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Degradation of Fluorene and Fluoranthene by the Basidiomycete *Pleurotus ostreatus*

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Abstract—The dependence of the degree of fluorene and fluoranthene degradation by the fungus *Pleurotus ostreatus* D1 on the culture medium composition has been studied. Polycyclic aromatic hydrocarbons (**PAH**s) have been transformed in Kirk's medium (under conditions of laccase production) with the formation of a quinone metabolite and 9-fluorenone upon the use of fluoranthene and fluorene as substrates, respectively. More complete degradation with the formation of an intermediate metabolite, phthalic acid that has undergone subsequent utilization, has occurred in basidiomycete-rich medium (under the production of both laccase and versatile peroxidase). The formation of phthalic acid as a metabolite of fluoranthene degradation by lignolytic fungi has been revealed for the first time. The data allow the supposition that both extracellular laccase and laccase on the mycelium surface can participate in the initial stages of PAH metabolism, while versatile peroxidase is necessary for the oxidation of the formed metabolites. A scheme of fluorene metabolism by *Pleurotus ostreatus* D1 is suggested.

Keywords: polycyclic aromatic hydrocarbons (PAHs), *Pleurotus ostreatus*, laccase, versatile peroxidase
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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds, the chemical structure of which includes two or more condensed benzene rings. The interest to PAH biodegradation in the environment is connected with its widespread occurrence, resistance to degradation, accumulation in soil and precipitation, and the presence of toxic, mutagenic, and carcinogenic properties [1–3]. The dumping and accidental transfer of these hydrophobic compounds into natural environments cause a serious problem, especially when when the natural capability of microflora for PAH biodegradation is insufficient for their removal or neutralization [2]. Bioremediation is the most efficient method for detoxification of these dangerous pollutants [1]. The development and efficient application of bioremediation technologies requires the comprehensive study of destructive organisms, metabolic pathways of PAH degradation, enzymatic systems that are involved in PAH degradation, and optimal conditions for biodegradation.

Basidiomycetes, which efficiently destruct lignocellulosic substrates, are one of the most active PAH degraders. It has been shown that, in addition to lignin, they can degrade a wide range of stable aromatic compounds, including pesticides, polychlorinated biphenyls, halogenated aromatic compounds, nitro- and amino substituted phenols, trinitrotoluene, synthetic dyes, and PAHs and their complex mixtures, for example, arochlors and creosote [4–6]. According to modern views, efficient degradation of lignin and xenobiotics is provided by the lignin-modifying system, the main component of which is comprised by the enzymes of the lignolytic complex [7–11]. The main lignolytic enzymes are lignin peroxidase (EC 1.11.1.14), Mn-peroxidase (EC 1.11.1.13), versatile peroxidase (EC 1.11.1.16), and laccase (EC 1.10.3.2) [7].

Tricyclic fluorene and tetracyclic fluoranthene, which are toxic, mutagenic, and carcinogenic, are poorly degradable PAHs, and the data on their decomposition are limited [12, 13]. Thus, fluorene degradation has been shown to occur under the action of *Agrocybe* sp. CU-43 [14], *Armillari* sp. F022 [15], *Pleurotus eringii* [12], and a number of other lignolytic fungi. Fluoranthene degradation is a serious problem due to its high hydrophobicity (its solubility does not exceed 0.26 mg/L) and, as a consequence, low bioavailability [3]. Thus, the degrees of its degradation by *Irpex lacteus* and *P. eringii* were 15 and 95%, respec-

tively [13, 16], with the formation of two identified metabolic products: 1-naphthalenecarboxylic and salicylic acids [13].

Consequently, the degradation of these toxicants by lignolytic fungi has remained understudied, especially in the case of metabolism and the role of lignolytic enzymes in the destructive process. We have previously shown that *Pleurotus ostreatus* D1 (Jacquin) P. Kummer metabolizes a number of tetracyclic PAHs (pyrene and chrysene) and actively produces laccase and versatile peroxidase [17, 18].

The purpose of the work is to study the degradation of fluorene and fluoranthene by *Pleurotus ostreatus* D1 with respect to the culture medium composition and to identify degradation products.

