ISSN 0003-6838, Applied Biochemistry and Microbiology, 2016, Vol. 52, No. 6, pp. 629–637. © Pleiades Publishing, Inc., 2016.
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Oil Degradation by Basidiomycetes in Soil and Peat at Low Temperatures

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Received April 5, 2016

Abstract—A total of 17 basidiomycete strains causing white rot and growing on oil-contaminated substrates have been screened. Three strains with high (*Steccherinum murashkinskyi*), average (*Trametes maxima*), and low (*Pleurotus ostreatus*) capacities for the colonization of oil-contaminated substrates have been selected. The potential for degrading crude oil hydrocarbons has been assessed with the use of fungi grown on nonsterile soil and peat at low temperatures. *Candida* sp. and *Rhodococcus* sp. commercial strains have been used as reference organisms with oil-degrading ability. All microorganisms introduced in oil-contaminated soil have proved to be ineffective, whereas the inoculation of peat with basidiomycetes and oil-degrading microorganisms accelerated the destruction of oil hydrocarbons. The greatest degradation potential of oil-aliphatic hydrocarbons has been found in *S. murashlinskyi*. *T. maxima* turned out to be the most successful in degrading aromatic hydrocarbons. It has been suggested that aboriginal microflora contributes importantly to the effectiveness of oil-destructing microorganisms. *T. maxima* and *S. murashkinskyi* strains are promising for further study as oil-oxidizing agents during bioremediation of oil-contaminated peat soil under conditions of low temperatures.

Keywords: polycyclic aromatic hydrocarbons, nonsterile soil, nonsterile peat, crude oil, basidial fungi, degradation of aliphatic and aromatic hydrocarbons

DOI: 10.1134/S0003683816060119

INTRODUCTION

Basidial fungi causing white rot are capable of degrading various oil hydrocarbons, including polycyclic aromatic hydrocarbons (**PAH**s) [1, 2]. The mechanisms of oil degradation by these basidiomycetes have been insufficiently studied. It has been shown that PAHs are oxidized under the effect of Mn-peroxidase (EC 1.11.1.13), lignin peroxidase (EC 1.11.1.14), and laccase (EC 1.10.3.2) [2–6]. Thus, it has been suggested that enzymes involved in the lignin-modifying system of fungi (**LMS**) [3–6] play a leading role in the process of oil degradation. PAHs may become completely mineralized under the joint effect of ligninolytic enzymes, cytochrome P-450 dependent monooxigenases (EC 1.14.14.1), and epoxide hydrolase (EC 3.3.2.9), which is evidence of the important role played by nonligninolytic enzymes in the process of their degradation by white rot fungi. It was shown that the combination of nonligninolytic enzymes and the LMS of two basidiomycetes (*Phanerochaete chrysosporium* and *Pleurotus ostreatus*) ensured complete mineralization of pyren and benz[a]pyrene [7]. At the same time, PAH aromatic rings may be oxidized by free hydroxyl radicals generated by white rot fungi [8].

Technogenic load intensification and sharp environmental degradation initiated the development of bioremediation technologies. Although biopreparations based on various strains of microorganisms, primarily bacteria and yeast, have been widely used for bioremediation of polluted environments [9], particular attention is being paid to the search for new oil-destructing microorganisms, such as the basidial fungi of white rot [10]. One of their major advantages is the ability to mineralize PAH containing four or more condensed aromatic rings, whereas bacteria decompose paraffin and naphtene hydrocarbons [2]. It was shown that a decrease of the PAH concentration in the environment is accompanied with repression of the synthesis of LMS enzymes taking part in their decomposition. Thus, basidial fungi are capable of degrading PAHs even at low concentrations [11]. The important feature of basidial fungi is their ability to grow and

Table 1. Strains of basial fungi used in the study

Collection number	Species	Family
LE-BIN0432	*Pleurotus ostreatus* (Jacq.) P. Kumm	*Pleurotaceae*
LE-BIN0677	*Coriolopsis caperata* (Berk.) Murrill	*Polyporaceae*
LE-BIN072	*Trametes hirsuta* Wulf. Ex. Fr	*Polyporaceae*
LE-BIN093	*Trametes ochracea* (Pers.) Gilb. & Ryvarden	*Polyporaceae*
LE-BIN1483	*Flammulina sp.*	*Physalacriaceae*
LE-BIN1795	*Xerula radicata* (Relhan) Dörfelt	*Physalacriaceae*
LE-BIN1911	*Trametes gibbosa* (Pers.) Fr.	*Polyporaceae*
LE-BIN1968	*Steccherinum murashkinskyi* (Burt), Maas, Geest.	*Steccherinaceae*
LE-BIN1998	*Antradiella faginea* Vampola & Pouzar	*Steccherinaceae*
LE-BIN2009	*Byssomerulius avellaneu* (Bres.) J. Erikss.&Hjortstam	*Phanerochaetaceae*
LE-BIN2013	*Junghuhnia nitida* (Pers.) Ryvarden	*Meruliaceae*
LE-BIN2032	*Tramates versicolor* (L.:Fr.)	*Polyporaceae*
LE-BIN2047	*Lenzites betulina* (L.: Fr.)	*Polyporaceae*
LE-BIN2124	*Peniophora lycii* (Pers.) Höhn. & Litsch.	*Peniophoraceae*
LE-BIN2322	*Antrodiella pallasii* Renvall, Johann. & Stenlid	*Phanerochaetaceae*
LE-BIN2738	*Steccherinum bourdotii* Saliba & A. David	*Steccherinaceae*
LE-BIN275	*Trametes maxima* (Mont.) David & Rajchenb	*Polyporaceae*

develop when the conditions are unfavorable. It was shown that *Lentinus* (*Panus*) *tigrinus* retained its ability to oxidize oil upon either a lack or excess of nitrogen in the axenic culture [12], and *P. chrysosporium* and *P. pulmonarius* effectively degraded PAHs in soil containing heavy metals, even at pH values that were unfavorable for their growth [13]. When ligninolytic basidiomycetes were studied as oil-degrading microorganisms, their degradation potential was estimated in either sterile conditions (model systems) [11, 14] or in soils under conditions favorable for fungal growth [2, 15]. The inability to extrapolate the obtained results into real contamination conditions restricts to a significant degree further development of this branch of bioremediation technologies [14]. According to modern views, temperature is the main physical factor determining the biodegradation rate of hydrocarbons [16]. It was demonstrated that the maximum rate of oil degradation in soil is observed at 30–40°C [9], whereas oil viscosity increases at lower temperatures; the vaporization rate of volatile components [17] and growth rate of microorganisms [18] decrease, which reduces the rate of decomposition of oil hydrocarbons in the environment.

