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Suppression of bioremediation by *Phanerochaete chrysosporium* by soil factors

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Abstract

The timing of onset of mineralization of pyrene by *Phanerochaete chrysosporium* correlated with the production of ligninase activity as monitored by the decolorization of the polymeric dye Poly R-478. A layer of native soil decreased the growth of *P. chrysosporium* by visual assessment of hyphal mass and measurement of ergosterol, a fungal membrane sterol. The native soil layer slowed the production of ligninase as determined by Poly R-478 decolorization and the rate of mineralization of pyrene was decreased. Inhibition of pyrene mineralization was also observed with layers of sterilized silt and clay but not by sand. Mineralization, fungal growth and rate of decolorization of the dye were improved when the native soil was sterilized by autoclaving. Suspensions of the native soil contained a consortium of bacteria and fungi. Several of the bacteria were antagonistic to the growth of *P. chrysosporium* on solid medium. The onset of dye decolorization by *P. chrysosporium* on a nitrogen-limited medium was delayed by certain bacteria. These findings suggest that in certain soils, bioremediation by *P. chrysosporium* could be suppressed by indigenous soil microbes as well as by the abiotic features of the soil composition.

1. Introduction

The efficacy of the white rot fungus *Phanerochaete chrysosporium* as a bioremediation agent in soil is of significance for its application in field sites. Degradation of pollutants by *P. chrysosporium* has been readily demonstrated under controlled laboratory conditions [1–5]. However, in field soil the existing microflora and soil composition may modify the effectiveness of *P. chrysosporium*. Soil inhibited the degradation of fluorene by *P. chrysosporium* [6] whereas synergism between soil organisms and *P. chrysosporium* was implied from studies of degradation of 2,4,5-trichlorophenoxyacetic acid [7] and phenanthrene [8].

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In this paper we investigate the effect of soil components and soil microbes on *P. chrysosporium* in systems using pyrene mineralization and polymeric dye decolorization as indicators of bioremediation potential. Correlation between polymeric dye decolorization and contaminant degradation by *P. chrysosporium* has been suggested. The rate of Poly R-478 decolorization in liquid media by *P. chrysosporium*, as well as other wood decay fungi, *Pleurotus ostreatus*, *Auricularia auriculajudae* and *Lentinus edodes*, followed a linear relationship with pentachlorophenol removal rates [9]. Also in liquid media, the rate of dye decolorization by several isolated white rot fungi was correlated strongly with phenanthrene mineralization rates [10]. Additional studies in liquid medium correlate polymeric dye decolorization with the lignin peroxidase system of *P. chrysosporium* [11, 12], veratryl alcohol oxidation [13] and the mineralization of ^{14}C-ring labelled synthetic lignin [14].

The extracellular lignin peroxidase system of *P. chrysosporium* has been implicated in the degradation of many recalcitrant pollutants [2, 3, 5, 15–17]. Before the use of dyes in our model systems, we established that decolorization of the dye Poly R-478 was catalyzed by purified lignin peroxidase. Also we confirmed that decolorization on solid medium correlated with growth conditions documented to promote or suppress the production of lignin peroxidase by *P. chrysosporium*.

We selected pyrene as the model pollutant. Pyrene is representative of a large class of potentially hazardous materials, the polycyclic-aromatic hydrocarbons. Degradation of pyrene occurs in soils [18] by isolated fungi [19] and by soil bacteria, including *Mycobacterium* [20] and *Xanthomonas* species [18]. Several studies show effective pollutant degradation in soil by *P. chrysosporium* requires amendments with plant-derived carbon sources [21–23]. The degradative pathway for pyrene mineralization by *P. chrysosporium* is not resolved. *P. chrysosporium* is reported to use the lignin peroxidase system in the degradation of benzo(a)pyrene [3, 4] but not phenanthrene [24] and the extracted enzyme will oxidize pyrene [11]. We asked whether in solid medium ligninase production, as assayed by dye decolorization, correlated with mineralization of pyrene by *P. chrysosporium*.

Two hypotheses were tested relative to the influence of soil on degradation of pyrene by *P. chrysosporium*. One hypothesis was that the percentage of sand, silt and clay that constitute the soil is influential. The second was that degradation would be effected by indigenous soil microbes. Our in vitro studies [25, 26] show certain soil-associated microbes are strong antagonists of *P. chrysosporium* growth. This finding is consistent with the ability of soil microbes to suppress growth of plant pathogenic fungi [27–30]. Microbial effects were examined by use of heat-sterilized soil and by investigating the potential for the indigenous soil bacteria to antagonize growth and effect the dye decolorization ability of *P. chrysosporium*.