METHODS

The work was carried out with the lignolytic fungus *Pleurotus ostreatus* D1 (Jacquin) P. Kummer [19] from the collection of the laboratory of microbiology in the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS). In order to obtain inoculate, the fungus was cultured for 7 days on a rotary shaker at 120 rpm and 26°C in flasks with a volume of 250 mL with 100 mL of modified basidiomycetes rich medium [20] with the following composition (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, $FeSO_4 \cdot 7H_2O$—0.01, $ZnSO_4 \cdot 7H_2O$—0.0028, $CaCl_2 \cdot 2H_2O$—0.033, glucose—10.0, peptone—10.0, pH 6.0.

PAH degradation was studied upon submerged cultivation in basidiomycete-rich medium as described above and in modified Kirk's medium [21] (g/L): KH_2PO_4—2.0, $MgSO_4$—0.348, $CaCl_2 \cdot 2H_2O$—0.143, NH_4NO_3—1.02, microelement solution—10 mL/L, and thiamine—0.5 mL/L. The microelement solution included (g/L): nitrilotriacetate—1.5, $MgSO_4 \cdot 7H_2O$—3.0, $MnSO_4 \cdot H_2O$—0.5, NaCl—1.0, $FeSO_4 \cdot 7H_2O$—0.1, $CoSO_4$—0.1, $CaCl_2$—0.082, $ZnSO_4$—0.1, $CuSO_4 \cdot 5H_2O$—0.01, $AlK(SO_4)_2$—0.01, H_3BO_4—0.01, $NaMoO_4$—0.01. Medium pH was maintained by 25 mM Na-tartaric buffer, pH 4.5, or 25 mM NaK-phosphate buffer, pH 6.0. Maltose (1%) was used as a carbon and energy source. Kirk's medium was supplemented with 0.1% Tween-80 in order to increase PAH solubility [22] and the production of lignolytic enzymes [23].

Media were inoculated with 7-day-old inoculum of the fungus and cultured at 26°C and 120 rpm. PAHs (fluorene or fluoranthene) or the supposed products of their degradation (9-hydroxy fluorene, 9-fluorenone or phthalic acid) were added into flasks in 3 days in 200 μL of chloroform or acetonitrile to a final concentration of 0.05 g/L. Control variants were supplemented with 200 μL of the solvent. Samples were taken in certain time periods (0, 7, 10, 14, and 21 days) in order to determine fungal dry biomass, PAH diminution, and formed metabolites.

The extraction of PAHs and their degradation products from the culture medium (CM) was performed with chloroform and ethyl acetate. PAHs were extracted three times with 5 mL of chloroform; the extracts were joined, evaporated to dryness, and analyzed by gas-liquid chromatography as described below. PAH degradation products were extracted from the CM with 30 mL of ethyl acetate. The procedure was repeated twice. Extracts were joined and evaporated to dryness; metabolites were determined by chromatographic methods (as described below).

In order to study PAH degradation by enzyme preparations, the fungus was cultured in basidiomycete-rich medium until maximum production of laccase (5–6 days) or versatile peroxidase (20 days). Then mycelium and medium were separated by filtration. The CM was collected after 5–6 days of culturing and used as a nonpurified enzyme preparation containing laccase, while the CM in 20 days represented a nonpurified enzyme preparation with the activity of versatile peroxidase. The reaction mixture with laccase preparation (10 U/mL) contained 50 mM phosphate buffer (pH 6.0), 1% acetonitrile, 20 μM 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (**ABTS**), and 20 μM of fluorene, fluoranthene, 9-hydroxy fluorene, 9-fluorenone or phthalic acid. The reaction with the versatile peroxidase preparation (10 U/mL) was performed in the same buffer containing 1% of acetonitrile, 20 μM of PAHs or their metabolites, 100 μM of $MnSO_4$, and 200 μM of H_2O_2. The reaction mixtures were incubated for 10 days at 29°C. Control samples were prepared analogously; however, the enzymes were inactivated by boiling for 10 min prior to the addition of PAHs or their degradation products.

Mycelium was harvested after 5–6 days of culturing and then washed with 50 mM phosphate buffer, pH 6.0, in order to remove extracellular enzymes. One gram of mycelium was incubated in 5 mL of the same buffer with 20 μM of fluorene, fluoranthene, 9-hydroxy fluorene, 9-fluorenone or phthalic acid for 10 days at 29°C. Control samples were prepared analogously; the mycelium was inactivated by boiling for 20 min prior to the addition of PAHs or their degradation products.

