Biodegradation (2008) 19:567–576
DOI 10.1007/s10532-007-9162-2

ORIGINAL PAPER

Conversion of polycyclic aromatic hydrocarbons by *Sphingomonas* sp. VKM B-2434

Mikhail Baboshin · Vladimir Akimov · Boris Baskunov · Timothy L. Born · Shahamat U. Khan · Ludmila Golovleva

Received: 7 September 2007 / Accepted: 15 October 2007 / Published online: 24 October 2007

Abstract A versatile bacterial strain able to convert polycyclic aromatic hydrocarbons (PAHs) was isolated, and a conversion by the isolate of both individual substances and PAH mixtures was investigated. The strain belonged to the *Sphingomonas* genus as determined on the basis of 16S rRNA analysis and was designated as VKM B-2434. The strain used naphthalene, acenaphthene, phenanthrene, anthracene and fluoranthene as a sole source of carbon and energy, and cometabolically oxidized fluorene, pyrene, benz[*a*]anthracene, chrysene and benzo[*a*]pyrene. Acenaphthene and fluoranthene were degraded by the strain via naphthalene-1,8-dicarboxylic acid and 3-hydroxyphthalic acid. Conversion of most other PAHs was confined to the cleavage of only one aromatic ring. The major oxidation products of naphthalene, phenanthrene, anthracene, chrysene, and benzo[*a*]pyrene were identified as salicylic acid, 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, *o*-hydroxyphenanthroic acid and *o*-hydroxypyrenoic acid, respectively. Fluorene and pyrene were oxidized mainly to hydroxyfluorenone and dihydroxydihydropyrene, respectively. Oxidation of phenanthrene and anthracene to the corresponding hydroxynaphthoic acids occurred quantitatively. The strain converted phenanthrene, anthracene, fluoranthene and carbazole of coal-tar-pitch extract.

Keywords Bioconversion · Biodegradation · PAHs · *Sphingomonas*

M. Baboshin · V. Akimov · B. Baskunov · L. Golovleva (✉)
G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms RAS, Prospekt Nauki, 5, Pushchino, Moscow region, Russia
e-mail: golovleva@ibpm.pushchino.ru

M. Baboshin · V. Akimov · L. Golovleva
Pushchino State University, Prospekt Nauki, 3, Pushchino, Moscow region, Russia

T. L. Born · S. U. Khan
Department of Chemistry and Biochemistry, George Mason University, 4400 University Drive, Fairfax, VA 22030-4444, USA

Introduction

Microbial conversion of PAHs is investigated with the purpose of development of bioremediation technologies (Sutherland et al. 1995) and chemical production by biotransformation (Parales et al. 2002). PAHs containing more than three aromatic rings, named high-molecular-weight PAHs, are particularly recalcitrant, and biodegradation of these chemicals is of special interest (Kanaly and Harayama 2000).

Microbial oxidation of complex PAH mixtures, such as oil and coal-tar derivatives, may be affected by various conditions. Cometabolism (Juhasz and Naidu 2000) and cross-induction (Molina et al. 1999) are favorable factors for biodegradation, while inhibition by the products of PAH transformation

(Casellas et al. 1998; Kazunga and Aitken 2000), competitive inhibition by PAHs themselves (Stringfellow and Aitken 1995) and low substrate bioavailability (Johnsen et al. 2005) may limit the rate and extent of PAH conversion. Versatile microbial strains capable of degrading various PAHs in mixtures are considered to be the most valuable for the purposes of biotechnology.

A number of isolated PAH-degraders belong to the genus *Sphingomonas* (Mueller et al. 1990; Dagher et al. 1997; Bastiaens et al. 2000; Ho et al. 2000; Willison 2004; Pinyakong et al. 2004; Zhong et al. 2007). They are characterized to a variable extent, but generally less than PAH-degrading actinobacteria.

This study describes the characterization of a *Sphingomonas* strain which used as a sole source of carbon and oxidized cometabolically a wide range of PAHs, including high-molecular-weight PAHs, both in the medium with crystalline substrate and in the medium with coal-tar-pitch extract.

Materials and methods

Chemicals

All PAHs were of the highest purity (>98%) available (Sigma-Aldrich, St. Louis, MO). Solvents were of chromatography grade. Diazomethane solution in diethyl ether was prepared by alkaline decomposition of *N*-nitrosomethylurea. All other chemicals used were of analytical reagent grade and purchased locally. Typical coal-tar pitch (pitch) was used for extract preparation. Pitch was provided by the Ukrainian State Research Institute for Carbochemistry (Kharkov, Ukraine). Ten grams of pitch was extracted with ethyl acetate (100 ml) in a closed flask four times over 24 h at 29°C with rotary shaking. The solvent was evaporated at 38°C under vacuum and the pitch extract appeared as a dark-brown viscous liquid with a weak characteristic smell.

