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Isolation and characterization of novel bacteria degrading polycyclic aromatic hydrocarbons from polluted Greek soils

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Abstract Three bacterial strains, designated as Wphe1, Sphe1, and Ophe1, were isolated from Greek soils contaminated with polycyclic aromatic hydrocarbon (PAH)-containing waste from the wood processing, steel, and oil refinery industries. Wphe1, Sphe1, and Ophe1 were characterized and identified as species of *Pseudomonas*, *Microbacterium*, and *Paracoccus*, respectively, based on Gram staining, biochemical tests, phospholipid analysis, FAME analysis, G+C content and 16S rRNA gene sequence analysis. The results of gas chromatography showed that strain Wphe1 degraded naphthalene, phenanthrene, and *m*-cresol over a wide temperature range; strain Sphe1 was a degrader of phenanthrene and *n*-alkanes; most interestingly, strain Ophe1 degraded anthracene, phenanthrene, fluorene, fluoranthene, chrysene, and pyrene, as well as cresol compounds and *n*-alkanes as sole carbon source. This is the first report of a representative of the genus *Paracoccus* capable of degrading PAHs with such versatility. These three strains may be useful for bioremediation applications.

Introduction

Recently, considerable efforts have focused on the isolation of microorganisms able to degrade polycyclic aromatic hydrocarbons (PAHs), in order to study the fate and accumulation of these compounds in natural environments. For instance, the US Environmental Protection Agency has monitored PAHs as priority pollutants in ecosystems since the 1970s (Kieth and Telliard 1979). PAHs are a group of compounds widely spread as pollutants, due to excessive use of products of hydrocarbon fuels, improper disposal of organic wastes from related industries, incomplete combustion of organic matter, etc. (Young and Cerniglia 1995; Nestler 1974; Beck et al. 1995). Many PAHs have highly toxic, mutagenic, or carcinogenic properties (Sato and Aoki 2002), but are quite recalcitrant to biodegradation. During the last few decades, a variety of bacteria capable of degrading PAHs, particularly low-molecular weight compounds (e.g. naphthalene and phenanthrene) have been discovered. Such bacteria belong to the genera *Agmenellum*, *Aeromonas*, *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Berjerinckia*, *Burkholderia*, *Corynebacterium*, *Cyclotrophicus*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Nocardioides*, *Pseudomonas*, *Lutibacterium*, *Rhodococcus*, *Streptomyces*, *Sphingomonas*, *Stenotrophomonas*, *Vibrio*, *Paenibacillus*, and others (Juhasz et al. 2000; Daane et al. 2002; Samanta et al. 2002; Van Hamme et al. 2003; and references there in). However, few bacteria are known to degrade higher-molecular weight PAHs, such as fluoranthene, pyrene, and benzo(α)pyrene. These include members of the genera *Bacillus*, *Burkholderia*, *Cycloclasticus*, *Flavobacterium*, *Pseudomons*, *Mycobacterium*, and *Stenotrophomonas* (Kanaly and Harayama 2000).

The present work describes the isolation and characterization of PAH-degrading bacteria from polluted Greek soils, including the first representative of *Paracoccus* able to utilize pyrene and other PAHs as sole carbon source.

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Materials and methods

Soil sampling

Soil samples were collected aseptically from three different polluted sites in Attica, Greece, in the vicinity of a steel industry, a wood processing plant, and an oil refinery, respectively. PAH-containing pollutants had been disposed without controls in these locations for more than 30 years.