GLC and HPLC were performed at the Simbioz core facilities with equipment for physico-chemical biology and nanobiotechnology (IBPPM RAS). GLC was carried out in a Shimadzu 2010 chromatograph (Japan) with a flame photometric detector. Substances were separated in a HP5 column (Agilent, United States) with helium as a carrier gas. A column temperature of 200°C was maintained for 3 min and then elevated to 270°C with a rate of 15°C/min; this temperature was maintained for another 2 min. PAH

degradation products were methylated with CH_3COCl prior to GLC.

HPLC was performed in a Dionex Ultimate 3000 system (Thermo Scientific, United States) with a Luna column (Phenomenex, United States) with C18(2) phase (the average size of particles was 5 μm), 4.6 × 150 mm. Chromatography was performed with isocratic elution. The mobile phase composition was 40% A and 60% B (component A was 100% MeCN, and component B was a solution of 500 μL of 50% H_3PO_4 in 500 mL MilliQ water to pH 2.5), with a flow rate of 0.5 mL/min; a volume of the introduced sample of 5 μL; and a column thermostate temperature of 30°C. Detection was performed with a diode array detector in a wavelength range of 200–400 nm, integration was implemented at 252 nm. Components were identified according to the retention times, which were compared with the corresponding values of standard samples.

TLC of CM extracts was performed in a benzene–ethanol (9 : 1) system. Silufol UV-254 plates with silica gel (Kavalier, Czech Republic) were used. The R_f of reaction products was determined as the ratio between the distance traveled by the substance and the distance travelled by the solvent.

The activity of enzymes was measured spectrophotometrically in an Evolution 60 spectrophotometer (Thermo Scientific, United States). The laccase activity was determined by the formation rate of ABTS oxidation product at 436 nm ($\varepsilon = 29300\ M^{-1}\ cm^{-1}$) [24]. The activity of versatile peroxidase was estimated by the formation of 2,6-dimethoxyphenol oxidation product at 468 nm ($\varepsilon = 27500\ M^{-1}\ cm^{-1}$) in the presence of 0.2 mM H_2O_2 and 0.5 mM $MnCl_2$ [25] and calculated from the difference in activity in the presence and in the absence of H_2O_2. The amount of the enzyme that catalyzes the formation of 1 μmol of the oxidation product per 1 min was taken as an activity unit. The activity of the enzymes was expressed in conditional units – μmol/min/mL of preparation (U/mL).

Vertical disc electrophoresis under nondenaturing conditions was performed according to Laemmli [26]. In order to determine the laccase activity, a gel was stained for 5–10 min with *o*-dianisidine (50 mg of *o*-dianisidine were dissolved in 1 mL of glacial acetic acid and diluted with 50 mL of 50 mM Na-tartaric buffer, pH 4.0). The peroxidase activity was revealed with the same reaction mixture, additionally supplemented with 0.5 mM $MnSO_4$ and 200 μM H_2O_2; the mixture was additionally incubated for 5–7 min.

All variants of the described experiments and analyses were done at least in triplicate. The results were statistically treated with Microsoft Excel 2003 and Origin 7 software.