Media

Media was prepared on the basis of mineral medium (MM) and contained the following components (g/l): NH_4NO_3—1.0, KH_2PO_4—1.0, K_2HPO_4—1.0, $MgSO_4$ $7H_2O$—0.2, $CaCl_2$—0.02, $FeCl_3$—two drops of a saturated solution, 50% KOH to pH 7.5. When axenic VKM B-2434 strain was incubated, the medium was supplemented with vitamin B_{12} (1 μg/l). Phenanthrene, fluoranthene, pyrene, fluorene and carbazole were added to 100 ml of MM (sterilized at 1 atm. for an hour) in acetone solution (50 g/l); anthracene, benz[*a*]anthracene and benzo[*a*]pyrene were added as 5 g/l solution in acetone; chrysene was added in dimethylformamide solution (5 g/l). A supplemented volume of 0.2–2.0 ml was appropriate for achievement of desirable PAH final concentrations of 0.01–1.0 g/l. Medium containing pitch extract was prepared in the following manner: silica gel powder (5 g, granular size 0.10–0.16 mm, repeatedly washed with water and acetone) was placed into a 750-ml flask and heated at 150°C for 2 h. After cooling, 0.2 g of pitch extract dissolved in 0.8 ml of acetone was placed on the surface of the silica gel. The flask was shaken vigorously to ensure an equal distribution of pitch extract. Sterile MM (100 ml) was then added to the flasks and they were inoculated with the appropriate microbial culture. The silica gel was used to prevent adherence of the substrate to the flask wall.

Strain

The bacterial strain was isolated from PAH-contaminated sediment of coke plant wastewater (Mariupol, Ukraine) by enrichment culture maintained in the medium with pitch extract as a sole source of carbon and energy. This strain, designated as VKM B-2434, was deposited in the All-Russian Collection of Microorganisms (VKM) with number VKM B-2434.

Incubation conditions

The growth medium was inoculated with 1 ml of a fresh culture grown in MM with 1 g/l sodium acetate, which corresponds to initial biomass concentration of about 2 mg/l. Culture incubation was performed in 750-ml flasks containing 100 ml of liquid medium at 29°C with rotary shaking (120 rpm).

Analytical methods

PAHs and their metabolites were extracted with ethyl acetate from culture medium acidified to pH 2 by

addition of hydrochloric acid. Extracts were dried over sodium sulfate and concentrated by rotary evaporation under vacuum at 38°C. Thin-layer chromatography was used for preparative isolation of metabolites. TLC was performed on "Merck" plates, which were developed in a mixture of benzene: dioxane:acetic acid (90:10:1 or 90:10:10). Spots corresponding to metabolites were marked under ultraviolet light, scraped from the plates and extracted with methanol. Metabolites were identified by electronic ionization mass-spectrometry with a "Finnigan MAT INCOS 50" quadrupole mass-spectrometer using an ionization energy of 70 eV and direct input of the sample into the ionization chamber. Quantitative analysis of PAHs contained in the extracts was performed by GC on a "Pye Unicam 104" (Philips, UK) chromatograph with flame-ionization detection using a column (1.5 m × 2 mm) with 3% SE-30 on G-AW-DMSC chromosorb. The injector and detector temperatures were maintained at 150 and 290°C, respectively. The column temperature was 180°C for analysis of acenaphthene, fluorene, phenanthrene and anthracene, 220°C for analysis of fluoranthene and pyrene, and 280°C for analysis of benz[*a*]anthracene, chrysene and benzo[*a*]pyrene. Analysis of pitch extract was carried out by GC-MS using a HP-GCD System (Hewlett-Packard, USA) equipped with a 30 m × 0.25 mm, 0.25 μm film thickness HP-5 capillary column. The inlet and transfer line temperatures were 250 and 290°C, respectively. The oven temperature was increased from 100 to 280°C at a rate of 10°C per minute with a 10 min hold.

Methylation was used for both identification of metabolites by mass-spectrometry and quantitative analysis by gas chromatography. Methylation was performed by addition of excess diazomethane solution in diethyl ether to the extract dissolved in methanol.

Preparative isolation of hydroxynaphthoic acids from cultures was performed according to the following method. Culture liquid was adjusted to pH 14 with 50% KOH and the precipitated biomass was separated by filtering through filter paper. The filtrate was then acidified with 10 M HCl to pH 1. The precipitated hydroxynaphthoic acid was separated by filtering through filter paper, washed with cold water and finally dried.

Absorption spectra of intermediates were performed on a "Shimadzu UV160" (Japan) spectrophotometer. Optical density of cultural medium (OD_{540}) was measured in a 0.3-cm length cuvette on a "KFK" (Russia) photocolorimeter using colourn filter with absorption maximum at 540 nm.

The growth economical coefficient was estimated on the basis of the slope of the graph in units of biomass yield versus initial substrate concentration. Biomass yield was measured gravimetrically after stationary-phase-culture centrifugation, washing of sediment with water, and drying at 105°C.