2. Procedures

2.1. Inhibition of veratryl alcohol oxidase activity of lignin peroxidase Poly R-478

The veratryl alcohol oxidase activity of lignin peroxidase H1, purified by FPLC and obtained from Dr. Steven D. Aust, Utah State University, was measured

spectrophotometrically [31]. The same reaction mixture was amended with defined concentrations of the dye Poly R-478 to observe inhibition of veratryl alcohol oxidation.

2.2. Maintenance of organisms

P. chrysosporium BKM-F-1767 ATCC #24725 was maintained by subculturing at 26 °C on potato dextrose agar (PDA) (Difco, Detroit, Michigan) amended with 1% agar. To maintain the ability of the fungus to cause wood decay, the fungus was inoculated onto moist wooden applicators (Baxter Scientific Products, A5000-1) after each seven passages through PDA. Mycelia were recovered from the wood three weeks after inoculation and transferred back to PDA plates. Inoculum for studies consisted of 1 × 1 cm blocks cut from growing mycelial tips of 2-day-old subcultures.

P. chrysosporium was grown on ground corn cobs to provide an inoculated matrix for mineralization studies. Cobs (50 ml) were autoclaved dry for 1 h on 2 consecutive days, inoculated with 20 ml of *P. chrysosporium* spore suspension (10^6 spores/ml) and grown under otherwise sterile conditions for 1 month at 26 °C in the dark. The inoculum was mixed by shaking to obtain uniformity and used directly.

Bacteria from the Timpanogos soil used in this study were grown on King's medium B agar plates [32] at 26 °C for 2 days prior to use in the studies.

2.3. Soil properties

The soil used in these studies was a Timpanogos silt loam collected in the grassland near Tremonton, Utah. The soil was sieved through a 2 mm mesh sieve and stored at room temperature for 1 yr in closed plastic containers prior to use. Soil characteristics were determined by the Utah State University Analytical Laboratories Soil Testing Lab, Logan, Utah (Table 1). Sterilized soil was prepared by autoclaving for 4 h on 2 consecutive days.

Bacteria were isolated from native soil using serial dilution onto King's medium B of 1 g soil in 100 ml 10 m*M* $MgCl_2$. Bacteria were streaked for isolation three successive times prior to storage at − 80 °C in 15% glycerol.

Table 1
Soil characteristics

Soil	pH	%O.C.[a]	CEC[b]	Field capacity	Texture
Timpanogos	7.2	1.78	20.8	20.6	silt loam (sand 25%, clay 18%, silt 57%)

[a] Percent organic carbon.
[b] Cation exchange capacity (meq/100 g).

2.4. Growth media for Poly R-478 dye decolorization and growth antagonism studies

In the plate studies, bacteria from 2-day-old cultures from King's medium B plates were suspended in 5 ml of sterile 10 m*M* $MgCl_2$.. Three aliquots of 10 µl of the resulting cell suspension were spotted in separate locations 1 cm from the edge of plates of PDA containing Poly R-478. *P. chrysosporium* inoculum, 1 × 1 cm agar blocks cut from the growing front of 2-day-old PDA plates grown at 26 °C, was placed mycelial side down in the center of the plates. Plates of *P. chrysosporium* alone and bacteria alone were prepared simultaneously. Growth of the organisms and their decolorization potential was assessed after incubation at 26 °C for 7 days.

2.5. The microcosms

Microcosms to examine simultaneous decolorization and mineralization by *P. chrysosporium* were established in two types of containers. Glass Erlenmyers (125 ml) were used for studies of decolorization and mineralization of ^{14}C-pyrene by *P. chrysosporium* on low and high nitrogen growth medium. Each flask contained 20 ml of defined medium [25] amended with 0.01% of the dye Poly R-478. The agar surface was inoculated with a 1 × 1 cm plug of *P. chrysosporium* from a 2-day-old PDA plate.

In the second system [26], Corning polystyrene tissue culture flasks (Corning Co. 25110-75 Corning NY) were laid flat and contained 40 ml of 1% water agar with no added nutrients but amended with 0.01% Poly R-478. These flasks were inoculated with 15 g of *P. chrysosporium* colonized-corn cobs.