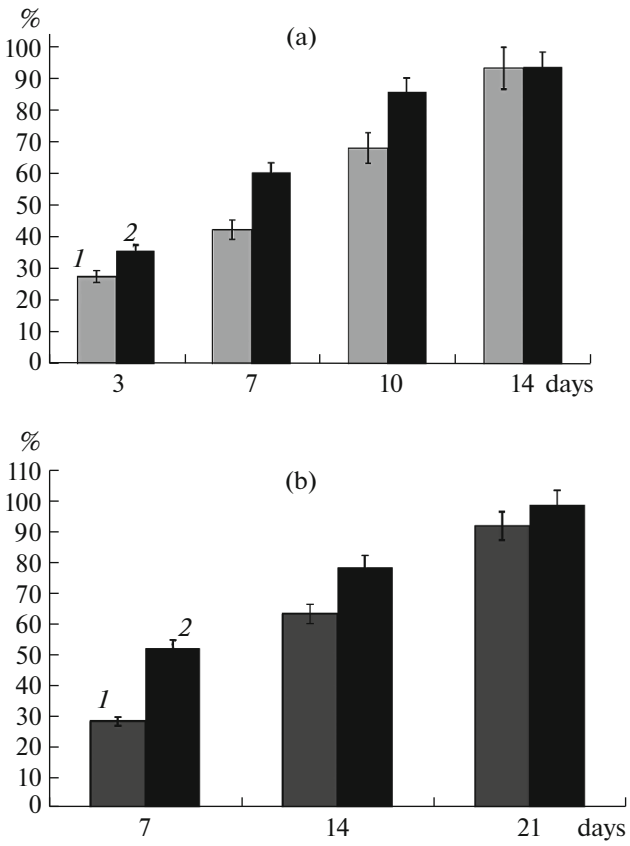

Fig. 1. Degradation (%) of (a) fluorene and (b) fluoranthene upon *P. ostreatus* D1 culturing in Kirk's medium at pH (*1*) 4.5 and (*2*) 6.0.

RESULTS AND DISCUSSION

Fluorene and fluoranthene degradation in Kirk's medium. Fluorene and fluoranthene degradation upon culturing of *P. ostreatus* D1 in Kirk's medium with maltose as the main carbon and energy source occurred in a similar way, regardless of the pH of the medium. Fluorene diminution reached 94% in 14 days of culturing (Fig. 1a). Moreover, both fluorene and its degradation products were nontoxic for the fungus; this was evident from the formation of equal amounts of mycelium of 444.8 and 430 mg in 100 mL of medium in the control variant and in the presence of PAH, respectively.

It should be noted that fluoranthene inhibited growth of *P. ostreatus* D1, because the mycelium mass after a week of culturing was ~42% of the control. However, PAH degradation in this case was high and reached 96% in 3 weeks (Fig. 1b). The sorption of both PAHs on mycelium did not surpass 5%; this is in accordance with our previous data obtained for the degradation of pyrene and chrysene [17, 18].

The CM changed color to yellow in the course of utilization of the studied PAHs by the fungus. The color persisted for the entire experiment and did not

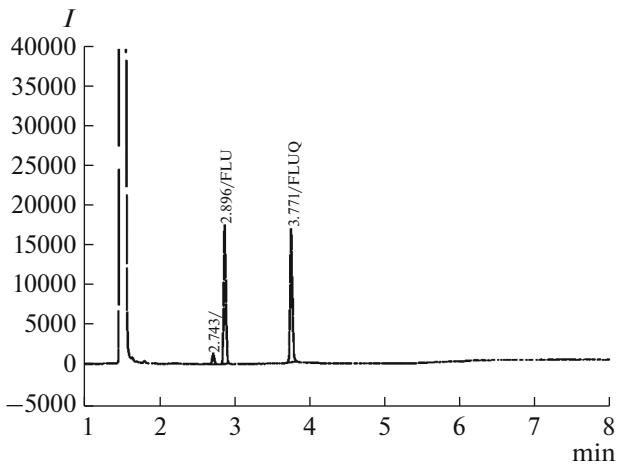

Fig. 2. GLC of *P. ostreatus* D1 culture medium extract after fluorene degradation in Kirk's medium.

disappear upon an increase in culturing time. The yellow color of medium disappeared upon the addition of a reducer dithionite; this indicates the possible presence of quinone intermediates. Similar data have been obtained by Hammel et al. [27] in the study of the degradation of another tetracyclic PAH, pyrene. In this case quinone metabolites also accumulated in medium, and their yellow color disappeared upon the addition of dithionite.

HPLC of *P. ostreatus* D1 CM extracts showed the presence of two peaks of fluorene degradation metabolites with retention times of 1.59 and 3.09 min. The comparison of these data with those for standard compounds allowed their identification as 9-hydroxyfluorene and 9-fluorenone. We should note the practically stoichiometric formation of these metabolites. The presence of 9-fluorenone was also confirmed by GLC (Fig. 2). The same metabolites had been previously found in the course of fluorene degradation by the fungi *P. ostreatus* [28] and *Agrocybe* sp. CU-43 [14].

Chromatographic methods allowed the revelation of the only fluoranthene degradation product by the studied fungus under the used conditions. GLC showed the presence of the main peak (fluoranthene) and an additional peak with a retention time of 5.08 min while TLC revealed a metabolite with $R_f = 0.9$. This metabolite presented in the CM over the course of the entire experimental. It seems that the corresponding quinone is one of the most probable fluoranthene degradation products.