Determination of 16S rRNA nucleotide sequence and phylogenetic analysis

Extraction of DNA was carried out as described previously (Sambrook et al. 1989). A segment of the 16S rRNA gene was amplified by PCR with the universal eubacterial primers 27f and 1492r in a GeneAmp PCR System 2700 (Applied Biosystems) thermocycler. Purified PCR product was sequenced using a CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter) and an automatic sequencer CEQ2000 XL (Beckman Coulter). The resulting 16S rDNA sequences were subjected to a BLAST search (http://www.ncbi.nlm.nih.gov/blast/) to determine similarities with sequences deposited in the GenBank database. The retrieved sequences were aligned using ClustalX 1.81 (Thompson et al. 1997) and the phylogenetic trees were constructed with the TREECON program (Van de Peer and De Wachter 1997).

16S rRNA nucleotide sequence of *Sphingomonas* sp. VKM B-2434 was deposited in GenBank with accession number EF128173.

Results

Characterization of the strain

The nucleotide sequence of a 1376 base region of the 16S rRNA gene from the VKM B-2434 strain was determined. A BLAST analysis of the nucleotide sequence indicated a close relationship to species of the genus *Sphingomonas* (*S. cloacae*, *S. chlorophenolicum*, *S. herbicidovorans* and *S. yanoikuyae*), with the highest identity to the *S. cloacae* strain—97.0% (Fig. 1).

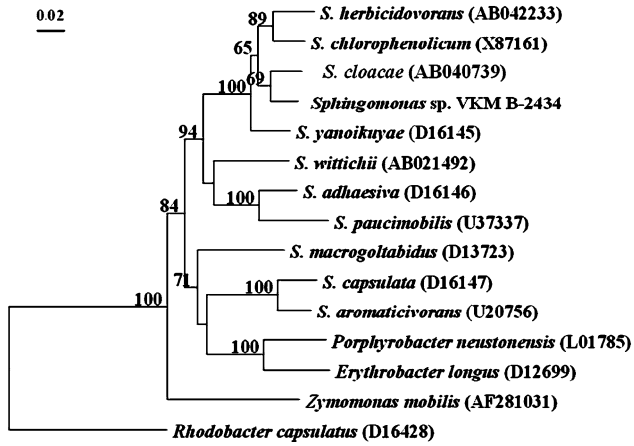

Fig. 1 Phylogenetic tree displaying the position of the VKM B-2434 strain among members of the genus *Sphingomonas*. The scale bar shows the distance equivalent to two substitutions per 100 nucleotides. Bootstrap values of 500 resamplings are shown at the branch points

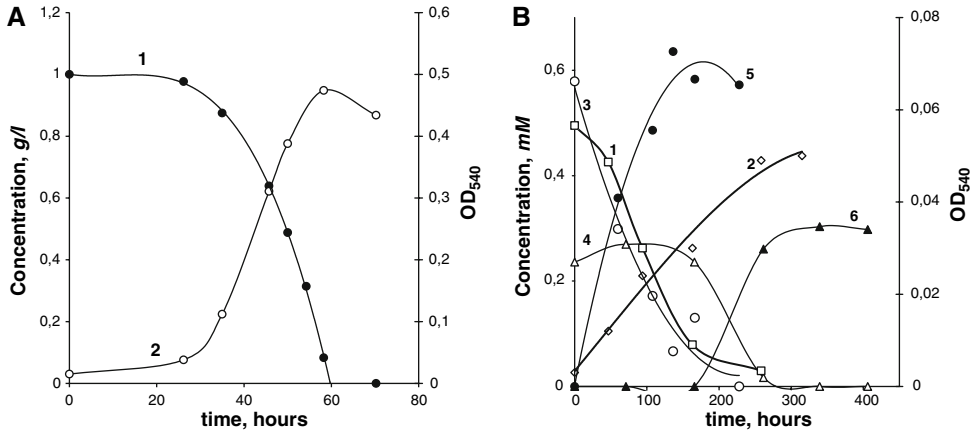

Fig. 2 Utilization of PAHs as a sole source of carbon and energy by *Sphingomonas* sp. VKM B-2434. (**a**) Degradation of acenaphthene (**1**) and growth of culture (**2**). The specific growth rate estimated by the substrate consumption curve was equal to 0.086 h^{-1}. (**b**) Degradation of fluoranthene (**1**) and growth of culture (**2**); conversion of phenanthrene (**3**) and anthracene (**4**); accumulation of 1-hydroxy-2-naphthoate (**5**) and 3-hydroxy-2-naphthoate (**6**) in process of phenanthrene and anthracene conversion, respectively. No changes of optical density (OD_{540}) were observed in sterile and carbon-free controls

The strain had relatively narrow metabolic capabilities. Most of the tested organic substrates (glucose, sucrose, citrate, malate, succinate, fumarate, hexadecane, propionate and benzoate) did not support growth. The only non-aromatic substance found that could be used as a sole source of energy and carbon was acetate. Catalytic quantities of vitamin B_{12} (less then 1 μg/l) were required for growth.

