World J Microbiol Biotechnol (2010) 26:205–211
DOI 10.1007/s11274-009-0161-9

ORIGINAL PAPER

# Influence of cultivation conditions on pyrene degradation by the fungus *Pleurotus Ostreatus* D1

**Natalia N. Pozdnyakova · Svetlana V. Nikiforova · Oleg E. Makarov · Marina P. Chernyshova · Kirill E. Pankin · Olga V. Turkovskaya**

Received: 26 January 2009 / Accepted: 20 August 2009 / Published online: 4 September 2009


**Abstract** For the first time the dependence of completeness of pyrene degradation by the white-rot fungus *Pleurotus ostreatus* D1 on cultivation conditions was found. In Kirk's medium about 65.6 ± 0.9% of the initial pyrene was metabolized after 3 weeks, with pyrene-4,5-dihydrodiol accumulating. This process was accompanied by laccase production only. In basidiomycetes rich medium, *P. ostreatus* D1 metabolized up to 89.8 ± 2.3% of pyrene within 3 weeks without pyrene-4,5-dihydrodiol accumulation throughout the time of cultivation. Phenanthrene and phthalic acid were identified as the metabolites produced from pyrene degradation under these conditions. Accumulation of phenanthrene with its subsequent disappearance was observed. One more metabolite probably was the product of phenanthrene degradation. Pyrene metabolism in basidiomycetes rich medium was accompanied first by laccase and tyrosinase production and later by versatile peroxidase production. The cell-associated activities of laccase, tyrosinase, and versatile peroxidase were found. The data obtained indicate that both enzymes (laccase and versatile peroxidase) are necessary for complete degradation of pyrene. Furthermore, both cell-associated and extracellular laccases can catalyse the first stages of pyrene degradation, and versatile peroxidase can be necessary for oxidation of the resulting metabolites.

N. N. Pozdnyakova (✉) · S. V. Nikiforova · O. E. Makarov · M. P. Chernyshova · K. E. Pankin · O. V. Turkovskaya
Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prospect Entuziastov, 410049 Saratov, Russia
e-mail: nataliapozdnyakova@yahoo.com

**Keywords** Biodegradation · Laccase · *Pleurotus ostreatus* D1 · Pyrene · Versatile peroxidase

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are components of coal tar, creosote, and crude oil and are formed by the incomplete combustion of organic material as a result of industrial and natural processes (Cerniglia 1992). With increasing molecular weight of PAHs, the solubility of these compounds decreases, this determines the possibility of their accumulation in the environment (Cerniglia 1992). Fluoranthene and pyrene are the primary four-ring PAHs present in creosote, and they have been found at many PAH-contaminated sites (Cerniglia 1992).

Several white-rot fungi, including *Irpex lacteus*, *Trametes versicolor*, *Phanerochaete chrysosporium*, and *Pleurotus ostreatus* can mineralize pyrene partially, with most of it being converted to hydroxylated soluble products (Song 1999). Yet, there is limited information about the metabolic pathways of pyrene degradation by white-rot fungi. The main metabolite of pyrene degradation by *P. ostreatus*, *Agrocybe aegerita*, and *Kuehneromyces mutabilis* was found to be pyrene-4,5-dihydrodiol (Bezalel et al. 1996b; Field et al. 1992; Song 1999).

Two most probable mechanisms of PAHs degradation have been discussed: one uses cytochrome *P*-450 (Bezalel et al. 1997) and the other uses extracellular ligninolytic enzymes (Bogan and Lamar 1996). Many authors believe that white-rot fungi metabolize PAHs through the extracellular ligninolytic enzymes, including lignin peroxidases, Mn-peroxidase, versatile peroxidase, and laccase. The precise role of these enzymes in PAH degradation has not yet been determined; however, it was shown that only

laccase-producing fungi can mineralize PAHs to $CO_2$ and $H_2O$ (Bogan and Lamar 1996; Field et al. 1992).

Pyrene is a substrate for all the three ligninolytic enzymes. The lignin peroxidase of *Ph. chrysosporium* oxidizes pyrene with the formation of pyrene-1,6- and pyrene-1,8-diols (Hammel et al. 1986). The oxidation of pyrene by Mn-peroxidase is coupled with lipid peroxidation (Sack et al. 1997). Pyrene was shown to be oxidized by *T. versicolor* laccase in the presence of 1-hydroxybenzotriazole (Majcherczyk et al. 1998) and by *Coriolus hirsutus* and *P. ostreatus* D1 laccases in the presence of ABTS (Cho et al. 2002; Pozdnyakova et al. 2006).