Degradation of the studied PAHs upon the culturing of *P. ostreatus* D1 in Kirk's medium was accompanied by the production of a single lignolytic enzyme—laccase—regardless of the pH of medium (Fig. 3). The control variant (without PAHs) had only trace amounts of this enzyme at pH 4.5; at a pH of 6.0, the laccase activity reached 20 U/mL in 10 days of culturing. Both studied PAHs induced the enzyme in experimental variants: at a pH of 4.5, its activity reached 40 and 100 U/mL in the presence of fluorene and fluoranthene, respectively; at a pH of 6.0, the presence of fluorene, fluoranthene, and/or their degradation products increased the production of the enzyme by 1.8–2.4 times as compared to the control without PAHs (Fig. 3a).

Electrophoresis under nondenaturing conditions revealed the presence of two isoenzymes (isoforms) of laccase at a pH of 4.5 in the presence of both fluorene and fluoranthene. Four isoforms were found at a pH of 6.0, regardless of the PAH used; moreover, two of

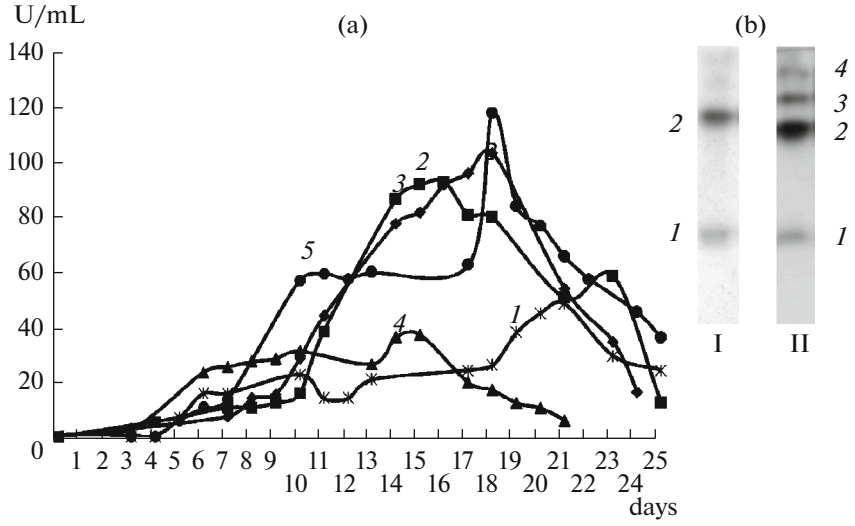

Fig. 3. Laccase production (U/mL) upon *P. ostreatus* D1 culturing in Kirk's medium. (a): (*1*) control without PAHs, pH 6.0, (*2*) fluorene, pH 4.5, (*3*) fluorene, pH 6.0, (*4*) fluoranthene, pH 4.5, (*5*) fluoranthene, pH 6.0. (b) *1–4*—laccase isoforms; I—control, II—in the presence of PAHs.

them (laccase 3 and 4, Fig. 3b) did not present in the control variant without PAHs.

Fluorene and fluoranthene degradation in basidiomycete-rich medium. The study of fluorene degradation upon *P. ostreatus* D1 culturing in rich medium containing yeast extract, peptone, and glucose revealed its almost complete consumption by the fungus in 14 days of culturing (Fig. 4). Similar to culturing in Kirk's medium, the degradation of this PAH occurred with the formation of 9-hydroxyfluorene and 9-fluorenone. However, metabolites did not accumulate in rich medium; according to GLC and HPLC data, the maximum quantities of 9-hydroxyfluorene and 9-fluorenone in 100 mL of CM did not exceed 1 and 0.4 mg, respectively. Only trace amounts of these substances were detected by the end of the experiment. Furthermore, GLC allowed the revelation of another metabolite that formed in 12–14 days of culturing, the retention time of which corresponded to phthalic acid. The results are in accordance with the literature data [14], which showed that 9-hydroxyfluorene and 9-fluorenone are not the final products of fluorene degradation by the fungus *Agrocybe* sp. CU-43.

Fluoranthene and its degradation products did not inhibit the growth of *P. ostreatus* D1 upon culturing in rich medium, in contrast to Kirk's medium. The biomass in the control and the experimental variants was approximately identical for 3 weeks of culturing and reached 540 mg/100 mL of medium by the end of the experiment. It was found that the fungus metabolized 89% of fluoranthene in 7 days of culturing under these conditions. The use of chromatographic methods allowed the determination of a metabolite with a retention time of 5.08 min (by GLC) and $R_f = 0.9$ (by TLC), which corresponded to the one detected in Kirk's medium. However, in contrast to Kirk's medium, this product did not accumulate in rich medium and could not be detected by the end of the experiment. GLC revealed one more metabolite that was formed in the course of fluoranthene degradation. It corresponded to phthalic acid, according to the retention time.