Prior to inoculation with the fungus, ^{14}C-pyrene (150 000 dpm) in 5 µl benzene was applied to the agar surface in both types of flasks. The ^{14}C-pyrene (Sigma Chemical Company, St. Louis, MO) was received in toluene at 32.3 m Ci/mmol, blown to dryness and resuspended in HPLC grade benzene. The benzene was flushed out by using the gas exchange system described below for 18 h at 23 °C. After fungal inoculation, flasks were incubated at 26 °C and sampled for $^{14}CO_2$ evolution daily using the system described below.

2.6. The sterile gas exchange system

All flasks were closed by rubber stoppers fitted with a sterile gas exchange system. Two sterile 20 gauge 1.5 in. syringe needles were inserted through the stoppers. Each needle was fitted with 0.2 µm sterile syringe filters (Nalgene 190-2520) and affixed to the top of the filters were two stopcocks valves (Cole-Parmer G-064-64-71, Cole-Parmer Instrument Co., Nile, Illinois). The valves were kept in the closed position except when the flasks were purged. Flasks were purged into a series of three traps after the method of Marinucci and Bartha [33]. Each trap consisted of a 20 ml borosilicate glass scintillation vial fitted with a #2 rubber stopper into which a 17 gauge 3 in. stainless needle was inserted as a gas inlet, and a 1.5 in. 20 gauge needle inserted as a gas outlet. The traps were connected with Tygon (R-3603) tubing connected to the plastic syringe needle ends with Cole-Parmer luer tapers (G-063-59-10). The first trap was 17 ml ethylene glycol monomethyl ether as a volatile organic

trap. The second and third traps were carbon dioxide traps and contained 17 ml 2 *M* monoethanolamine in methanol [34].

Flasks were purged periodically with house air which was filtered for volatile organics by passage through a series of 3 dense foam plugs. Ten headspace volumes (2500 ml) of scrubbed house air were passed through each flask at 250 ml per minute. Headspace was sampled periodically with passage through the three trapping systems. After purging, the contents of the trap vials were subdivided and 10 ml Ready Safe scintillation cocktail (Beckman Instr., Inc., Fullerton, CA) added to each vial. The vials were shaken and counted the following day. Background counts of 70 dpm were subtracted from each vial after counting.

2.7. Soil amendments to the microcosms

Timpanogos soil was used directly or after sterilization by autoclaving twice for 4 h on 2 consecutive days. 15 g soil was overlayed on the pyrene-contaminated Poly R-478 agar and shaken to produce an even layer. An inoculum of 15 g ground corn cobs previously inoculated with *P. chrysosporium* was added on top of the soil layer and shaken slightly for even distribution. Control flasks received no soil or no inoculum. Flasks were incubated at 26 °C and subjected periodically to headspace analysis and daily for visual dye decolorization. At the end of the studies, to check for ^{14}C incorporation into carbonates in the soil, flask contents were acidified with 20 ml 4.0 *M* HCl and subjected to headspace analysis for 2 h.

In other studies a layer of sand, silt or clay was used to replace the soil. Sand (particle size 355 μm) was purchased and autoclaved at 121 °C for 1 h on 2 consecutive days before use. Silt (particle size <45 μm) was obtained by sieving and sedimentation of a high silt soil. To remove organic material a suspension of silt (50 g) in 30% H_2O_2 (20 ml) was heated to boiling for 5 min, cooled and reheated twice [35]. The H_2O_2 was removed by extensive washes with sterile distilled water. Clay (particle size <1 μm) was obtained commercially as Bentonite. Silt and clay were sterilized by autoclaving at 121 °C for 1 h on two consecutive days.

2.8. Measurement of ergosterol

Corn cobs with and without inoculations of *P. chrysosporium* were extracted for ergosterol by the method of Woods [36]. The ergosterol was quantified spectrophotometrically. Controls of authentic ergosterol added to non-inoculated corn cobs were prepared to confirm the spectral analysis.

2.9. Preparation of corn cob leachate

Dry corn cobs (200 g) were treated with 850 ml sterile distilled water for 5 h at 22 °C. The mixture was filtered through cheese cloth and centrifuged at 10 000 g for 14 min. The supernatant was sterilized at 121 °C for 20 min prior to inoculation with pseudomonads. Cultures were grown at 26 °C on a rotary shaker at 100 rpm. Absorbances at 600 nm were measured daily.