Utilization of PAHs as a sole source of carbon and energy

Sphingomonas sp. VKM B-2434 completely converted acenaphthene (more than 1 g/l), phenanthrene (up to 400 mg/l), anthracene (up to 80 mg/l), and fluoranthene (up to 100 mg/l) during incubation in MM with vitamin B_{12} and the respective PAH (Fig. 2). While conversion of naphthalene, phenanthrene, anthracene and fluoranthene was accompanied by a little accumulation of biomass (less than 0.05 OD units), a significant growth was observed when the strain was incubated in the medium with acenaphthene (Fig. 2a), the economical coefficient was 0.30 ± 0.04 ($P = 0.95$). Several products of PAH oxidation were identified (Table 1), and a metabolic diagram was proposed (Fig. 3). Oxidation of phenanthrene and anthracene was a highly selective process. As shown in Fig. 2b, approximately equivalent quantities of

Table 1 Products of PAH conversion by *Sphingomonas* sp. VKM B-2434

Substrate	Product	Identification
Naphthalene	Salicylic acid	UV-spectrum is identical to UV-spectrum of standard sample: three maxima at ~210, ~235 and ~300 nm (pH 2); and three isosbestic points at ~228, ~258 and ~298 nm
Phenanthrene	1-Hydroxy-2-naphthoic acid	Mass-spectrometric data[a]: M^+ 188(35), 189(3), 171(13), 170(100), 142(15), 115(61), 114(11). UV-spectrum and R_t(TLC) are identical to those of a standard sample
Anthracene	3-Hydroxy-2-naphthoic acid	Mass-spectrometric data[a]: M^+ 188(35), 189(4), 171(10), 170(90) 143(11), 142(100), 115(23), 114(84), 113(30). UV-spectrum and R_t(TLC) are identical to those of a standard sample
Fluoranthene	2-Hydroxy-1-acenaphthoic acid	Mass-spectrometric data[a]: M^+ 214(44), 196(100), 168(22), 140(54), 139(61), 115(13)
	Naphthalene-1,8-dicarboxylic acid	Mass-spectrometric data[b]: M^+ 198(55), 170(19), 154(100), 126(97)
Acenaphthene	Naphthalene-1,8-dicarboxylic acid	Mass-spectrometric data[c]: M^+ 244(39), 213(100), 185(27), 170(35), 154(11), 126(16), 114(19)
	3-Hydroxyphthalic acid	Mass-spectrometric data[a,c]: M^+ 224(19), 193(100), 165(6), 163(8), 161(13), 150(12), 134(9), 120(15)
Pyrene	Pyrene-4,5-dihydrodiol	Mass-spectrometric data: M^+236(69), 219(46), 218(100), 205(17), 189(67), 187(11), 176(12), 109(9), 95(25) UV-spectrum was identical to that of *cis*-4,5-pyrene dihydrodiol, published previously (Rehmann et al. 1998)
	Hydroxypyrene	Mass-spectrometric data: M^+218(100), 189(47), 187(11), 109(9), 94(18)
Fluorene	Fluorenone	UV-spectrum and R_t(TLC and GC) are identical to those of a standard sample
	Hydroxyfluorenone[d]	Mass-spectrometric data: M^+ 196(100), 168(34), 139(31), 113(6), 98(9), 84(7)
	Hydroxyfluorenone[d]	Mass-spectrometric data: M^+ 196(100), 168(6), 139(20), 113, 98, 84(7)
Benz[*a*]anthracene	Unidentified[e]	Mass-spectrometric data[a,c]: M^+252(56), 220(100), 192(50), 173(16), 164(38), 163(40), 135(8), 111(12), 60(45)
Chrysene	*o*-Hydroxyphenanthroic acid	Mass-spectrometric data[a,c]: M^+ 252(38), 220(100), 192(11), 164(30), 163(24), 149(28), 110(12), 60(55)
Benzo[*a*]pyrene	*o*-Hydroxypyrenoic acid	Mass-spectrometric data[a,c]: M^+276(46), 244(100), 216(17), 188(45), 187(18), 149(34), 94(20), 60(53)

[a] *Ortho*-effect was observed at the fragmentation of molecular ion

[b] Analyzed in the form of anhydride

[c] Analyzed after methylation

[d] Two different isomeres of hydroxyfluorenone were isolated

[e] According to mass-spectrometric data it can be *o*-hydroxyanthroic and *o*-hydroxyphenanthroic acids

1-hydroxy-2-naphthoate and 3-hydroxy-2-naphthoate were formed during phenanthrene and anthracene oxidation, respectively. Hydroxynaphthoic acids were isolated from the culture liquid by precipitation at low pH with hydrochloric acid. According to the TLC, HPLC, and mass spectrometry data, pure substances were obtained, and the yield of these compounds was about 70–80% as compared with the theoretical yield.

Co-metabolic conversion of PAHs

In the presence of fluoranthene (at an initial concentration of 100 mg/l) the culture was able to oxidize pyrene (27 mg/l at an initial concentration 50 mg/l), benz[*a*]anthracene (8 mg/l at an initial concentration 10 mg/l), fluorene (100 mg/l) and its heterocyclic analog carbazole (100 mg/l); fluoranthene was converted completely at a concentration of 100 mg/l.