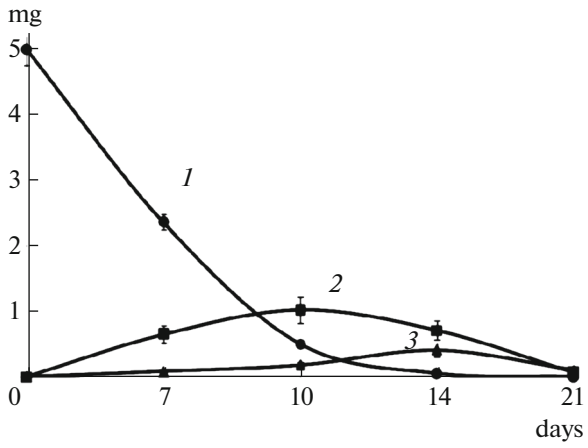

Fig. 4. Fluorene degradation (*1*) and the formation of (*2*) 9-hydroxyfluorene and (*3*) 9-fluorenone upon *P. ostreatus* D1 culturing in rich medium.

P. ostreatus D1 produced two lignolytic enzymes—laccase and versatile peroxidase—upon culturing in rich medium in the presence of fluorene and fluoranthene. The production of laccase started in 3–5 days, while the production of versatile peroxidase shifted to 10–14 days (Fig. 5a). The activity of both enzymes did

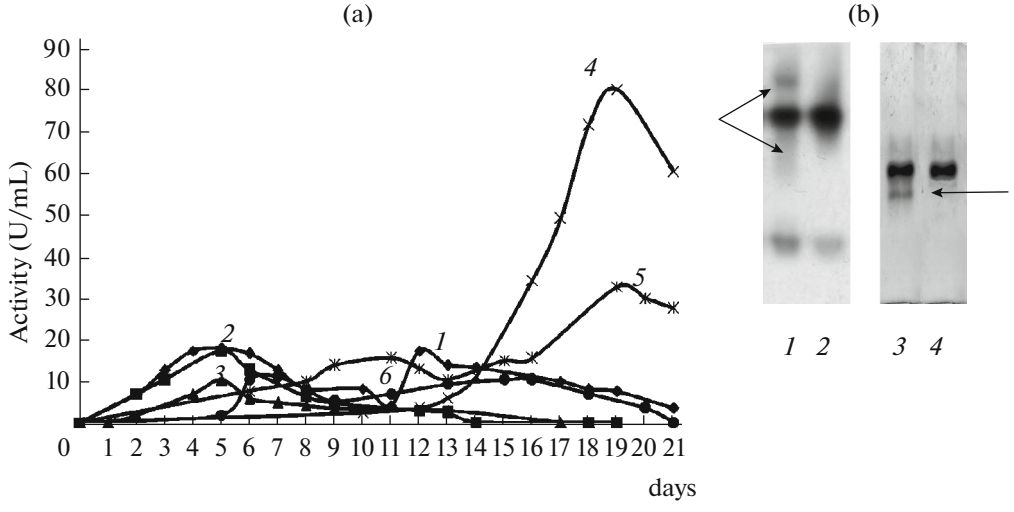

Fig. 5. Activity dynamics (a) and the electrophoregram (b) of lignolytic enzymes from CM of *P. ostreatus* D1 cultured in rich medium. (a): *1*—laccase (control); *2*, *3*—laccase, fluoranthene, and fluorene, respectively; *4*—versatile peroxidase; *5*, *6*—versatile peroxidase, fluoranthene, and fluorene, respectively. (b): *1*—laccase in the presence of PAHs, *2*—laccase (control), *3*—versatile peroxidase in the presence of PAHs, *4*—versatile peroxidase (control); additional forms of laccase and versatile peroxidase are shown by arrows.