The purpose of this work is to screen basidiomycetes causing white rot upon their growth on oil-contaminated substrate, as well to estimate the oil-degrading activity of the selected strains in oil-contaminated soil and peat at low temperatures.

METHODS

Strains of basidial fungi. Basidiomycete strains from the collection of the Komarov Botanical Institute, Russian Academy of Sciences, were used in this study (Table 1). The basidiomycete cultures were a film of well-developed white aerial mycelium with a noncolored reversum. All strains were kept in tubes at 4°C on wort-agar slants prepared via the dilution of beer wort with water in the ratio of 1 : 4. The obtained cultures were stored without transfer for 6–12 months without any loss of biosynthetic activity.

Oil-destructing microorganisms. The reference microorganisms used were commercial biological preparations based on the *Rhodococcus* sp. actinobacterium and the *Candida* sp. yeast from the Bioros biopreparation (Scientific and Research Institute of Natural Gases and Gas Technologies, Russia).

Samples of soil, peat, and oil. Soil samples were taken in Moscow region and identified as Retisol by international classification [19]. It was a medium-textured loam with a water-extract acidity of (pH_{water}) 6.9, organic carbon (C_{org}) and nitrogen content 18.0 and 0.86 g/kg, respectively. The peat (Yamalo-Nenets autonomous okrug, Russia) was a sphagnum peat with decomposition degree 40%, pH_{water} 3.8, C_{org} 437.0 g/kg, N_{org} 3.9 g/kg. The soil and peat were passed through a sieve (mesh size 2 mm). The crude oil (Enty-Purovskoe deposit, Yamalo-Nenets autonomous okrug) had a density of 861 kg/m^3.

Screening of basidiomycete strains. Strains were grown on an agarized nutrient medium containing aqueous wort solution (1 : 4, vol/vol) and 2% agar. The medium was sterilized (30 min, 0.5 atm) and transferred to sterile Petri dishes. The strains were inoculated by the method of blocks from the agarized medium with subsequent growth in the dark at 28°C until the agarized medium was completely overgrown by fungal mycelium. The cultivation time varied depending on the strain and was 7–10 days.

To identify the ability of basidiomycete strains to grow on oil-contaminated substrate, we used an

agarized Czapek-Dox medium of the following composition (g/L): sucrose—30.0, $NaNO_3$—3.0, $MgSO_4$—0.5, KCl—0.5, $Fe_2(SO_4)_3$—0.01, K_2HPO_4—1.0, and agar-agar—13.0. To prepare the medium, the components above were dissolved in water at pH 7.3 ± 0.2 and sterilized for 30 min at 1 atm. and 121°C. The Czapek-Dox medium was supplemented with oil up to a final concentration of 3% (wt), sterilized (5 h, 75°C), and transferred to sterile Petri dishes. Inoculation was performed by the method of a block of basidiomycete strains grown on wort-agar.

The growth rate of strains of basidial fungi on oil-contaminated substrate was determined by the diameter of colonies (mm) 7, 10, and 21 days after inoculation.

Oil degradation. To estimate the oil degradation in soil and peat, which were artificially contaminated with crude oil, we used reference oil-destructing microorganisms and strains of the following basidiomycetes: *P. ostreatus*, *S. murashkinskyi,* and *T. maxima*.

The material for further inoculation of basidiomycete strains (grinded by ceramic beads) was grown by the superficial method on nutrient medium at 25–27°C and pH 6.0 as previously described [20]. Cultivation was performed until the mycelium on the nutrient medium reached the weight of 4 g/L for 6–14 days depending on the strain.

The pH values optimal for the growth of *Candida* sp. and *Rhodococcus* sp. are 4.0–4.2 and 7.0, respectively. Sterile distilled water (10 mg/mL) was added to a sterile tube with lyophilized culture and aerated for 2 h on a Rotamix rotation mixer (Elmi, Latvia) at the rate of 90 rpm. Cultures were grown under sterile conditions in a LabGard NU-437-300E safety cabinet (NuAire, United States).

To prepare a medium contaminated artificially with oil, a Petri dish was supplemented with 15 g of nonsterile soil or 1.5 of nonsterile peat. Oil (to a final concentration of 30 g/kg) and distilled water were then added up to the level of normal capacity (**NC**) as according to [21]. The amounts of distilled water were 3.2 and 5.0 mL per dish for soil and peat, respectively. The material for inoculation (2 mL per dish) contained about 8 mg of mycelium or 20 g of the culture of oil-destructing microorganisms.

After inoculation, the microorganisms were incubated for 90 days at 4°C. This temperature corresponded to the mean annual temperature in the layer of 0–25 cm of peat soils in the south taiga zone of Western Siberia, which varies within the range of 3.5–4.2°C [22]. Cultivation was then continued under conditions optimal for the development of soil microflora for 120 days at 25°C and an NC of 60%. After that, the residual content of aliphatic hydrocarbons (**AlHC**s) of oil was determined by gas chromatography with mass spectrometry-based detection (**GC-MS**), and the content of aromatic hydrocarbons (**ArHC**s) was determined by UV spectrophotometry.