3. Results

3.1. Lignin peroxidase interaction with Poly R-478

Poly R-478 inhibited the ability to detect the oxidation of veratryl alcohol by the purified H1 isozyme (Fig. 1). Coincidentally the dye was decolorized from red through orange to yellow.

3.2. Dye decolorization and mineralization of pyrene by P. chrysosporium

Decolorization of Poly R-478 was observed on low but not high nitrogen medium inoculated with *P. chrysosporium* (Fig. 2). Decolorization was initiated by day 6 and

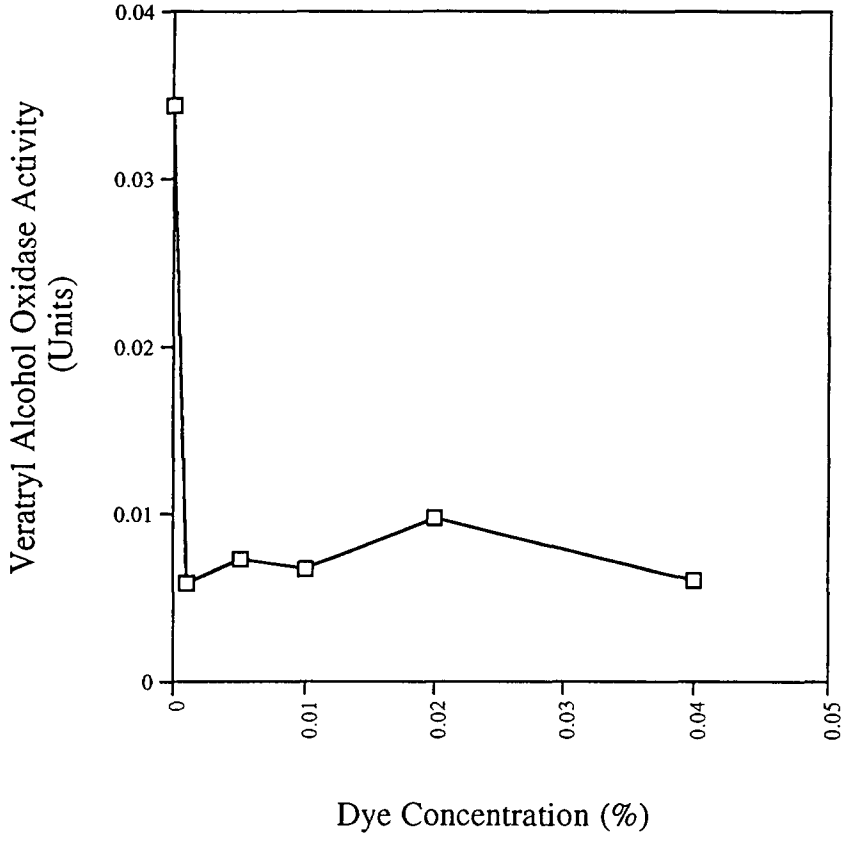

Fig. 1. Prevention of the detection of the veratryl alcohol oxidase activity of lignin peroxidase H1 by Poly R-478. Poly R-478 was added over a concentration range to reaction mixtures containing purified lignin peroxidase H1 as described in procedures. Activity was determined by measuring veratryl alcohol oxidation.