Current studies of PAHs degradation by white-rot fungi are directed to investigating in detail the metabolic pathways and the enzymes involved in them. The aim of this work was to study the influence of cultivation conditions on pyrene degradation by the fungus *Pleurotus ostreatus* D1.

## Materials and methods

### Chemicals

Pyrene, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,6-dimethoxyphenol (DMOP) were from Fluka (Switzerland). Polyoxyethylene sorbitol monooleate (Tween-80) and acetonitrile were from Sigma–Aldrich (Germany). All the other chemicals were from Reachim Co. (Russia).

### Organism and culture conditions

*Pleurotus ostreatus* D1 was obtained from the Laboratory of Microbiology and Mycology of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (Nikitina et al. 2003). The fungus was grown at 29°C in basidiomycetes rich medium or in Kirk's medium with our modifications. The composition of basidiomycetes rich medium (pH 6.0) was as follows (g/l): $NH_4NO_3$, 0.724; $KH_2PO_4$, 1.0; $MgSO_4{\cdot}7H_2O$, 1.0; KCl, 0.5; yeast extract, 0.5; $FeSO_4{\cdot}7H_2O$, 0.001; $ZnSO_4{\cdot}7H_2O$, 0.0028; $CaCl_2{\cdot}2H_2O$, 0.033; D-glucose, 10.0; peptone, 10.0 (Bezalel et al. 1997). The composition of Kirk's medium was as follows (g/l): $KH_2PO_4$, 0.2; $MgSO_4 \times 7H_2O$, 0.05; $CaCl_2$, 0.01; thiamine, 0.0025; $NH_4NO_3$, 0.724; D-glucose, 10.0 (Kirk et al. 1986). pH (6.0) of Kirk's medium was supported by 50 mM NaK-phosphate. Pyrene was added on day 2 of cultivation as a chloroform solution to a final concentration of 50 mg/l (100 µl of stock solution per Erlenmeyer flask). Cultivation was done at 29°C with shaking (150 rev/min). Residual pyrene and its degradation products were extracted from the acidified cultivation medium (pH 2.0) by ethyl acetate (equal volume, three times). Resulting extracts were evaporated to 500 µl. Three experiments for each cultivation medium were run separately, and each experiment was performed in triplicate. A control (pyrene-free, containing 100 µl of chloroform) was added to each experiment.

### Pyrene degradation by intact mycelium

Mycelium was separated from the culture medium by filtration after cultivation with or without pyrene. The mycelium was washed several times with 50 mM phosphate buffer (pH 6.0) until no enzyme activity was detectable in the washing buffer. Then, the mycelium was incubated in the same buffer with 20 µM pyrene for 10 days at 29°C. Pyrene and its degradation products were extracted as described above. Control samples were prepared identically, except that the cells had been inactivated by boiling for 20 min before pyrene addition. All experiments were done in triplicate.

### Pyrene degradation by crude enzymes

As a source of extracellular enzymes, the cultivation medium was used. Experiments with crude laccase (1 U/ml) were done in 50 mM phosphate buffer (pH 6.0) containing 1% (v/v) acetonitrile and 20 µM pyrene. Experiments with versatile peroxidase (1 U/ml) were done in the same buffer containing 1% (v/v) acetonitrile, 20 µM pyrene, with or without 100 µM $MnSO_4$, and 200 µM $H_2O_2$, with or without 2 mM Tween-80. The tubes were incubated for 10 days at 29°C. Pyrene and its degradation products were extracted as above. Controls were prepared identically, except that the enzymes had been inactivated by boiling for 20 min before pyrene addition. All experiments were run in triplicate.

### Measurement of pyrene and its degradation products

Pyrene and its degradation products were analysed with an HPLC system (GPC, Laboratorni Přistroje Praha, Czech Republic) at isocratic elution (3 ml/min; acetonitrile:$H_2O$, 70:30, v/v), by using with a UV-detector at 254 nm. A Supelcosil$^{TM}$ LC-PAH (5 cm × 4.6 mm, 3 µm) column was used. The sample volume was 20 µl.

Thin-layer chromatography (TLC) was performed with a benzene:ethanol (9:1, v/v) solvent system and Silufol UV-254 plates ("Kavalier", Czech Republic).