Identification of AlHCs in oil products by GC-MS. The AlHCs of oil products were extracted from the soil or peat using hexane. The extracts were purified on Al_2O_3 according to [23]. Chromatographic separation on a MDN-1 column (hard-bonded methylsilicone 30 m × 0.25 mm) (Supelco, Japan) was carried out with the help of a GC 2010 chromatograph (Shimadzu, Japan) with a GCMS-QP 2010 mass detector in the regime of temperature gradient (exposure 100°C 1 min, then 10°C/min up to 200°C, 20°C/min to 260°C, exposure 2 min 260°C) at the following temperatures: injector, 200°C; interface, 210°C; and detector, 200°C. Helium with a flow of 1.2 cm^3/min and a flow pressure of 1 : 2 was used as a carrier gas. The parameters of the mass detector are the registration regime (MIC) and a mass/charge of 51, 71, 85. GC-MS analysis of the studied sample showed that C13 (7%) and C15 (4%) alkanes prevailed among linear AlHCs; the minimum content was found for C22 (2%).

Identification of aromatic hydrocarbons in the oil by UV spectrophotometry. The solution of the studied oil sample in hexane was characterized by three well-defined shoulders with maxima at 203, 228, and 264 nm (Fig. 1). Oil absorption in the range of 200–210, 220–230, and 230–270 nm is determined by the absorption of aromatic hydrocarbons (**ArHC**s) with benzene, naphthalene, and tricyclic (or more) structures [24]. Based on the absorption of these ranges in the local maxima at 203, 228, and 264 nm in the hexane extracts from soil and peat obtained according to [23], the content of derivatives of benzene, naphthalene, and tricyclic structures, respectively, was estimated. The optical density of hexane extracts was determined in a cuvette with an optical pathway length of 10 mm with a 512 UV/Vis spectrophotometer (Portlab, England).

Statistical data treatment. All experiments were performed in three replicates. Statistical processing of the screening results was carried out with the help of one-way analysis of variance with calculation of the least significant difference (**LSD**) according to [25].

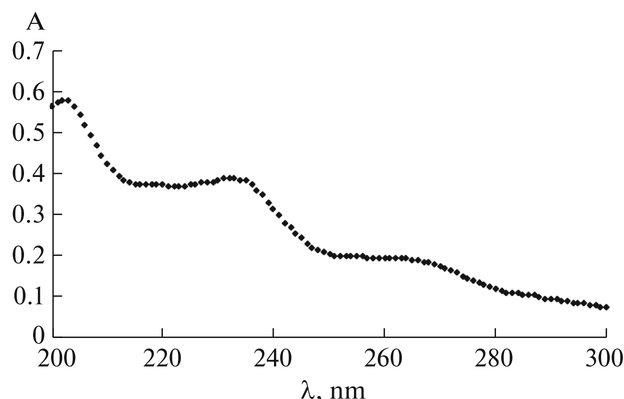

Fig. 1. Absorption spectrum of a hexane solution of the studied oil in the range of 200–300 nm.

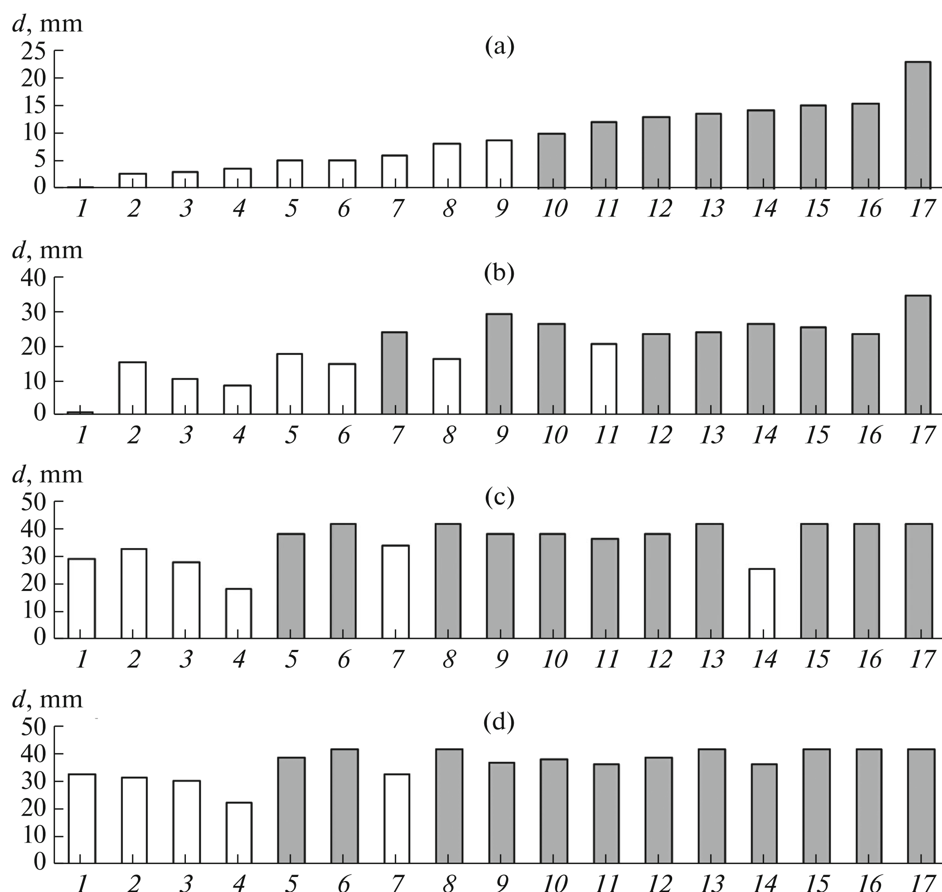

Fig. 2. Diameter of colonies of basidial fungi of the strains *B. avellaneus* (*1*), *S. bourdotii* (*2*), *T. gibbosa* (*3*), *C. caperata* (*4*), *L. betulina* (*5*), *A. pallasii* (*6*), *P. ostreatus* (*7*), *A. faginea* (*8*), *T. maxima* (*9*), *T. hirsuta* (*10*), *T. ochracea* (*11*), *J. nitida* (*12*), *Flammulina* sp. (*13*), *T. versicolor* (*14*), *X. radicata* (*15*), *Pen. lycii* (*16*), *S. murashkinskyi* (*17*) during growth on oil-contaminated agar on day 7 (a), 10 (b), 17 (c), and 21 (d) of cultivation. Strains with above-average colony diameters are shown with gray color.