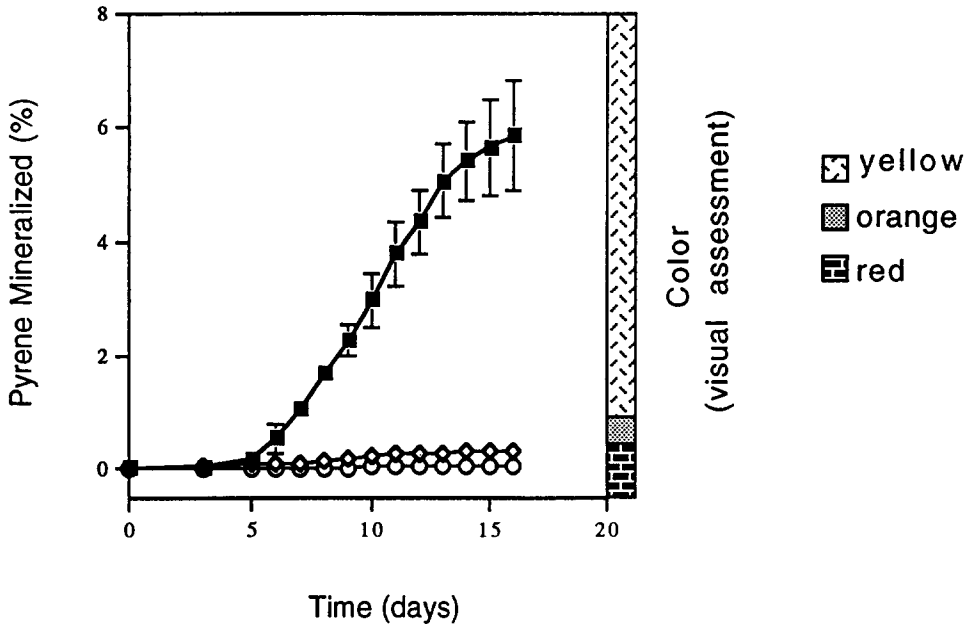

Fig. 2. ^{14}C-pyrene mineralization by *P. chrysosporium* grown on low nitrogen (2.4 m*M* NH_4Cl, ■), and high nitrogen (48 m*M* NH_4Cl, ◇) media. The control flasks contained sterile low nitrogen medium with no inoculum of *P. chrysosporium* (○). Right Y-axis shows the observable decolorization of Poly R-478 by *P. chrysosporium* from red through orange to yellow. Data are the averages from each of 5 flasks. The standard error of the means is shown for each data point.

was complete by day 9 in low nitrogen medium (Fig. 2). Error bars lie within the symbols. No decolorization occurred on the high nitrogen medium although growth of the fungal mycelium was greater than that on the low nitrogen medium. Mineralization of pyrene also commenced after 6 days of growth of *P. chrysosporium* on low nitrogen medium with about 6% mineralization by 15 days (Fig. 2). No mineralization was observed in inoculated flasks with high nitrogen medium or in flasks that lacked *P. chrysosporium* (Fig. 2). No radioactivity over 0.01% of the added pyrene was detected in the volatile organic trap or in the second carbon dioxide trap.

4. Effect of soil layers on pyrene mineralization

4.1. The Timpanogos soil

An inoculum of *P. chrysosporium* on corn cobs mineralized over 5% of ^{14}C-pyrene in 22 days (Fig. 3). Error bars lie within the symbols. Coincident with the onset of mineralization was the decolorization of the Poly R-478 present in the water agar layer upon which the pyrene was added (Fig. 3). The addition of a layer of native

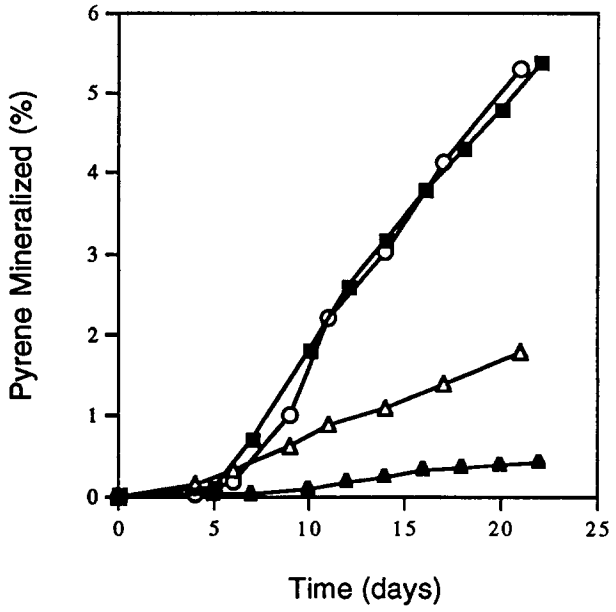

Fig. 3. Effects on pyrene mineralization of a layer of native or sterilized Timpanogos soil between *P. chrysosporium* and ^{14}C-pyrene. Data are from two separate experiments. *P. chrysosporium*, pregrown on ground corn cobs, was placed directly on the pyrene-contaminated water agar surface (○ and ■) or on top of 15 g of an overlay of native (▲) or sterilized (△) Timpanogos soil. All experiments were performed in Corning flasks as described in procedures.

Timpanogos soil between the pyrene-water agar surface and the corn cob inoculum decreased mineralization to 0.4% at day 22 (Fig. 3). The Poly R-478 dye was decolorized only in patches at this time.