GC-MS analyses were performed with a Termo Finifan "Trace DSQ" device (ThermoElectron Corp., Austin, USA) equipped with a turbo-molecular pump, with ionization by electron impact and with an MS library (NIST-02 and Wiley). Compounds were separated by using an RT-FAME capillary column (30 m × 0.25 mm ID; 0.25-µm film

thickness) and helium as the carrier gas. The column temperature was kept at 50°C for 3 min, programmed to 280°C at a rate of 20°C $min^{-1}$, and kept at 280°C for 10 min. The mass spectrometer was operated at an electron ionization energy of 70 eV. The injector and analyser temperatures were set at 250 and 280°C, respectively.

### Enzyme activity measurements

Laccase activity was measured by the oxidation rate of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) at 436 nm ($\varepsilon = 29{,}300\ M^{-1}\ cm^{-1}$) according to Niku-Paavola et al. (1988). Versatile peroxidase was measured by the oxidation rate of 2,6-dimethoxyphenol (DMOP) with $H_2O_2$ and without $Mn^{2+}$ at 468 nm ($\varepsilon = 14{,}800\ M^{-1}\ cm^{-1}$) according to Heinfling et al. (1998). Peroxidase activity was calculated as the difference between the values for DMOP oxidation with and without $H_2O_2$. Tyrosinase was measured by the oxidation rate of L-dihydroxyphenylalanine (L-DOPA) at 475 nm ($\varepsilon = 3{,}700\ M^{-1}cm^{-1}$) according to Pomerantz and Murthy (1974). One unit of enzyme activity (U/ml) is defined as the amount of enzyme that oxidizes 1 μM of substrate per min.

### Emulsifying activity measurements

Emulsifier activity was measured according to Cooper and Goldenberg (1987) by adding 6 ml of kerosene to 4 ml of aqueous sample and vortexing at high speed for 2 min. Measurements were made 48 h later. The emulsion index ($E_{48}$) is the height of the emulsion layer, divided by the total height, multiplied by 100.

## Results and discussion

### Pyrene degradation under fungal-growth conditions in Kirk's medium

At the first stage, pyrene degradation was studied in Kirk's medium, which is frequently used for xenobiotic degradation by white-rot fungi (Bumpus 1989; Dhawale et al. 1992; Morgan et al. 1991). Under these conditions, *P. ostreatus* D1 degraded pyrene by 46.0 ± 0.9% over 14 days. The first stages gave rise to toxic metabolites inhibiting fungal growth (the mycelium dry weight was less then 50% of the pyrene-free control). These metabolites were then degraded, and the mycelium weight observed in the experiment became the same as that in the pyrene-free control. Under these conditions, about 65.6 ± 0.9% of the initial pyrene was metabolized after 3 weeks of cultivation (Fig. 1).

During pyrene degradation, the cultivation medium became yellow. This color remained throughout cultivation, probably because of accumulation of quinone metabolites. Literature data indicate that white-rot fungi metabolize PAHs to corresponding quinones, which color the cultivation medium yellow (Dhawale et al. 1992; Lambert et al. 1994). We found that the yellow color of the cultivation medium disappeared after dithionite addition, probably as a result of quinone reduction. The same results were obtained by Hammel et al. (1986), who showed that dihydrodiol is the main product of pyrene oxidation by lignin peroxidase from *Ph. chrysosporium* and that the yellow color disappears after dithionite addition.

Thin-layer chromatography (TLC) showed the presence of several metabolites, one of which was the main metabolite, which was present in the medium throughout cultivation. This metabolite had an $R_f$ of 0.94, as found by TLC, and a retention time of 14 min, as found by HPLC. Its absorption spectrum differed markedly from that of the parent substance. The typical pyrene peaks at 260, 272, 304, 318, and 334 nm disappeared and the peaks at 256, 295, and 347 nm appeared, which, according to the data of some authors, are presented in the absorption spectrum of pyrene-4,5-dihydrodiol (Lange et al. 1994). GC–MS revealed a molecular ion at *m/z* 236 ($M^+$) and fragment ions at *m/z* 218 ($M^+$-$H_2O$), 189 ($M^+$-$H_2O$-CHO), 176 ($M^+$-$CH_2C(OH)_2$), and 94 ($M_{189}{}^{++}$). Comparison of the data obtained with the MS data of well-known pyrene metabolites (Bezalel et al. 1996b; Lange et al. 1994) permitted us to identify this product as pyrene-4,5-dihydrodiol. According to the data of different authors, pyrene-4,5-dihydrodiol is the main metabolite of pyrene degradation by white-rot fungi (Field et al. 1992; Sack et al. 1997).