The significance level is $\alpha = 0.05$. Microsoft Excel 2007 and Statistica 8.0 programs were used.

RESULTS AND DISCUSSION

Strain growth on oil-contaminated substrate. The studied strains of basidial fungi were characterized by their different capacities for growth on oil-contaminated substrate (Fig. 2). An above-average size of fungal colonies (10 mm) was registered in such genera as *Trametes* and *Steccherinum*; the exception was *S. bourdotii* and *T. gibbosa*, in which the diameter of colonies was not larger than 4 mm. The size of colonies in the studied fugal strains during the period from days 7 to 10 increased by 2.4 times on average. It has been shown that the maximum growth was observed in *S. bourdotii* and *P. ostreatus* strains (five- and fourfold increases, respectively). *S. murashkinskyi* exhibited an even growth; the colonies of this fungus increased by about 1.5 times. The data showed that the studied strains of basidial fungi differed by the rate of adaptation to oil contamination and, consequently, the lag period. Therefore, *P. ostreatus* was assigned to the group of strains with a colony diameter that exceeded the average value, and *T. ochracea* was placed in the group of strains with a below-average colony size. The reduction in the growth rate of all basidiomycetes was observed on day 17 (Fig. 2), and the diameter of their colonies from days 10 to 17 increased by 27 times on average. It can be suggested that this fungal strain had a longer lag period under the conditions of oil contamination. The colony diameters of strains of *A. pallasii*, *A. faginea*, *Flammulina* sp., *X. radicata*, *Peniophora lycii*, *S. ochraceum*, and *S. murashkinskyi* reached their maximum on day 17.

In a summary of the data on the growth dynamics of the studied strains of basidial fungi, it can be noted that *S. murashkinskyi* was characterized by a more pronounced ability to colonize when grown on oil-contaminated substrate: the largest colonies were observed on day 17 of cultivation; mycelium covered the entire surface available for growth. The growth rate under the conditions of oil contamination was higher than that for fungi of the genera *Trametes* and *Pleuro-*

Table 2. Separation of basidial fungi strains into groups with the same ability to grow on oil-contaminated agar.

Strain	Average colony diameter, mm	Homogenous groups							
		I	II	III	IV	V	VI	VII	VIII
C. caperata	13.5	+							
T. gibbosa	15.9	+							
B. avellaneus	18.2	+							
S. bourdotii	20.8		+						
L. betulina	22.5		+	+					
P. ostreatus	24.5		+	+	+				
T. versicolor	25.9			+	+				
A. pallasii	26.1			+	+				
T. ochracea	26.6			+	+	+			
J. nitida	27.1				+	+	+		
A. faginea	27.3				+	+	+		
T. hirsuta	28.5				+	+	+		
T. maxima	28.6				+	+	+		
Flammulina sp.	30.6					+	+		
P. lycii	31.0					+	+	+	
X. radicata	31.4						+	+	+
S. murashkinskyi	35.6								+

"+" shows the group to which the strain belongs.

tus characterized, as according to the available data, by a high capacity for oil destruction [2, 11]. The observed disagreement is probably explained by the absence of any direct interaction between the ability of strains to colonize oil-contaminated substrate in the first days of growth (lag phase) and the capacity of these strains for oil destruction, which is commonly measured after longer periods of cultivation lasting for at least 30 days. *B. avellaneus* can be characterized as a strain with the least expressed capacity for colonization: the diameter of its colonies was minimal in the first days of observation (lag phase), and the growth was almost totally inhibited by day 21 (stationary growth phase).

Based on the variance analysis, in which the colony sizes in the studied strains of basidiomycetes were considered a resultant feature and the strains were considered a factor, the groups of strains with the same ability to grow on oil-contaminated agar were isolated (Table 2).

The group of strains with low colonization ability (groups I and II) included such strains as *C. caperata*, *T. gibbosa, B. avellaneus, S. bourdotii*, *L. betulina*, and *P. ostreatus*. The group with average colonization ability (groups III–VI) involved the majority of studied strains (Table 2). The high colonization ability (groups VII and VIII) was found in *S. murashkinskyi*, *P. lycii*, and *X. radicata*.

The most strongly pronounced capacity for colonization was observed in *S. murashkinskyi*. Thus, it was selected for experiments on oil degradation. For comparison, the experimental scheme also included strains with the average (*T. maxima*) and low (*P. ostreatus*) capacity for colonization.

Oil degradation by basidiomycetes in soil and peat. It was shown that, as commercial microorganisms were introduced in soil and peat, the concentration of oil HC in them on day 210 of cultivation decreased significantly; at the same time, the degradation of ArHC was more efficient than that of AlHC (Fig. 3). The calculated amount of residual AlHC and ArHC in the soil and peat was about 49 and 19%, 43 and 24%, respectively (Fig. 3), which was consistent with the range of decomposition values of oil HC [9]. The data prove the high oil-degrading activity of microorganisms in nonsterile samples of soil and peat. The degree of AlHC degradation in soil in the presence of strains of both basidial fungi and nonoxidizing microorganisms involved in commercial preparations remained low compared to the control variant (Fig. 4a and 5a). Thus, the residual content of AlHCs in the presence of basidial fungi did not reliably differ from the control value and even exceeded the control values when commercial microorganisms were used. This may have resulted from the partial suppression of aboriginal microflora.