The visible growth of the white mycelium on the corn cobs overlayering the native soil was less than that on corn cobs added directly over the agar surface. Ergosterol measured in extracts from the infested corn cobs at 22 days showed reduced levels in the corn cob-inoculum overlayed onto the native soil than when layered directly on the agar (Table 2). Because ergosterol is associated with fungal mycelium, these findings agree with the visual assessment that fungal growth is reduced by the native soil.

A layer of sterilized Timpanogos soil between the agar surface and the corn cob inoculum reduced the level of mineralization but to a lesser extent than the native soil (Fig. 3). The sterilized soil layer permitted greater mycelial growth of *P. chrysosporium* on the corn cobs as determined by eye and by ergosterol measurement (Table 2).

In a control study in which the inoculum of *P. chrysosporium* was omitted but in which the agar surface was overlayed with native soil, pyrene was gradually mineralized (Fig. 4). Acidification of the flask contents after 22 days did not release $^{14}CO_2$.

Table 2
Ergosterol content of corn cobs with and without an inoculum of *P. chrysosporium* and exposure to soil

Treatment	Relative units of ergosterol
Non inoculated, exposed to water agar	0.17 (± 0.01)
Inoculated, exposed to water agar	0.53 (± 0.2)
Inoculated, exposed to sterilized soil	0.42 (± 0.2)
Inoculated, exposed to native soil	0.36 (± 0.2)

Corn cobs were or were not preinoculated with *P. chrysosporium*. The cobs were layered onto water agar or native or sterilized Timpanogos soil under otherwise sterile conditions and incubated at 26 °C for 22 days. Extracts were prepared as described in Procedures to measure the ergosterol content. Data are the means of two separate samples.

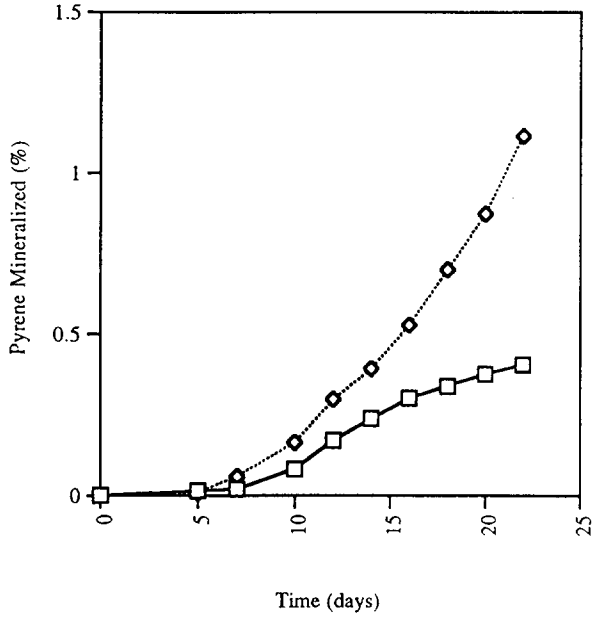

Fig. 4. Mineralization of pyrene by native Timpanogos soil or by native Timpanogos soil plus *P. chrysosporium*. Data are for two separate studies. The native soil was placed directly onto the pyrene contaminated-water agar surface. Soil was overlayed with an inoculum of *P. chrysosporium* pregrown on corn cobs in one set of flasks and corn cobs without inoculation in a second set of flasks, as described in procedures. (□) *P. chrysosporium* + native soil; (◇) native soil.

5. Soil components

Overlays of sand, silt or clay onto the pyrene-water agar surface differentially affected the mineralization of pyrene (Fig. 5). Mineralization in the presence of sand