Studying the time course of pyrene degradation by *P. ostreatus* D1 under these conditions showed that pyrene-4,5-dihydrodiol was the main metabolite, which

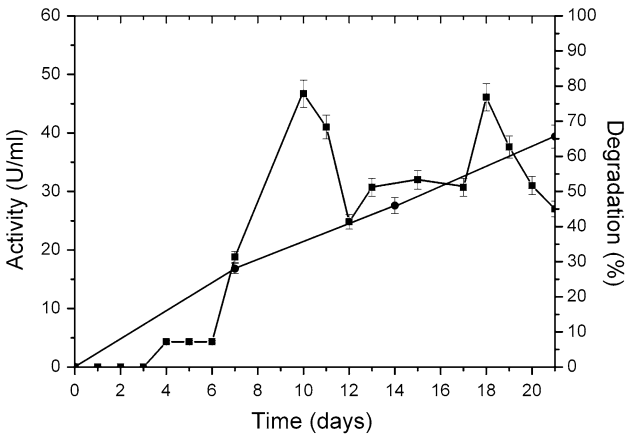



**Fig. 1** Pyrene degradation (●) and laccase production (■) by *P. ostreatus* D1 in Kirk's medium

accumulated throughout cultivation. The presence of additional spots on a thin-layer chromatogram ($R_f = 0.47$, 0.36, 0.28, and 0.24), which were absent during the first days of cultivation, suggested that pyrene-4,5-dihydrodiol could be metabolized further. Unfortunately, under the conditions used by us, these products were formed in amounts insufficient for their isolation and identification.

PAHs degradation by white-rot fungi is connected with ligninolytic enzyme production (Moen and Hammel 1994). We studied extracellular enzyme production during cultivation of *P. ostreatus* D1 in the presence of pyrene under these conditions. Pyrene and/or its degradation products induced only laccase. Laccase was produced throughout cultivation, reaching the maximum on days 10 and 19 (Fig. 1).

Based on the data obtained, we assume that pyrene-4, 5-dihydrodiol accumulation may have resulted from the absence of ligninolytic peroxidases in the cultivation medium. In this case, in the presence of ligninolytic peroxidases, the pyrene degradation should be more complete. For verification of this hypothesis, basidiomycetes rich medium, supporting peroxidase production by *P. ostreatus* (Martinez et al. 1996), was used.

## Pyrene degradation under fungal-growth conditions in basidiomycetes rich medium

The first stages of pyrene degradation during growth of *P. ostreatus* D1 gave rise to toxic metabolites inhibiting fungal growth (the mycelium dry weight was about 53% of the pyrene-free control). These metabolites were then degraded, and the mycelial weight observed in the experiment became the same as that in the pyrene-free control. Under these conditions, about 89.8 ± 0.8% of the initial pyrene was metabolized after 3 weeks of cultivation.

Our data agree with the results of Bezalel et al. (1996a), showing that under the same conditions, another *P. ostreatus* strain metabolized about 91% of pyrene and mineralized 0.4% of pyrene. However, in contrast to those authors, we did not find pyrene-4,5-dihydrodiol at any point in cultivation. TLC revealed five products of pyrene degradation by *P. ostreatus* D1. GC-MS of the product with an $R_f$ of 0.86 (retention time, 11.23 min) revealed a molecular ion at *m/z* 177($M^+$) and fragment ions at *m/z* 175 ($M^+$-2H), 151 ($M^+$-$C_2H_2$), 75 ($M_{151}{}^{++}$), and 88 ($M^{++}$). These data suggest the presence in this compound of a three-ring phenanthrene structure. The identity of the $R_f$ value, the UV-absorption spectrum, and GC-MS retention time for this metabolite to those of authentic phenanthrene (GC-MS retention time 11.235 min) support this hypothesis.