The estimation of the residual ArHC content of (Fig. 6a–6c) showed an absence of the degree of their degradation, as did AlHC during the introduction of basidial fungi or industrial oil destructors in soil.

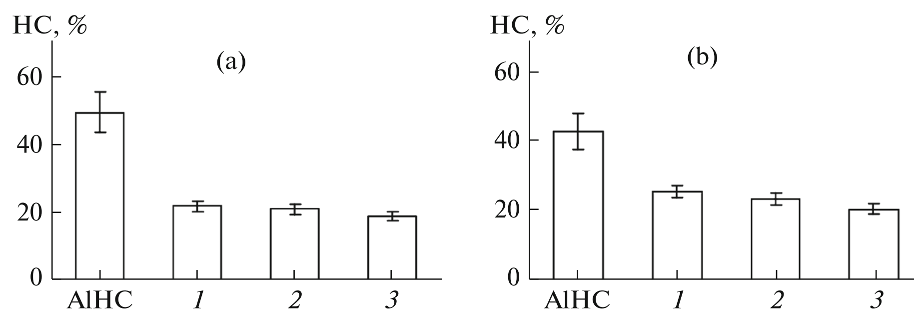

Fig. 3. content (% of the initial value) of benzene and its homologs (*1*), naphthalene and its homologs (*2*), and tricyclic compounds and their homologs (*3*) of oil in soil (a) and peat (b) after 210 days of cultivation without introduction of microorganisms.

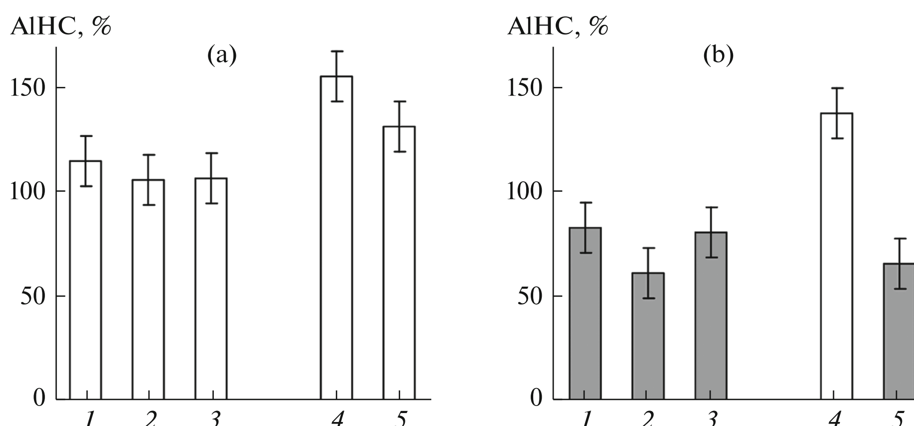

Fig. 4. Oil AlHC content (% of control) in soil (a) and peat (b) after 210 days of cultivation in the presence of the strains *P. ostreatus* (*1*), *T. maxima* (*2*), *S. murashkinskyi* (*3*), *Candida* sp. (*4*), and *Rhodococcus* sp. (*5*). The controls were soil and peat without introduced microorganisms. Values significantly lower than control (α = 0.05) are shown with gray color. LSD = 15%.

The data on the efficiency of use of oil-destructing strains in nonsterile soil are contradictory [11, 15, 26–28]. Thus, the comparative study of the oil-destructing activity of three white rot species (*P. chrysosporium*, *T.* (*Coriolus*) *versicolor*, and *P. ostreatus*) showed that the degree of HC degradation by these strains at an initial oil concentration in soil of 32 g/kg over 12 months was 69, 53, and 78%, respectively [26]. Another strain of basidial fungi, *P. tuber-regium*, completely colonized the oil-contaminated soil and efficiently mobilized the aliphatic and aromatic oil fractions (by 70%) [27]. However, it was shown in the literature [11, 15, 28] that the efficiency of oil degradation by basidiomycetes and other oil-destructing microorganisms may decrease in nonsterile soil compared to sterile soil; sometimes, there was no effect from introducing microorganisms. The absence of any increase in the degree of oil biodegradation in soil as a result of the introduction of basidiomycetes probably proved the low competitive ability of these strains as compared to the aboriginal microflora [15, 28]. It was shown that the cultivation of *P. chrysosporium* at pH 7.4 on oil-contaminated soil did not cause the release of laccase and arylalcohol oxidase (EC 1.1.3.7) [29]. Therefore, the soil pH determines the synthesis of fungal extracellular enzymes tht commonly cause oil HC degradation.

The introduction of basidiomycetes or oil-destructing microorganisms in peat, in contrast to soil, promoted the acceleration of oil-HC decomposition (Table 2). Thus, in the presence of *P. ostreatus*, *T. maxima*, *Rhodococcus* sp., and *S. murashkinskyi*, characterized by different capacities for colonization, there was accelerated oil AlHC decomposition (Figs. 4b and 5b). However, oil AlHC degradation did not occur in the course of inoculation of *Candida* sp. in peat.