Enrichment and isolation of bacterial strains

A soil sample (100 g) from each site was suspended in 500 ml of liquid minimal medium (MM) containing (per liter): $Na_2HPO_4 \cdot 2H_2O$ 8.5 g, KH_2PO_4 3.0 g, NaCl 0.5 g, NH_4Cl 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2$ 14.7 mg. MM also contained trace elements as follows (per liter): $CuSO_4$ 0.4 mg, KI 1.0 mg, $MnSO_4 \cdot H_2O$ 4.0 mg, $ZnSO_4 \cdot 7H_2O$ 4.0 mg, H_3BO_3 5.0 mg, $H_2MoO_4 \cdot 2H_2O$ 1.6 mg, $FeCl_3 \cdot 6H_2O$ 2.0 mg. Each soil-MM suspension was supplemented with 0.01% (w/v) phenanthrene as enrichment substrate and incubated with shaking at 180 rpm at 30°C in the dark. After 30 days continuous shaking without further transfers, the cultures were allowed to stand for 3 h to sediment the soil particles. Under these culturing conditions, turbidity was observed, as compared to similar suspensions kept at 2°C without shaking over the same period. An aliquot (200 μl) from each culture was transferred and spread on solid MM plates supplemented with phenanthrene, sprayed on the surface of the medium, as the sole carbon source (Kiyohara et al. 1982). After incubation for 7 days, colonies, especially those forming clear zones on the spray-coated plates, were selected as candidate phenanthrene-degrading strains (Kiyohara et al. 1982). All isolates were stored as liquid cultures containing 20% glycerol (v/v) at −80°C.

Carbon source utilization

In addition to phenanthrene, the purified strains were also tested for growth on one of the following compounds at 0.01% (w/v): naphthalene, acenaphthene, anthracene fluoranthene, fluorene, and pyrene, several *n*-alkanes, as well as other related substrates which were added as sole carbon sources to liquid MM (Table 1). Chrysene and benzo(α)pyrene were provided at 0.002% (w/v). Liquid MM (200 ml in 1-l conical flasks) containing the appropriate PAH or other carbon source was inoculated with the test strain and incubated in an orbital shaker as described above. Growth was followed by measuring the increase of biomass production (total protein).

For solid MM media, PAHs (except naphthalene) were dissolved in 5% (w/v) in diethyl-ether (0.2% for chrysene) and sprayed on the surface of the medium using a Desaga spraying device (Desaga, Germany). Naphthalene was provided as crystals directly placed on the plate lid. Biodegradation of PAHs in solid media was followed by the formation of a clear zone around the growing colonies or the appearance of pigments. All chemicals were of analytical grade and obtained from Sigma or Fluka.

PAH degradation assays

Each liquid culture (10 ml MM+0.01% PAH, or 0.04% naphthalene, or 0.002% chrysene) was inoculated with 100 μl pre-culture (Luria broth, OD_{600}=0.7, about 10^8 cells/ml) of each strain and incubated as described above. After the desired period of incubation, the entire culture was extracted twice with 5 ml dichloromethane. Organic-phase extractions were combined, dried over anhydrous sodium sulfate, and 1.0 μl of the organic phase was analyzed by gas chromatography (GC) (Shimadzu), FID mode, using a fused silica capillary column (30-m length × 0.25-mm ID, 0.25-μm film thickness, QUADREX) and nitrogen as the carrier gas. The temperature program was set as follows: 80°C for 1 min, then increasing by 5°C/min up to 240°C for 5 min. Degradation rate was estimated by calculating the GC profile of substrate PAH. Cultures inoculated with boiled dead cells were used in parallel as the abiotic negative controls.

Biochemical tests

Morphologic and phenotypic identification of the PAH-degrading bacteria was initially done by Gram-staining, antibiotic resistance

Table 1 Substrate profiles of the isolated polycyclic aromatic hydrocarbon (PAH)-degrading strains. Strains were grown in liquid minimal medium with the corresponding compound as sole carbon source as described in "Materials and methods." Growth was estimated by the biomass produced (mg total protein) after cultivation for 1 week. ++ Good growth (more than 0.3 mg/ml), + growth (0.1–0.3 mg/ml), +/− poor growth (less than 0.1 mg/ml), − no growth (initial inoculum 0.005 mg/ml)