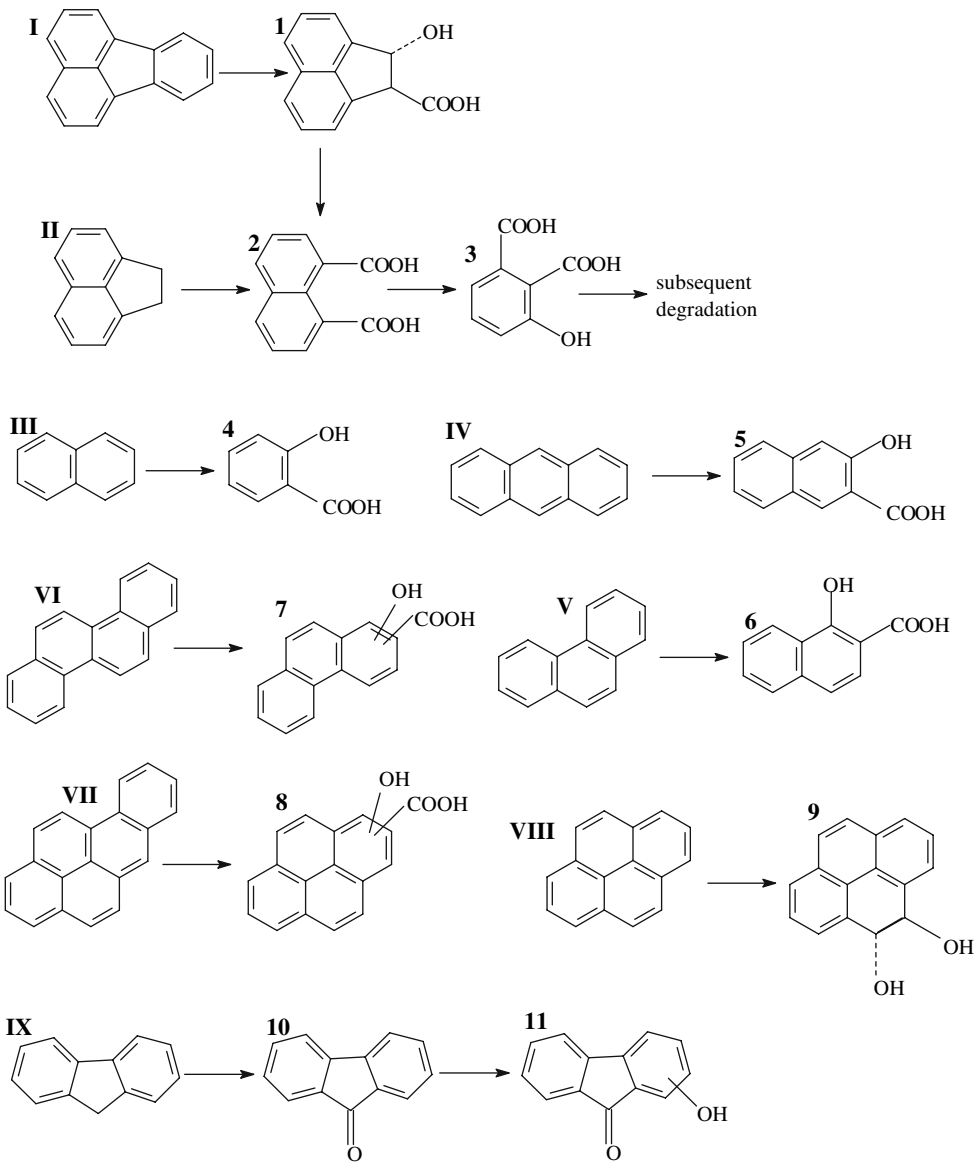

Fig. 3 Metabolic pathways of conversion of fluoranthene (**I**), acenaphthene (**II**), naphthalene (**III**), anthracene (**IV**), phenanthrene (**V**), chrysene (**VI**), benzo[*a*]pyrene (**VII**), pyrene (**VIII**) and fluorene (**IX**) by *Sphingomonas* sp. VKM B-2434. **1**, 2-hydroxy-1-acenaphthoic acid; **2**, naphthalene-1,8-dicarboxylic acid; **3**, 3-hydroxyphthalic acid; **4**, salicylic acid; **5**, 3-hydroxy-2-naphthoic acid; **6**, 1-hydroxy-2-naphthoic acid; **7**, *o*-hydroxyphenanthroic acid (both 1-hydroxy-2-phenanthroic acid and 2-hydroxy-1-phenanthroic acid according to mass-spectrometric data); **8**, *o*-hydroxypyrenoic acid (both isomers:1-hydroxy-2-pyrenoic acid and 2-hydroxy-1-pyrenoic acid according to mass-spectrometric data); **9**, 4,5-dihydroxypyrenediol; **10**, fluorenone; **11**, hydroxyfluorenone (two different isomeres)

The culture, growing in MM with phenanthrene, acenaphthene or acetate, also converted the PAHs mentioned above to a significant extent. Since no aromatic metabolites derived from acetate, use of acetate as a co-substrate gave us an opportunity to isolate and identify metabolites of fluorene, pyrene, benz[*a*]anthracene, chrysene and benzo[*a*]pyrene (Table 1, Fig. 3). Conversion of fluorene was accompanied by the accumulation of fluorenone followed by complete removal of the latter with the appearance of hydroxyfluorenone in the medium. Products of pyrene oxidation were identified as pyrene-4,5-

dihydrodiol and hydroxypyrene. An intermediate of benz[*a*]anthracene bioconversion was either *o*-hydroxyanthroic or *o*-hydroxyphenanthroic acid.