After the inoculation of peat with all of the studied basidiomycetes on day 210 of cultivation, there was higher degradation of paraffines C17–C22 and alkane isomers C17–C22 (with retention periods of 11.0, 12.63, 13.36, and 13.73), as well as C17–C22 linear and branched alkenes (Fig. 5b). Basidial fungi caused total degradation of the aliphatic compounds, C19–C21 alkenes (with retention periods of 12.10, 12.38, and 14.03) (Fig. 5b). However, the degree of degradation of C13–C16 linear alkanes from the long chain decreased in the presence of *T. maxima* and *P. ostreatus* (Fig. 5b). No reduction was observed in the deg-

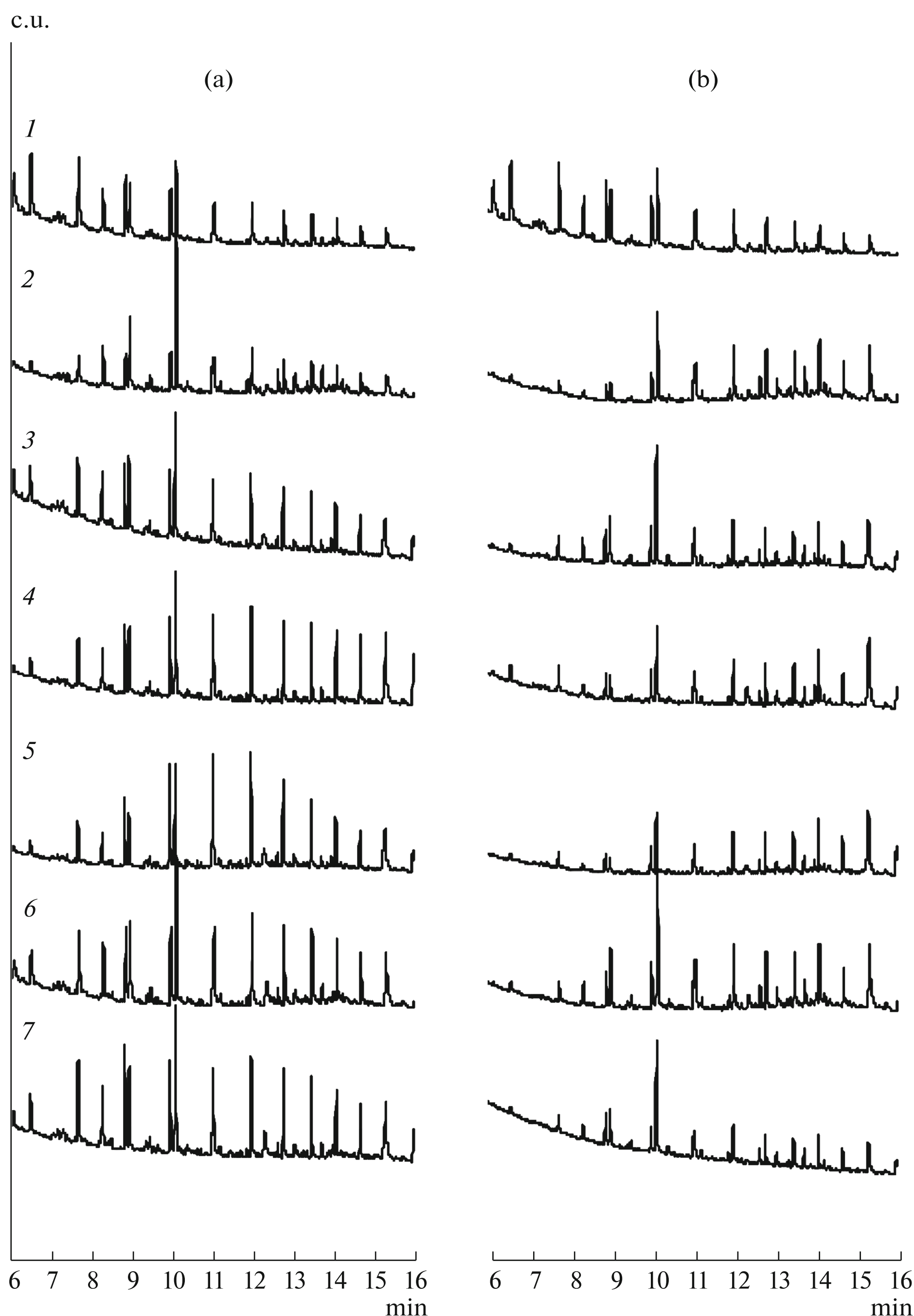

Fig. 5. Chromatograms of hexane extracts of oil-contaminated soil (a) and peat (b) from the beginning of the experiment (*1*), after 210 days of cultivation without introduction of microorganisms (*2*), and after 210 days of cultivation in the presence of the strains *P. ostreatus* (*3*), *T. maxima* (*4*), *S. murashkinskyi* (*5*), *Candida* sp. (*6*), and *Rhodococcus* sp. (*7*).

radation rate of these alkanes upon the introduction of *S. murashlinskyi*. This indicated that *S. murashlinskyi* had the highest degradation potential in relation to oil AlHC.

The introduction of *P. ostreatus* in peat did not change the content of ArHC. In the presence of *T. maxima* and *S. murashkinskyi*, there was a decrease in all ArHC fractions in peat (Figs. 6d–6f). The highest degree of ArHC degradation was observed when *T. maxima* was introduced in peat, in which the content of benzene homologs, naphthalene, and tricyclic compounds was 66, 59, and 60% of the control values, respectively.

The inoculation of *Candida* sp. in peat also resulted in a decrease of ArHCs compared to the control values. There was no such effect of this microorganism in