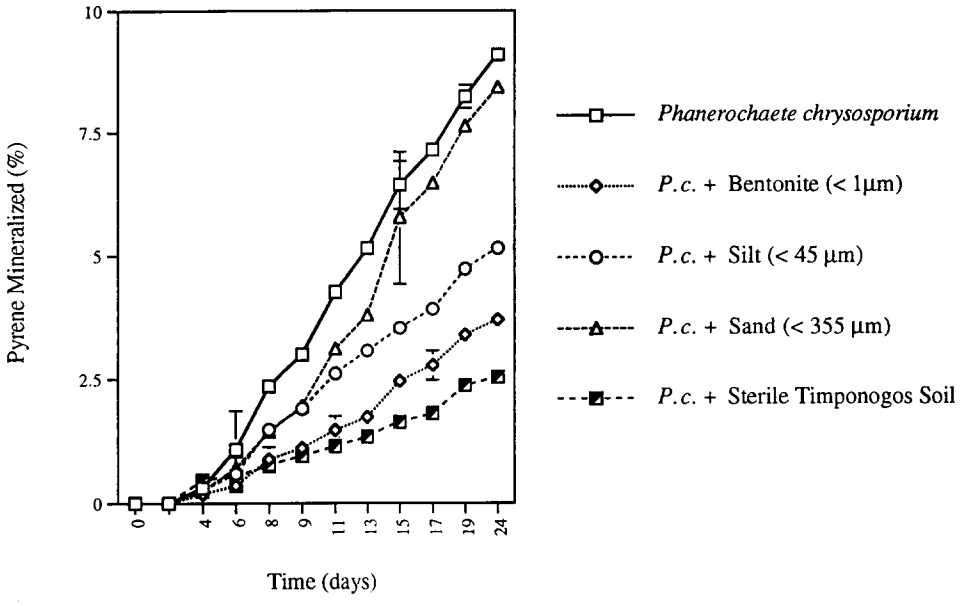

Fig. 5. Effects on pyrene mineralization of a layer of sterilized sand, silt or clay between the inoculum of *P. chrysosporium* and ^{14}C-pyrene. Data are from two separate experiments. *P. chrysosporium*, pregrown on ground corn cobs, was placed directly upon the pyrene-contaminated water agar surface or on top of an overlay of sterilized bentonite, silt, sand, or sterile Timpanogos soil, as described in procedures.

was similar to control studies where the corn cob inoculum was placed directly upon the agar surface. With silt, the mineralization was depressed. Clay caused an even stronger inhibition in mineralization.

5.1. Effects of bacteria from the native soil

Bacteria of different appearance on King's medium B were isolated from the Timpanogos native soil. No bacteria were isolated from the heat sterilized soil. Certain bacteria strongly inhibited the growth of the fungus (Table 3). Some but not all of the antagonists produce fluorescent green pigments on King's medium B indicative of fluorescent pseudomonads (Table 3).

These bacteria differentially influenced the extent of decolorization of Poly R-478 (Table 3). The antagonistic isolates limited decolorization to the zone occupied by the fungal mycelium. Isolate 7 totally prevented decolorization although there was no antagonism of growth of the fungus. None of the bacteria alone caused decolorization of the dye.

Three of the of the fluorescent pseudomonads tested were able to grow on the cold water leachate from the corn cobs. Data from one of the isolates, antagonistic isolate 11, are shown in Table 4.

Table 3
Effect of bacterial isolates from Timpanogos soil on decolorization of Poly R-478 by *P. chrysosporium*, and growth of the fungus

Bacterial isolate	Fluorescence of bacteria	Antagonism at 7 days	Decolorization 14 days
1	F	+	I
8	F	+	I
2	F	+ +	I
11	F	+ +	I
13	F	+ + +	I
5	F	−	C
6	F	−	C
7	F	−	—
12	F	−	C
15	F	−	C
3	N	−	I
4	N	−	C
9	N	−	C
10	N	−	C
14	N	−	C
Whatman	N	+ + +	I
none	NA	NA	C

Bacterial isolates and *P. chrysosporium* were inoculated onto PDA amended with Poly R-478 as described in procedures. Plates were maintained at 26 °C. Fluorescence of bacteria on King's medium B plates is recorded as F for yellow–green fluorescence and N for no fluorescence. Antagonism was recorded on the scale of + + + for zones of growth inhibition of *P. chrysosporium* of 2 cm, + + for inhibition of 1 cm and + for inhibition of 0.5 cm or − for no antagonism. Decolorization was scored C for complete, I as incomplete and − as none. Data are the results of two separate studies.
NA = not appropriate.

Table 4
Growth of fluorescent pseudomonads on extracts from corn cobs

Medium pH	Cell density A 600 nm		
	day 1	day 2	day 3
5	0.08	0.1	0.13
6	0.65	1.1	0.85
7	0.63	1.0	0.89

A fluorescent pseudomonad (isolate 11) with antagonism against *P. chrysosporium* was inoculated into corn cob leachate prepared by cold water extraction. The pH of the leachate was adjusted to pH 5, 6 or 7 prior to inoculation.