The time course of phenanthrene appearance and disappearance was studied (Fig. 2). The maximal quantity of phenanthrene was found on day 7 of cultivation. After that,

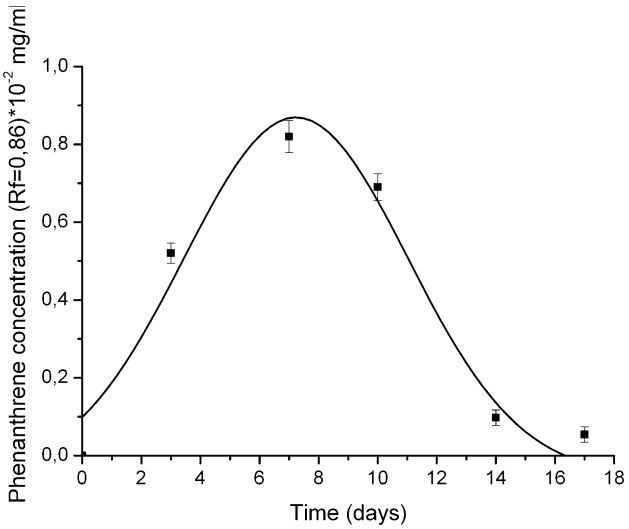
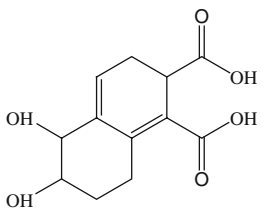



**Fig. 2** Time course of appearance and disappearance of the phenanthrene metabolite during pyrene degradation in basidiomycetes rich medium

it disappeared completely, and as a result of subsequent degradation, another metabolite was found that had a GC-retention time of 11.98 min. GC-MS data and the proposed structure of this product are shown in Fig. 3.

The third metabolite was isolated by preparative TLC ($R_f = 0.23$). Comparison of the Rf value and GC-retention time (8.48 min) of this product with those of the authentic standard permitted us to identify it as phthalic acid. The GC-MS retention time of authentic phthalic acid was 8.475 min. Studies of phthalic acid degradation under different conditions (Kirk's medium and basidiomycetes rich medium) showed that this compound can be metabolized by *P. ostreatus* independently of the cultivation conditions used. About 65.6 ± 1.8 and 92.4 ± 0.11% of the parent phthalic acid was metabolized during 14 days in Kirk's medium and basidiomycetes rich medium, respectively.

During shake cultivation of *P. ostreatus* D1 in the presence of pyrene, foam production was observed. Our data agree with the results of Song (1999), who showed foam production during cultivation of *Ph. chrysosporium* in the presence of pyrene. The culture-liquid emulsion index ($E_{48}$) obtained in the presence of pyrene exceeded that in the pyrene-free control by more than three times.




Molecular ion at *m/z* 254($M^+$) and fragment ions at *m/z* 253 ($M^{+\bullet}$-H), 235 ($M^{+\bullet}$-$H_2O$), 222 ($M^{+\bullet}$-$CH_2OH$), 204 ($M^{+\bullet}$-$C_4H_8O_3$), 169, 153 ($M^{+\bullet}$-$CH_3O$-$C_4H_5O$), 148($M^{+\bullet}$-$C_4H_8O_3$), 69, and 55

**Fig. 3** The proposed structure of the metabolite with a retention time of 11.98 min

We speculate that foam formation by *P. ostreatus* D1 results from surfactant formation and that this surfactant is responsible for pyrene solubilization in an aqueous medium.

We also studied extracellular enzyme production during pyrene degradation by *P. ostreatus* D1 under these conditions. In basidiomycetes rich medium, pyrene and/or its degradation products induced both laccase and versatile peroxidase. Laccase was produced throughout cultivation, reaching the maximum on days 3 to 4. The data obtained contradict the results of Bezalel et al. (1996a), who showed that under the same conditions, laccase production by a different strain of *P. ostreatus* was independent of the presence of pyrene (Bezalel et al. 1996a). Versatile peroxidase production started after day 7, when about 10% pyrene was determined in the cultivation medium (Fig. 4). Apparently, the pyrene-degradation metabolites induce versatile peroxidase production, which, in part, can be involved in the last steps of pyrene degradation. The involvement of Mn-peroxidase in PAHs degradation by *Agrocybe praecox* and *Stropharia coronilla* was found earlier by Steffen et al. (2003), who speculated that Mn-peroxidase could catalyse some stages resulting in mineralization of these compounds. However, no literature data are available on the involvement of versatile peroxidase in PAH degradation by white-rot fungi.

Furthermore, we found that besides laccase and versatile peroxidase, *P. ostreatus* D1 produced some tyrosinase during cultivation in the presence of pyrene (Fig. 4). This finding was unexpected because we had not found any literature data on tyrosinase production during PAH degradation.