Despite considerably less activity towards chrysene and benzo[*a*]pyrene in comparison with other PAHs, we succeeded in isolating the products of chrysene and benzo[*a*]pyrene oxidation, which were shown to be *o*-hydroxyphenanthroic acid and *o*-hydroxypyrenoic acid, respectively, on the basis of mass-spectrometric data (Table 1). Absorption spectrum of the chrysene metabolite dimethyl derivative in methanol had maxima at 374, 356, 340, 301, 290 and 259 nm; absorption spectrum of the benzo[*a*]-pyrene metabolite dimethyl derivative in methanol had maxima at 424, 402, 348, 332, 318, 268 and 259 nm.

Conversion of polycyclic aromatic compounds of pitch extract

Sphingomonas sp. VKM B-2434 completely converted phenanthrene, fluoranthene and carbazole of pitch extract during incubation for 1 month in the medium with 2 g/l of pitch extract; a considerable decrease in the content of anthracene and conversion of pyrene also was observed (Fig. 4). Decrease of benz[*a*]anthracene, chrysene and benzo[*a*]pyrene was insignificant.

Discussion

Cultivation in a medium containing an extract of coal-tar pitch was used for the growth of enrichment culture, and a bacterial strain capable of degrading a diversity of PAHs was isolated. The new strain differed significantly by 16S rRNA nucleotide sequence (Fig. 1), physiological characteristics (narrow spectrum of used carbon sources, vitamin B_{12} requirement) and PAH conversion (discussed below) from the *Sphingomonas* strains known to degrade PAHs, such as *S. paucimobilis* EPA505 (Mueller et al. 1990), *S. yanoikuyae* 107 (Dagher et al. 1997), *Sphingomonas* sp. CO6 (Ho et al. 2000) and *Sphingomonas* sp. CHY-1 (Willison 2004).

Sphingomonas sp. VKM B-2434 showed a broad substrate specificity towards PAHs, and all tested PAH substrates were converted by the strain to a certain extent. Acenaphthene and fluoranthene were degraded via naphthalene-1,8-dicarboxylic acid and 3-hydroxyphthalic acid, which were previously described as metabolites (Weissenfels et al. 1991; Selifonov et al. 1993). Acenaphthene was converted

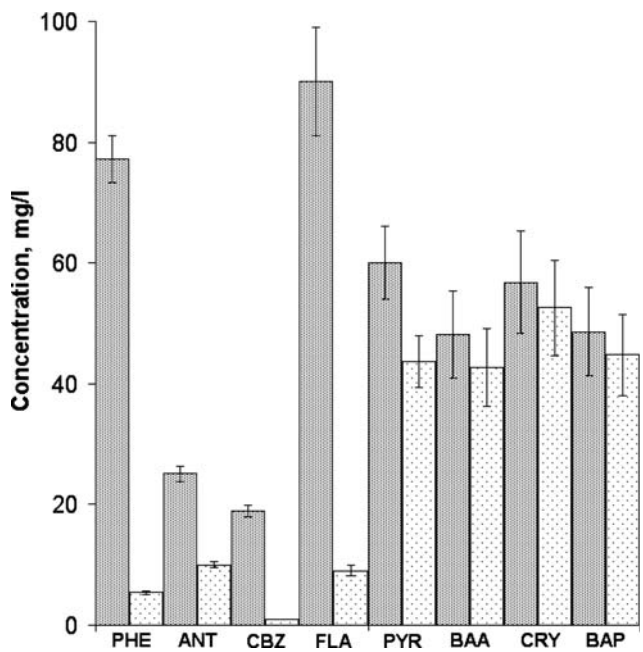

Fig. 4 Conversion of polyaromatic compounds—components of pitch extract by *Sphingomonas* sp. VKM B-2434. PHE, phenanthrene; ANT, anthracene; CBZ, carbazole; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CRY, chrysene; BAP, benzo[*a*]pyrene. Culture was incubated for 1 month. Residual PAH concentrations in sterile control (dark bars) and in *Sphingomonas* sp. VKM B-2434 culture (light bars) are shown in the figure. Averages are from triplicate experiment (±SD)

at a considerably higher concentration than fluoranthene, and the difference apparently concerned with the initial reactions of fluoranthene metabolism. 2-Hydroxy-1-acenaphthoic acid, which was known previously as a fluoranthene metabolite (Story et al. 2001), was the only intermediate of the initial reactions of fluoranthene metabolism isolated in this study.

While acenaphthene and fluoranthene were converted to a monoaromatic compound, which presumably was further degraded, conversion of most other PAHs was confined to the cleavage of only one aromatic ring. Phenanthrene and anthracene were quantitatively converted by *Sphingomonas* sp. VKM B-2434 to their corresponding hydroxynaphthoic acids, which were obtained with high yield. These products are usual intermediates of bacterial PAH metabolism (Habe and Omori 2003) and supposed to be degraded further by other bacteria present in PAH-contaminated ecosystems.