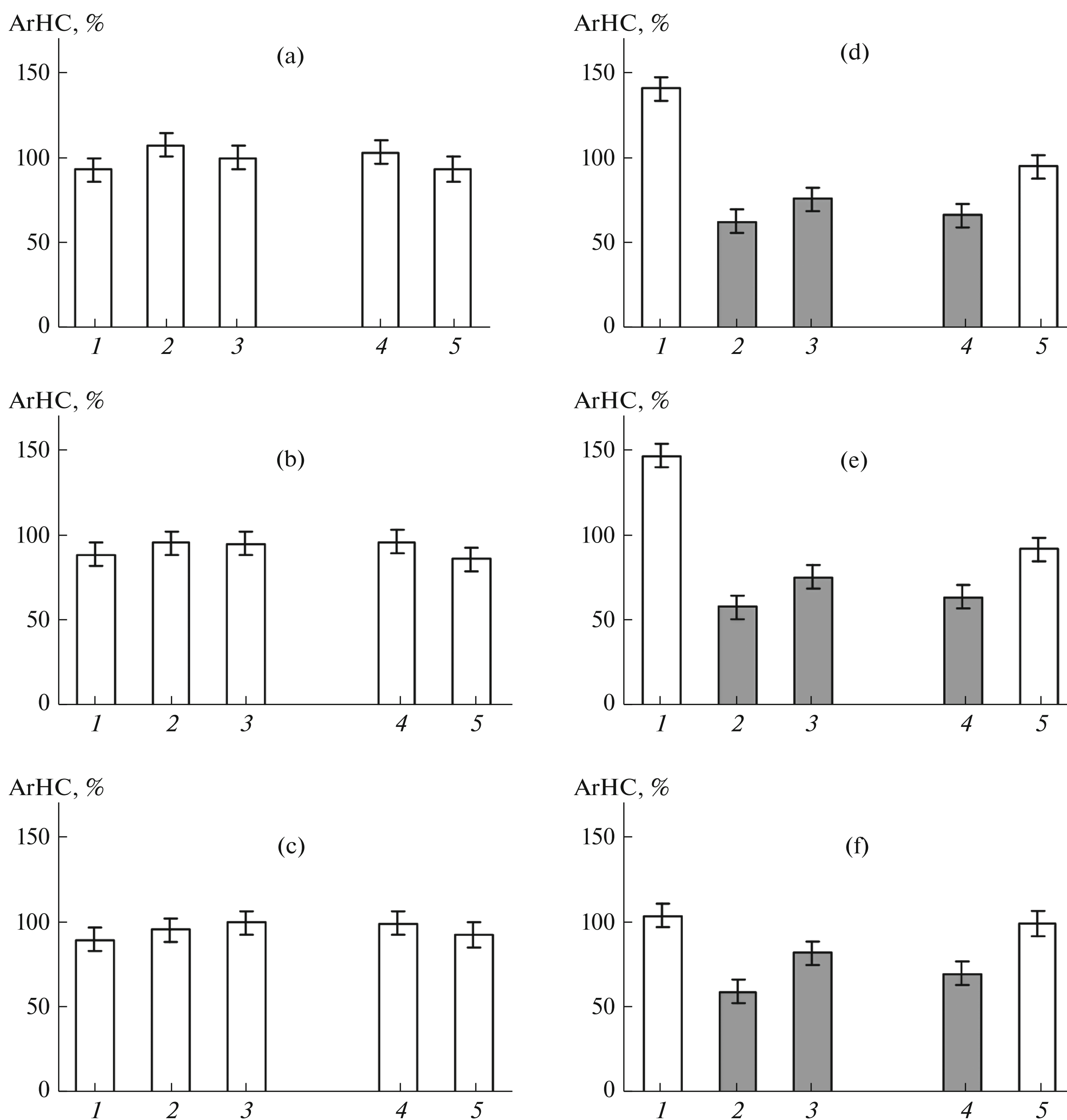

Fig. 6. Content (% of control) of benzene and its homologs (a, d), naphthalene and its homologs (b, e), and tricyclic compounds and their homologs (c, f) in soil and peat after 210 days of cultivation in the presence of the strains *P. ostreatus* (*1*), *T. maxima* (*2*), *S. murashkinskyi* (*3*), *Candida* sp. (*4*) and *Rhodococcus* sp. (*5*). The controls were soil and peat without introduced microorganisms. Values significantly lower than control ($\alpha = 0.05$) are shown with gray color. LSD = 18%.

soil. These differences might have been caused by the environmental pH, which was more favorable for this microorganism, as well as the high activity of aboriginal microflora in nonsterile conditions [30]. The introduction of *Rhodococcus* sp. did not decrease the ArHC content in peat, which was consistent with the data on preferred degradation of AlHC by *Rhodococcus* sp. [31]. As is known, actinobacteria of the genus *Rhodococcus* are psychrotolerant and psychrotrophic microorganisms capable of oil degradation at low temperatures [31]. An important role is played by the release of biosurfactants, which intensifies upon a deficit of nitrogen [32]. Since the ratios of C : N in soil and peat varied from 21 to 112, it can be suggested that the increase in the degree of oil degradation in peat in the presence of *Rhodococcus* is determined by the effect of surfactants, which promote better oil bioavailability; the temperature decrease did not play any key role.

Therefore, the results proved the inefficiency of using such basidiomycetes as *P. ostreatus, T. maxima*, and *S. murashkinskyi* to degrade crude oil in soil at low temperatures. This is probably associated with their low competitive ability in relation to the aboriginal microflora. Upon the inoculation of peat, all of the

studied microorganisms presented oil-oxidizing activity. The most efficient strains were *T. maxima* and *S. murashkinskyi*, in the presence of which active degradation of both AlHCs and ArHCs occurred. Differences in the efficiency of oil degradation by the studied strains of basidiomycetes at a low temperature in soil and peat showed that the decrease in HC degradation in nonsterile substrates may be caused by not only an unfavorable temperature but also the level of medium acidity and the C : N ratio. It can be assumed that the efficiency of oil degradation under these conditions is influenced by the composition and oil-oxidizing activity of the aboriginal microflora.

Further study of the possible use of *T. maxima* and *S. murashkinskyi* basidiomycetes is needed to develop technologies for the bioremediation of oil-contaminated peat soils under the conditions of low temperatures.

ACKNOWLEDGMENTS

The study was supported by the Russian Science Foundation (project no.16-14-00081).

We are grateful to E.L. Listov (Scientific and Research Institute of Natural Gases and Gas Technologies, Gazprom, Russia) for kindly providing us with *Rhodococcus* sp. and *Candida* sp. strains, as well as G.V. Matyshak (Department of Soil Science, Moscow State University) for the peat sample and N.Yu. Grechishcheva (Gubkin Russian State University of Oil and Gas) for the oil sample.