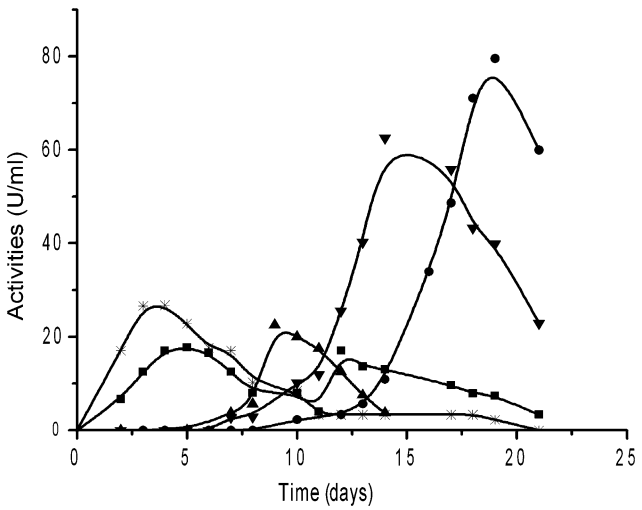



**Fig. 4** Extracellular enzymes production during pyrene degradation by *P. ostreatus* D1: ■, laccase (control); *, laccase (with pyrene); ●, versatile peroxidase (control); ▼, versatile peroxidase (with pyrene); ▲, tyrosinase (with pyrene)

## Pyrene degradation in the presence of tyrosine

The involvement of tyrosinase in pentachlorophenol degradation by *Amylomyces rouxii*, in the absence of lignin peroxidase in the cultivation medium, was found by Montiel et al. (2004). They showed that *A. rouxii* metabolized up to 85% of pentachlorophenol in the presence of tyrosine and about 50% of it without tyrosine in the cultivation medium.

We assumed that tyrosinase production by *P. ostreatus* D1 in the presence of pyrene could also be involved in PAHs degradation by this fungus. Within 14 days of cultivation, the fungus metabolized about 79.4 ± 6.5% of pyrene, whereas in the presence of 0.1 M tyrosine the metabolization averaged 76.1 ± 4.2%. The presence of tyrosine in the cultivation medium did not influence fungal growth or pyrene degradation. The data obtained do not allow a conclusion to be drawn about the involvement of tyrosinase in pyrene degradation by *P. ostreatus* D1.

## Pyrene degradation by crude enzymes

Studies of the role of extracellular enzymes in the metabolism of xenobiotics usually begin with an investigation of degradation of these compounds by the culture medium, which is a crude enzyme preparation. In such studies, Bezalel et al. (1996a) showed that a laccase-containing culture liquid did not oxidize PAHs and assumed that extracellular enzymes are not involved in the initial attack on PAHs (Bezalel et al. 1996a). However, subsequent work by Sack et al. (1997) showed that a crude Mn-peroxidase of *Nematoloma frowardii* completely oxidized pyrene in the presence of glutathione. Almost complete removal of pyrene by a crude Mn-peroxidase from *Stropharia coronilla* in the presence of Tween-80 was shown by Steffen et al. (2003).

We studied pyrene degradation by the crude enzymes of *P. ostreatus* D1. The pyrene decrease was 74.2 ± 2.7% when crude laccase was incubated with 20 μM pyrene. GC-MS of the product of oxidation (retention time 12.26 min) showed a molecular ion at *m/z* 218 ($M^+$) and fragment ions at *m/z* 188 ($M^{+\cdot}$-$CH_2O$), and 176 ($M^{+\cdot}$-$CH_2CO$). Comparison of these results with literature data (Lange et al. 1994) permitted us to identify this product as a pyrene-4,5-oxide.

Crude versatile peroxidase oxidized pyrene with or without $Mn^{2+}$. It reduced the pyrene concentration by 34 ± 3.5% in the presence of $H_2O_2$. Addition of $Mn^{2+}$ to the reaction mixture slightly increased the oxidation. Addition of Tween-80 resulted in a 2.5-fold increase in pyrene removal (83.5 ± 0.7%). Our data agree with the findings of Moen and Hammel (1994) and Bogan and Lamar (1995), who showed increases in Mn-peroxidase

activity in the presence of Tween-80 and suggested that PAHs oxidation and unsaturated hydrocarbon chain peroxidation are coupled reactions.