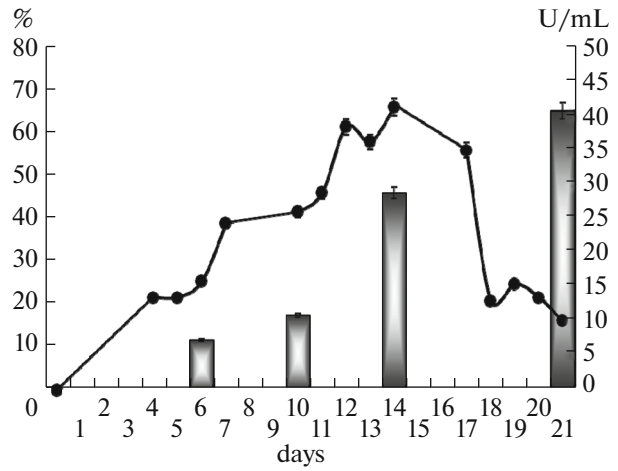

Fig. 6. Phthalic acid degradation (histogram) and laccase production upon *P. ostreatus* D1 culturing in Kirk's medium.

not exceed the control in the case of fluoranthene and was remarkably lower in the case of fluorene. Since the initial substance was depleted in CM in 7–8 days but its quinone metabolites were present, it was evident that they inhibited the further production of lignolytic enzymes. The production of versatile peroxidase in the presence of fluoranthene started a week earlier than in the control; this was possibly caused by the induction of the enzyme by degradation products of the studied PAH (Fig. 5a). Similar results were obtained by Novotny et al. [16] upon the culturing of the fungus *Irpex lacteus* in the presence of fluoranthene. On the basis of the study of the dynamics of extracellular enzymes, we can suppose that, as in the case of other PAHs, laccase participates at the initial stages of fluoranthene degradation, while versatile peroxidase is evidently involved in oxidation of its degradation products.

Nondenaturing electrophoresis revealed the presence of additional forms of laccase and peroxidase upon *P. ostreatus* D1 culturing in the presence of fluorene and fluoranthene (Fig. 5b), which coincided with isoforms that had been previously discovered in the studies of other PAHs under the same conditions [17, 18]. These forms were not found in the control (without PAHs).

Degradation of PAH main metabolites. The study of the destructive activity of *P. ostreatus* D1 towards the revealed fluorene metabolites, 9-hydroxyfluorene and 9-fluorenone, showed that they were utilized by the fungus only upon culturing in rich medium (under the conditions of the production of laccase and versatile peroxidase). In this case 9-hydroxyfluorene diminution was 45% with the formation of 9-fluorenone, and 9-fluorenone degraded by 48% in 14 days of culturing. The product of deeper PAH degradation, phthalic acid, was also metabolized by *P. ostreatus* D1: its diminution was 65 and 92% in 14 days in Kirk's medium (Fig. 6) and rich medium, respectively. It is strongly probable that the phthalic acid formed in the course of PAH degradation can be incorporated into the basic metabolism [29].

Oxidation of fluorene, fluoranthene, and their main metabolites by intact mycelium and nonpurified enzyme preparations of laccase and versatile peroxidase. We showed that, despite different times and conditions of culturing and the low level of enzyme production at particular stages, fluorene and fluoranthene were almost completely degraded. Moreover, the absence of remarkable sorption on mycelium proves that enzymatic degradation occurs, which causes deep decomposition of the toxicants. In order to verify this supposition, we performed experiments with nonpurified concentrates of CM that differed in the composition of lignolytic enzymes.

We studied the oxidation of fluorene, fluoranthene, 9-hydroxyfluorene, 9-fluorenone, and phthalic acid by nonpurified enzyme preparations of laccase and versatile peroxidase (table). Incubation with laccase resulted in fluorene and fluoranthene decreases of 52.5 and 28.4%, respectively. We revealed the only product of fluorene oxidation by laccase, 9-fluorenone; fluoranthene oxidation products were not revealed under these conditions. The metabolites of these PAHs (9-hydroxyfluorene, 9-fluorenone, and phthalic acid) were not transformed by laccase. Versatile peroxidase decreased the concentration of fluorene and fluoranthene by 89.6 and 61.3%, respectively, in the presence of H_2O_2 and $MnCl_2$. The study of metabolite oxidation showed that they could be substrates for this enzyme (table). 9-hydroxyfluorene oxidation was 46.6%; it resulted in the formation of 9-fluorenone. The diminution of 9-fluorenone and phthalic acid was 56.3 and 60.6%, respectively.

