* (ATCC 66463). Both of these organisms possessed low hyphal extension rates and were sensitive to creosote. The usefulness of tolerance to contaminant and hyphal extension rate, in the present study, to predict the ability of basidiomycetes to deplete PCP in soil but not PAHs in wood illustrates that different attributes of fungal growth and pollutant-degrading abilites will be important under different conditions.

The results of the present work indicate that simple measures of contaminant-degrading ability or tolerance to a pollutant(s), while important, will not alone provide sufficient information for selection of fungi for use in bioaugmentation of contaminated soils. As was demonstrated in the present work for *P. sordida,* one fungus may be a superior performer under one set of contaminant-media conditions (e.g., PCP-contaminated soil) and be average or mediocre under another set of conditions (e.g., creosote-treated wood). More information on the bioremediation performance of well characterized fungi on soils that represent a range of soil physical, chemical, and biological characteristics and pollutants is necessary to build a data base of information from which to base selection of superior fungal strains to evaluate for application to specific contaminant-media conditions.

Such a data base would ideally include fungal characteristics such as growth rates, growth temperature ranges and optima, sensitivities to and abilities to degrade specific contaminants, bioremediation performance in well characterized media and relative competitive abilities. The later is a key factor that has been almost completely ignored in the evaluation of fungi for use in bioaugmentation. The ability of a fungus to colonize a contaminated soil volume and to execute contaminant degradation with little or minimal effects from indigenous microbes is at least as important as the ability to degrade the contaminant. Important media characteristics would include pH, levels of available nutrients, particularly C and N, identification of contaminants and their concentrations and identification of indigenous microbial populations. More information on the performance of fungi

under well characterized contaminant-media conditions, may allow the identification of general attributes that will permit the efficient selection of appropriate fungi for use under specific contaminant-media conditions. Therefore future studids concerned fungal contaminant degradation in complex media should be designed with additional purpose of adding information to such data base.

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