### Pyrene degradation by an intact mycelium

The involvement of different enzyme groups in PAH degradation by white-rot fungi remains little studied. In our opinion, current efforts do not account for one more enzyme pool, including cell-associated laccases. Information on these laccases and on their possible functions is scarce (Schlosser et al. 1997). We assume that cell-associated laccase can be involved in the initial attack on the PAH molecule.

We studied the activity of intact mycelium of *P. ostreatus* D1. The mycelium obtained by fungal cultivation in a pyrene-free medium contained cell-associated laccase [1.24 U/(g moist mycelium wt)], but that obtained in a pyrene-containing medium had 3.4 times more cell-associated laccase. Besides cell-associated laccase, we found the activities of cell-associated versatile peroxidase and tyrosinase. Cultivation of the fungus in the pyrene-containing medium resulted in increases in cell-associated enzyme activities: a doubling for versatile peroxidase and by 1.5 times for tyrosinase. Pyrene incubation with washed mycelium resulted in oxidation of 37.2 ± 2.8% of pyrene. TLC showed the presence of several degradation products, but their concentrations were insufficient for isolation and identification purposes.

## Conclusions

For the first time the dependence of the completeness of pyrene degradation by the white-rot fungus *Pleurotus ostreatus* D1 on cultivation conditions was found. Our data, alone and when compared with the literature findings, suggest that the initial attack on the pyrene molecule may be catalyzed by cell-associated enzymes (at least by laccase), because some time is required for the extracellular enzymes to appear in the culture medium at concentrations sufficient for substrate degradation. Apparently, both cell-associated and extracellular laccases can catalyse the first stages of pyrene degradation resulting in pyrene-4,5-dihydrodiol formation. Cultivation under conditions of laccase production only resulted in accumulation of this product. In basidiomycetes rich medium, when both ligninolytic enzymes were produced, pyrene degradation was complete, with phenanthrene and phthalic acid being formed as intermediates. Phthalic acid, in turn, can be involved in basal metabolism. Versatile peroxidase production under these conditions is involved in oxidation of the metabolites being formed. Biosurfactant production late in pyrene degradation may indirectly corroborate the involvement of versatile peroxidase in degradation of this xenobiotic. To our knowledge, this is the first report on the involvement of versatile peroxidase in PAHs degradation by white-rot fungi. The data presented here give a new insight into the main reactions involved in degradation of high-molecular-weight PAHs.

**Acknowledgments** We thank Dr. V. E. Nikitina for kindly providing us with *P. ostreatus* D1. We are grateful to Dmitry N. Tychinin for his assistance in preparation of the English text of this paper. This work was supported by the federal target-oriented programme "Issledovaniya I razrabotki po prioritetnym napravleniyam razvitiya nauchno-tekhnologicheskogo kompleksa Rossii na 2007–2012 gody" (Research and Developments on Priority Directions in the Development of Russian's Science and Technology Complex for 2007–2012)—State contract no. 02.512.11.2210.

## References

Bezalel L, Hadar Y, Cerniglia C (1996a) Mineralization of polycyclic aromatic hydrocarbons by the fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62:292–295

Bezalel L, Hadar Y, Fu PP, Freeman JP, Cerniglia CE (1996b) Initial oxidation products in the metabolism of pyrene, anthracene, fluorine, and dibenzothiophene by the white-rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62:2554–2559

Bezalel L, Hadar Y, Cerniglia CE (1997) Enzymatic mechanism involved in phenanthrene degradation by the white-rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 63:2495–2501

Bogan BW, Lamar RT (1995) One-electron oxidation in the degradation of creosote polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl Environ Microbiol 61:2631–2636

Bogan B, Lamar RT (1996) Polycyclic aromatic hydrocarbon-degrading capabilities of *Phanerochaete laevis* HHB-1625 and its extracellular ligninolytic enzymes. Appl Environ Microbiol 62:1597–1603

Bumpus JA (1989) Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl Environ Microbiol 55:154–158

Cerniglia CF (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351–368

Cho S-J, Park SJ, Lim J-S, Rhee YH, Shin K-S (2002) Oxidation of polycyclic aromatic hydrocarbons by laccase of *Coriolus hirsutus*. Biotechnol Lett 24:1337–1340

Cooper DG, Goldenberg BG (1987) Surface-active agents from two *Bacillus* species. Appl Environ Microbiol 53:224–229