The strain oxidized naphthalene, phenanthrene, anthracene, benz[*a*]anthracene, chrysene, and benzo[*a*]pyrene in a similar manner, realizing *ortho*-cleavage of an aromatic ring. The observed reactions were presumably performed by the same enzymatic system, as shown for other PAH-degrading bacteria (Habe and Omori 2003).

Some of benzo[*a*]pyrene bacterial metabolites have been identified previously (Schneider et al. 1996; Moody et al. 2004), but this is the first report of formation of *o*-hydroxypyrenoic acid as a product of benzo[*a*]pyrene bioconversion. Chrysene-3,4-dihydrodiol was suggested to be the initial intermediate of the chrysene degradation pathway (Boyd et al. 1997), and *o*-hydroxyphenanthroic acid was probably a subsequent chrysene metabolite. A product of the benz[*a*]anthracene oxidation was also tricyclic hydroxycarboxylic acid, such the benz[*a*]anthracene metabolites described previously (Mahaffey et al. 1988). The *o*-hydroxycarboxylic acids, produced from different PAHs by *Sphingomonas* sp. VKM B-2434, seem to be suitable for subsequent enzymatic attack, unlike products of PAHs transformation by some other microorganisms, such as quinones (Cerniglia 1997) and monosubstituted PAH derivatives (Schneider et al. 1996; Moody et al. 2004).

Sphingomonas sp. VKM B-2434 co-metabolically oxidized fluorene, similar to other bacterial strains (Grifoll et al. 1992, 1994; Trenz et al. 1994; Casellas et al. 1998). Although fluorene co-metabolism was often confined to methylene group oxidation resulting in formation of toxic and recalcitrant fluorenone, the strain under study realized more extensive metabolism of fluorene. The culture also transformed pyrene under co-oxidative conditions, and this ability seems to be typical for sphingomonads (Ho et al. 2000), while nocardioform bacteria can use pyrene as a sole source of carbon (Rehmann et al. 1998). Hydroxypyrene, which was purified from the culture medium together with pyrene dihydrodiol, presumably was a product of a non-enzymatic dehydration of the latter. Similarly, the two different isomers of hydroxyfluorenone, detected in fluorene-incubated culture, probably derived from the corresponding fluorenone dihydrodiol. Products of transformation of fluorene (Casellas et al. 1998) and pyrene (Kazunga and Aitken 2000) were shown to inhibit the bacterial degradation of other PAHs, but *Sphingomonas* sp. VKM B-2434 can convert PAHs in mixture at a significant concentration that indicated no appreciable inhibition effect.

The presence of a surfactant in culture medium is often required for efficient PAH degradation by sphingomonads (Mueller et al. 1990; Shi et al. 2001), while the VKM B-2434 strain converted PAHs without surfactant addition.

Sphingomonas sp. VKM B-2434, transforming a wide range of polycyclic aromatic compounds, belongs to versatile PAH-degrading bacterial strains. It can be used for degradation of PAHs in contaminated soils and sediments, where the initial reactions of PAH oxidation often limit the rate of bioremediation. The strain also may be useful for chemical production by bioconversion, as it performed a selective oxidation of only one aromatic ring in most cases. In addition, the strain is good for investigation of PAH mixture bioconversion and high-molecular-weight PAHs degradation.

Acknowledgments This work was supported financially by Russian Education Grant N P1,2,06; RFBR-Ural N 07-04-97625, and NATO Collaborative Linkage Grant, ESP.NR.NRCLG981949.

References

Bastiaens L, Springael D, Wattiau P, Harms H, deWachter R, Verachtert H, Diels L (2000) Isolation of adherent

polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers. Appl Environ Microbiol 66:1834–1843

Boyd DR, Sharma ND, Agarwal R, Resnick SM, Schocken MJ, Gibson DT, Sayer JM, Yagi H, Jerina DM (1997) Bacterial dioxygenase-catalysed dihydroxylation and chemical resolution routes to enantiopure *cis*-dihydrodiols of chrysene. J Chem Soc Perkin Trans 1:1715–1723

Casellas M, Grifoll M, Sebate J, Solanas AM (1998) Isolation and characterization of a fluorenone-degrading bacterial strain and its role in synergistic degradation of fluorene by a consortium. Can J Microbiol 44:734–742

Cerniglia CE (1997) Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J Ind Microbiol Biotechnol 19:324–333

Dagher F, Deziel E, Lirette P, Paquette G, Bisaillon J-G, Villemur R (1997) Comparative study of five polycyclic aromatic hydrocarbon degrading bacterial strains isolated from contaminated soils. Can J Microbiol 46:368–377

Grifoll M, Casellas M, Bajona JM, Solanas AM (1992) Isolation and characterization of a fluorene-degrading bacterium: identification of ring-oxidation and ring-fission products. Appl Environ Microbiol 58:2910–2917