6. Discussion

Our findings on solid medium support previous studies in liquid culture [11, 12, 37] that dye decolorization is an effective measure of lignin peroxidase activity during

growth of *P. chrysosporium*. The similarity of the pH optima of dye decolorization and veratryl alcohol oxidase activity of purified isozyme H1 suggest that the dye functions in the complex redox reactions catalyzed by the peroxidase enzymes. This concept is supported by the observed prevention by the dye of the detection of veratryl alcohol oxidation. This effect may be similar to the action of EDTA which is proposed to act as an electron donor in cycling the veratryl alcohol cation radical back to the alcohol and preventing aldehyde formation [38]. Physiologically we find decolorization of Poly R-478 only occurs under growth at low but not high nitrogen, conditions under which the fungus produces lignin peroxidases [17].

Using the dye Poly R-478 we observe that decolorization is coincident with pyrene mineralization on solid, low nitrogen medium by *P. chrysosporium*. Also because no decolorization or mineralization occurred with growth on high nitrogen medium,we suggest that mineralization of pyrene requires lignin peroxidase as a rate limiting first step. This possibility is supported by the observation that isolated lignin peroxidase will oxidize pyrene [11].

Native soil inhibited both the decolorization of Poly R-478 and pyrene mineralization by *P. chrysosporium*. Sterilization of the soil, a process that destroys the indigenous microflora, partially reduced the inhibition in mineralization caused by the soil layer and permitted greater growth of the mycelium of *P. chrysosporium*. The native soil had an abundant microflora. Certain bacteria from the Timpanogos soil were strongly inhibitory to the growth of the fungus in vitro and effected the pattern and rate of decolorization of Poly R-478 by *P. chrysosporium*. Isolates that prevented complete decolorization included fluorescent pseudomonads. Inhibition of fungal growth may be related to the production of antibiotics by the bacteria, a phenomenon previously observed [25]. Competition for nutrients may be another contributing factor [39]. The reduced rate of decolorization may correlate with growth inhibition of the fungus or to effects on the activity of the ligninase complex. *P. chrysosporium* produces extracellularly a complex system of metabolites (hydrogen peroxide, veratryl alcohol and oxalate) required for the ligninase system to function [17]. We speculate that any removal of these components by the bacteria [39] would limit peroxidase activity.

Successful bioremediation by *P. chrysosporium* in soil is dependent upon carbon amendments [7, 21–23]. We propose that certain amendments could also act in the soil as nutritional sources for bacteria with potential to inhibit *P. chrysosporium*. We find that leachates from our carbon amendment, corn cobs, permitted growth of bacteria isolated from the Timpanogos soil. Whether other amendments, such as wood chips or sawdust, support growth of potential antagonistic awaits determination. It is possible that the phenolics present in these materials would have antibacterial activity.

In addition to the microbial component, the soil composition could modify the effectiveness of *P. chrysosporium* in pollutant degradation. The inhibitory Timpanogos soil has a high silt and clay content. We find depression of pyrene mineralization by *P. chrysosporium* by overlays of clay and silt particles but not by sand. Partitioning of the pyrene into the clay may be involved in reducing availability to the fungus [40, 41]. Altered fungal growth in clays has been observed in other systems and

has been related to several factors [39]. For example, modification of pH [39] may be an important feature in restricting general metabolism and more specifically reducing ligninase activity because the enzyme has an acidic pH optimum. Our findings that heat sterilization of the soil did not fully restore the potential of *P. chrysosporium* to minimalize pyrene are consistent with abiotic factors, stemming from the soil composition, playing a significant role in controlling the degree of mineralization.

It is interesting that the native soil alone increases in its potential to degrade pyrene with time. These data are consistent with observations that pyrene can be transformed by microbes present in the soil [18–20]. The reduced rate of degradation in the native soil amended with *P. chrysosporium* compared with native soil without the fungal inoculum suggests that *P. chrysosporium* may exert effects on the soil indigenous microbes.

6. Conclusions

The effectiveness of bioremediation in field soils by *P. chrysosporium* may be suppressed by biotic and abiotic features of the soil. The ability of indigenous soil microbes to antagonize the growth and/or the function of the ligninase complex is a possibility. The physical components, e.g. the clays or silt composition, of the soil structure could also be inhibitory. Examination of soils which permit successful bioremediation with *P. chrysosporium* will indicate whether these problems existed in the soils. If the soils possess antagonists and/or have a high clay or silt composition, the success of bioremediation suggests that under certain circumstances the fungus can negate their effects.

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