Dhawale SW, Dhawale SS, Dean-Ross D (1992) Degradation of phenanthrene by *Phanerochaete chrysosporium* occurs under ligninolytic as well as nonligninolytic conditions. Appl Environ Microbiol 58:3000–3006

Field JA, de Jong E, Costa GF, de Bont JAM (1992) Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white-rot fungi. Appl Environ Microbiol 58:2219–2226

Hammel KE, Kalyanaraman B, Kirk TK (1986) Oxidation of polycyclic aromatic hydrocarbons and dibenzo[p]dioxins by *Phanerochaete chrysosporium* ligninase. J Biol Chem 261: 16948–16952

Heinfling A, Martinez MJ, Martinez AT, Bergbauer M, Szewzyk U (1998) Purification and characterization of peroxidases from dye-decolorizing fungus *Bjerkandera adusta*. FEMS Microbiol Lett 165:43–50

Kirk TK, Croan S, Tien M, Murtagh K, Farrell RL (1986) Production of multiple ligninases by *Phanerochaete chrysosporium* effect of selected growth condition and use mutant strain. Enzyme Microb Technol 8:27–32

Lambert M, Kremer S, Sterner O, Anke H (1994) Metabolism of pyrene by the basidiomycete *Crinipellis stipitaria* and identification of pyrenequinones and their hydroxylated precursors in strain JK375. Appl Environ Microbiol 60:3597–3601

Lange B, Kremer S, Sterner O, Anke H (1994) Pyrene metabolism in *Crinipellis stipitaria*: identification of trans-4, 5-dihydro-4, 5-dihydroxypyrene and 1-pyrenylsulfate in strain JK364. Appl Environ Microbiol 60:3602–3607

Majcherczyk A, Johannes C, Huttermann A (1998) Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of *Trametes versicolor*. Enzyme Microb Technol 22:335–341

Martinez MJ, Ruiz-Duenas FJ, Guillen F, Martinez AT (1996) Purification and catalytic properties of two manganese peroxidase isoenzymes from *Pleurotus eryngii*. Eur J Biochem 237:424–432

Moen MA, Hammel KE (1994) Lipid peroxidation by the manganese peroxidases of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl Environ Microbiol 60:1956–1961

Montiel AM, Fernandez FJ, Marcial J, Soriano J, Barrios-Gonzalez J, Tomasini A (2004) A fungal phenoloxidase (tyrosinase) involved in pentachlorophenol degradation. Biotechnol Lett 26:1353–1357

Morgan P, Lewis T, Watkinson RJ (1991) Comparison of abilities of white-rot fungi to mineralize selected xenobiotic compounds. Appl Microbiol Biotechnol 34:693–696

Nikitina V, Marinina N, Boldyrev V, Ozerov R (2003) Characteristics of some wild-growing strains of veshenka with the purpose of their use in practical fungi-growing. Bull Bot Gard Saratov State Univ 2:169–176 (in Russian)

Niku-Paavola M-L, Karhunen E, Salola P, Paunio V (1988) Ligninolytic enzymes of white-rot fungus *Phlebia radiate*. Biochem J 254:877–884

Pomerantz SH, Murthy VV (1974) Purification and properties of tyrosinases from *Vibrio tyrosinaticus*. Arch Biochem Biophys 160:73–82

Pozdnyakova NN, Rodakiewicz-Nowak J, Turkovskaya OV, Haber J (2006) Oxidative degradation of polyaromatic hydrocarbons catalyzed by blue laccase from *Pleurotus ostreatus* D1 in the presence of synthetic mediators. Enzyme Microb Technol 39:1242–1249

Sack U, Hofrichter M, Fritsche W (1997) Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of *Nematoloma frowardii*. FEMS Microbiol Lett 152:227–234

Schlosser D, Grey R, Fritsche W (1997) Patterns of ligninolytic enzymes in *Trametes versicolor*. Distribution of extra- and intracellular enzyme activities during cultivation on glucose, wheat straw and beech wood. Appl Microbiol Biotechnol 47:412–418

Song H-G (1999) Comparison of pyrene degradation by white-rot fungi. World J Microbiol Biotechnol 15:669–672

Steffen K, Hatakka AI, Hofrichter M (2003) Degradation of benzo[a]pyrene by the litter-decomposing basidiomycete *Stropharia coronilla*: role of manganese peroxidases. Appl Environ Microbiol 69:3957–3964