REFERENCES

1. Bamforth, S.M. and Singleton, I., *J. Chem. Technol. Biotechnol.*, 2005, vol. 80, pp. 723–736.
2. Pozdnyakova, N.N., Nikitina, V.E., and Turkovskaya, O.V., *Appl. Biochem. Microbiol.*, 2008, vol. 44, no. 1, pp. 69–75.
3. Acevedo, F., Pizzul, L., Castillo, M., Cuevas, R., and Diez, M., *J. Hazard. Mater.*, 2011, vol. 185, pp. 212–219.
4. Pozdnyakova, N., *Biotechnol. Res. Int.*, 2012, Article ID 243217.
5. Zeng, J., Lin, X., Zhang, J., Zhu, H., Chen, H., and Wong, M., *Appl. Microbiol. Biotechnol.*, 2013, vol. 97, no. 7, pp. 3183–3194.
6. Hadibarata, T. and Kristanti, R.A., *Fungal Biol.*, 2014, vol. 118, no. 2, pp. 222–227.
7. Bezalel, L., Hadar, Y., and Cerniglia, C.E., *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 7, pp. 2495–2501.
8. Bogan, B.W. and Lamar, R.T., *Appl. Environ. Microbiol.*, 1995, vol. 61, no. 7, pp. 2631–2635.
9. Das, N. and Chandran, P., *Biotechnol. Res. Int.*, 2011, Article ID 941810.
10. Magan, N., Fragoeiro, S., and Bastos, C., *Mycobiology*, 2010, vol. 38, no. 4, pp. 238–248.
11. Kokanina, A.V., Marchenko, M.Yu., Barkov, A.V., Leont'eva, M.I., Avtonomova, A.V., Vinokurov, V.A., and Krasnopol'skaya, L.M., *Bashkir. Khim. Zh.*, 2010, vol. 17, pp. 123–129.
12. Covino, S., Svobodova, K., Kresinova, Z., Petruccioli, M., Federici, F., D'Annibale, A., Cvancarova, M., and Cajthaml, T., *Bioresour. Technol.*, 2010, vol. 104, pp. 3004–3012.
13. Quaratino, D., Mincione, E., and Petruccioli, M., *Biotechnol. Bioengin.*, 2005, vol. 90, pp. 723–731.
14. Sasek, V., *The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solution*, Sasek, V., Glaser, J.A., and Baveye, Ph., Eds., Dordrecht: Kluwer Academic Publishers, 2003.
15. Bishnoi, K., Kumar, R., and Bishnoi, N., *J. Sci. Ind. Res.*, vol. 67, pp. 538–542.
16. Jonathan, D.V., Ajay, S., and Owen, P.W., *Microbiol. Mol. Biol. Rev.*, 2003, vol. 67, pp. 503–549.
17. Atlas, R.M., *J. Appl. Microbiol.*, 1975, vol. 30, no. 3, pp. 396–403.
18. Venosa, A.D. and Zhu, X., *Spill Sci. Technol.*, vol. 8, no. 2, pp. 163–178.
19. World Reference Base for Soil Resources 2014. *A Framework for International Classification, Correlation and Communication, Word Soil Resource Report 106*, FAO, Rome, 2014.
20. Koroleva-Skorobogat'ko, O., Stepanova, E., Gavrilova, V., Morozova, O., Lubimova, N., Dzchafarova, A., Yaropolov, A., and Makower, A., *J. Biotechnol. Appl. Biochem.*, 1998, vol. 28, pp. 47–54.
21. Zhurbitskii, Z.I., *Teoriya i praktika vegetatsionnogo metoda (Theory and Practice of Vegetative Method)*, Moscow: Nauka, 1968.
22. Dyukarev, E.A. and Golovatskaya, E.A., *Geogr. Prir. Resur.*, 2013, no. 1, pp. 65–71.
23. Drugov, Yu.S. and Rodin, A.A., *Analiz zagryaznennoi pochvy i opasnykh otkhodov. Prakticheskoe rukovodstvo* (Analysis of Contaminated Soil and Hazardous Waste: A Practical Guide), Moscow: BINOM. Laboratoriya znanii, 2007.
24. Neff, J.M. and Anderson, J.W., *Bull. Environ. Contam. Toxicol.*, 1975, vol. 14, pp. 122–128.
25. Dmitriev, E.A., *Matematicheskaya statistika v pochvovedenii (Mathematical Statistics in Soil Science)*, Moscow: MGU, 2010.
26. Yateem, A., Balba, M.T., Al-Awadhi, N., and El-Nawawy, A.S., *Environ. Int.*, 1998, vol. 24, pp. 181–187.
27. Ogbo, E. and Okhuoya, J., *African J. Biotechnol.*, 2008, vol. 4, pp. 4291–4297.
28. Bento, F.M., Camargo, F.A.O., Okeke, B.C., and Frankenberger, W.T., *Biores. Technol.*, 2005, vol. 96, pp. 1049–1055.
29. D'Annibale, A., Ricci, M., Leonardi, V., Quaratino, D., Mincione, E., and Petruccioli, M., *Biotechnol. Bioeng.*, 2005, vol. 90, pp. 723–731.
30. Gargouri, B., Mhiri, N., Karray, F., Aloui, F., and Sayadi, S., *BioMed Res. Int.*, 2015.
31. Margesin, R., Moertelmaier, Ch., and Mair, J., *Int. Biodeterior. Biodegrad.*, 2013, vol. 84, pp. 185–191.
32. Pacheco, G.J., Ciapina, E.M.P., de Barros, G.E., and Junior, N.P., *Braz. J. Microbiol.*, 2010, vol. 41, pp. 685–693.

Translated by A. Karmazina