Oxidation of PAHs and their metabolites by *P. ostreatus* enzyme preparations

Enzyme preparation	Diminution (%)				
	fluorene	fluoranthene	9-hydroxy fluorene	9-fluorenone	phthalic acid
Mycelium	42.0 ± 6.2	27.1 ± 4.1	0	0	0
Laccase	52.5 ± 2.4	28.4 ± 5.5	0	0	0
Versatile peroxidase	89.6 ± 6.3	61.3 ± 8.6	46.6 ± 3.0	56.3 ± 9.9	60.3 ± 8.5

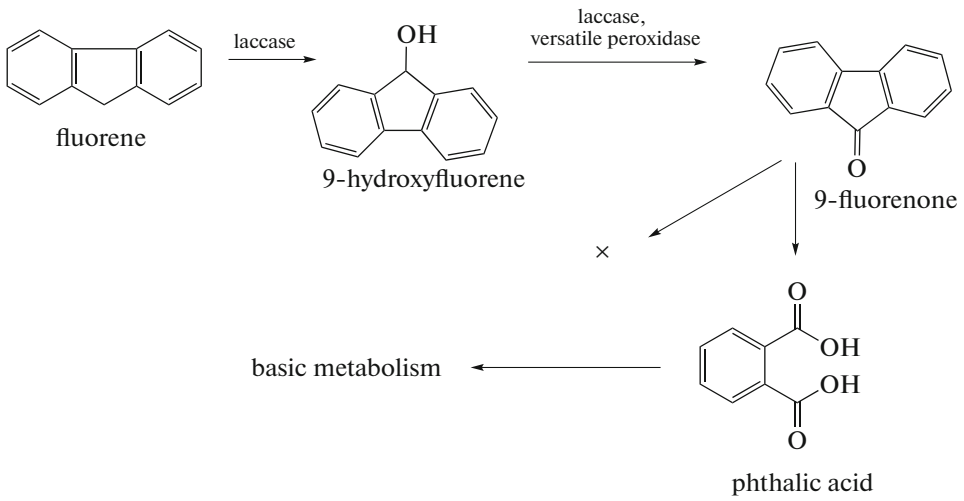

Fig. 7. Supposed scheme of fluorene metabolism by *Pleurotus ostreatus* D1.

The estimation of the participation of lignolytic enzymes in PAH degradation does not take into account the laccase pool on the surface of mycelium. The information on these laccases and their possible functions is insufficient [30]. A laccase sorbed on the mycelial surface may participate in the initial attack of a PAH molecule, since the secretion of extracellular laccase into CM takes time. Indeed, intact mycelium of *P. ostreatus* D1 obtained upon culturing in basidiomycete-rich medium without PAHs decreased the concentrations of fluorene or fluoranthene by 42.0 and 27.1%, respectively, upon incubation for 7 days (table). GLC revealed the presence of a peak in a fluoranthene oxidation product with a retention time of 5.08 min that was analogous to that found upon culturing of the fungus in both media. Fluorene oxidation by intact mycelium caused the formation of 9-fluorenone.

Consequently, the results of the study show that the level of PAH degradation and the composition of the extracellular enzyme complex produced in their presence depend on the culturing conditions. Thus, PAH degradation in Kirk's medium was accompanied by the production of only laccase over the course of the entire experiment, while the same process in rich medium caused the production of both laccase and peroxidase. In the first case PAH quinone metabolites accumulated, while the degradation was deeper in the second case with the formation of phthalic acid, which can be involved in the basic metabolism (Fig. 7).

Laccase was produced by the fungus only at the beginning of degradation, while versatile peroxidase was synthesized at later stages. Although fluorene and fluoranthene are available for oxidation by the enzyme, peroxidase was not evidently necessary for the initial attack of PAH molecules and it participated in the oxidation of the formed metabolites. The dynamics of laccase production allows the supposition that it is exactly this enzyme that can catalyze not only the initial stages of a PAH molecule transformation but also the later stages that cause aromatic ring cleavage.

We showed the effect of the culturing conditions of the fungus *P. ostreatus* D1 on the composition of the enzyme complex and the related degree of fluorene and fluoranthene degradation. In addition to the scientific importance, the results of the study have great practical importance for the development of technologies for the purification of polluted objects. The addition of the corresponding substrate, especially at the initial stages of bioremediation, stimulated not only the rate but also the degree of PAH degradation that occurs with the formation of intermediate metabolites, which can be further incorporated into the basic metabolism.

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