Grifoll M, Selifonov SA, Chapman PJ (1994) Evidence for a novel pathway in the degradation of fluorene by *Pseudomonas* sp. strain F274. Appl Environ Microbiol 60:2438–2449

Habe H, Omori T (2003) Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci Biotechnol Biochem 67:225–243

Ho Y, Jackson M, Yang Y, Mueller JG, Pritchard PH (2000) Characterization of fluoranthene- and pyrene-degrading bacteria isolated from PAH-contaminated soils and sediments. J Ind Microbiol Biotechnol 24:100–112

Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133:71–84

Juhasz AL, Naidu R (2000) Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of microbial degradation of benzo[*a*]pyrene. Int Biodeterior Biodegradation 45:57–88

Kanaly RA, Harayama S (2000) Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J Bacteriol 182:2059–2067

Kazunga C, Aitken MD (2000) Products of incomplete metabolism of pyrene by polycyclic aromatic hydrocarbon-degrading bacteria. Appl Environ Microbiol 66: 1917–1922

Mahaffey WR, Gibson DT, Cerniglia CE (1988) Bacterial oxidation of chemical carcinogens: formation of polycyclic aromatic acids from benz[*a*]anthracene. Appl Environ Microbiol 54:2415–2423

Molina M, Araujo R, Hodson RE (1999) Cross-induction of pyrene and phenanthrene in a *Mycobacterium* sp. isolated from polycyclic aromatic hydrocarbon contaminated river sediments. Can J Microbiol 45:520–529

Moody JD, Freeman JP, Fu PP, Cerniglia CE (2004) Degradation of benzo[*a*]pyrene by *Mycobacterium vanbaalenii* PYR-1. Appl Environ Microbiol 70:340–345

Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH (1990) Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl Environ Microbiol 56:1079–1086

Parales RE, Bruce NC, Schmid A, Wackett LP (2002) Biodegradation, biotransformation, and biocatalysis (B3). Minireview. Appl Environ Microbiol 68:4699–4709

Pinyakong O, Habe H, Kouzuma A, Nojiri H, Yamane H, Omori T (2004) Isolation and characterization of genes encoding polycyclic aromatic hydrocarbon dioxygenase from acenaphthene and acenaphthylene degrading *Sphingomonas* sp. strain A4. FEMS Microbiol Lett 238:297–305

Rehmann K, Noll HP, Steinberg CEW, Kettrup AA (1998) Pyrene degradation by *Mycobacterium* sp. Strain KR2. Chemosphere 36:2977–2992

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Schneider J, Grosser R, Jayasimhulu K, Xue W, Warshawsky D (1996) Degradation of pyrene, benz[*a*]anthracene, and benzo[*a*]pyrene by *Mycobacterium* sp. strain RJGII-135, isolated from a former coal gasification site. Appl Environ Microbiol 62:13–19

Selifonov SA, Slepenkin AV, Adanin VM, Grechkina GM, Starovoytov II (1993) Catabolism of acenaphthene by strains of *Alcaligenes eutrophus* and *Alcaligenes paradoxus*. Mikrobiologija 62:120–128

Shi T, Fredrickson JK, Balkwill DL (2001) Biodegradation of polycyclic aromatic hydrocarbons by *Sphingomonas* strains isolated from the terrestrial subsurface. J Ind Microbiol Biotechnol 26:283–289

Story SP, Parker SH, Hayasaka SS, Riley MB, Kline EL (2001) Convergent and divergent points in catabolic pathways involved in utilization of fluoranthene, naphthalene, anthracene, and phenanthrene by *Sphingomonas paucimobilis* var. EPA505. J Ind Microbiol Biotechnol 26:369–382

Stringfellow WT, Aitken MD (1995) Competitive metabolism of naphthalene, methylnaphthalene and fluorene by phenanthrene-degrading pseudomonads. Appl Environ Microbiol 61:357–362

Sutherland JB, Rafii F, Khan A, Cerniglia CE (1995) Mechanisms of polycyclic aromatic hydrocarbons degradation. In: Young LY, Cerniglia CE (eds) Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss, New York

Thompson JD, Plewniak TJ, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Trenz SP, Engesser KH, Fisher P, Knackmuss H (1994) Degradation of fluorene by *Brevibacterium* sp. strain DPO 1361: a novel C–C bond cleavage mechanism via 1,10-dihydroxyfluorene-9-on. J Bacteriol 176:789–795

Van de Peer Y, De Wachter R (1997) Construction of evolutionary distance trees with TREECON. Comput Appl Biosci 13:227–230

Weissenfels WD, Beyer M, Klein J, Rehm HJ (1991) Microbial metabolism of fluoranthene: isolation and identification of ring fission products. Appl Microbiol Biotechnol 34:528–535

Willison JC (2004) Isolation and characterization of a novel sphingomonad capable of growth with chrysene as sole carbon and energy source. FEMS Microbiol Lett 241: 143–150

Zhong Y, Luan T, Wang X, Lan C, Tam NFY (2007) Influence of growth medium on cometabolic degradation of polycyclic aromatic hydrocarbons by *Sphingomonas* sp. strain PheB4. Appl Microbiol Biotechnol 75:175–186