Substrate	Wphe1	Sphe1	Ophe1
Naphthalene	++	−	−
Acenaphthene	−	−	−
Anthracene	−	−	+[a]
Phenanthrene	+[a]	+[a]	++[a]
Fluorene	−	−	+[a]
Fluoranthene	−	−	+[a]
Chrysene	−	−	+[a]
Pyrene	−	−	+[a]
Benzo(α)pyrene	−	−	−
Toluene	−	−	−
Benzene	−	−	−
Xylene	−	−	−
Phenol	−	−	−
m-Cresol	+/−	−	+
o-Cresol	−	−	+
p-Cresol	−	−	+
Methanol	−	−	++
Ethanol	−	−	++
1,2-Dichlorobenzene	−	−	−
2,4-Dinitrophenol	−	−	−
Pentachlorophenol	−	−	+/−
Catechol	+	−	−
Salicylic acid	+	+	+
Biphenyl	−	−	−
Quinoline	−	−	−
Hexane	−	+	+
Dodecane	−	+	+
Hexadecane	−	+	+
Octadecane	−	+	+
Octacosane	−	+	+
Indole	+	−	−
1-Hydroxy-2-naphthoic acid	+/−	+	+/−
9-Hydroxyfluorene	−	−	+
Phthalic acid	+	+	+
2-Carboxybenzaldehyde	−	+	+
9,10-Phenanthrenequinone	−	+	−
9-Fluorenone-1-carboxylic acid	−	−	−
Protocatechuic acid	−	+/−	+/−
9-Fluorenone	−	−	−

[a]Colonies forming halo on solid minimal medium supplemented with the corresponding substrate as sole carbon source (halo was not visible in naphthalene solid cultures because this substrate is highly volatile and therefore was provided as crystals on the lid of the Petri dish)

tests, and other classical tests. Nineteen biochemical tests (Cappuccino and Sherman 1996) were done as follows: oxidase, catalase and urease activity, Voges–Proskauer and methyl red reaction, dextrose, lactose and sucrose fermentation, hydrogen sulfide and indole production, citrate use, gelation liquefaction, starch, lipid and casein hydrolysis, nitrate reduction, lysine, arginine, and ornithine decarboxylation.

Enzyme assays

Cells were grown in liquid MM with phenanthrene as sole carbon source (200-ml cultures as described above) and pelleted by centrifugation. They were then suspended in 10-mM Tris–HCl, pH 7.5, containing 1-mM dithiothreitol, disrupted in a mini Bead Beater (Biospec Products, Oklahoma, USA) using zirconium beads (0.15-mm diameter) and centrifuged at 12,000 *g* for 30 min. The supernatant was used as the crude cell-free extract in the enzyme assays. 1-Hydroxy-2-naphthoate hydroxylase and salicylate hydroxylase activities were estimated with 1-hydroxy-2-naphthoate or salicylate as substrate, respectively, according to the method of Yamamoto et al. (1965), by measuring the decrease of NADH at 340 nm. 2-Carboxybenzaldehyde dehydrogenase was determined with 2-carboxybenzaldehyde as substrate by following NADH formation, as described by Kiyohara and Nagao (1978). Changes in NADH concentrations were monitored following the absorbance at 340 nm in a Shimadzu UV-Visible spectrophotometer. Specific activity is defined as μmol NADH per min per mg protein. Boiled crude extracts of cells were used as negative controls. Catechol 2,3-dioxygenase activity was identified as described by Zukofski et al. (1983). Colonies of cells that express catechol 2,3-dioxygenase become yellow within seconds after spraying with catechol solution (0.5 M), a colorless substrate that is converted by this enzyme to 2-hydroxymuconic semialdehyde. Naphthalene dioxygenase was detected by the formation of blue indigo pigmentation on MM plates containing 1 mM indole (Ensley et al. 1983).

Phospholipid and FAME analyses

Cells grown on Luria Broth were extracted according to the method of Bligh and Dyer (1959) as modified by Kates (1972). The total lipids were dissolved in chloroform/methanol (2:1) and stored under nitrogen at −20°C for phospholipid and fatty acid analysis. Phospholipids were detected essentially as previously described (Koukou et al. 1990) with the following modifications: the phospholipid fraction was separated by two-dimensional thin-layer chromatography on silica gel 60 F_{254} (Merk) with chloroform/methanol/water (65:25:4) in the first dimension and chloroform/acetic acid/methanol/water (72:25:5:1.5) in the second. Fatty acid methyl esters (FAMEs) were prepared according to the method of Morrison and Smith (1964) and analyzed by GC as described for the degradation assays, except that the oven temperature program was: 80°C for 1 min, increasing by 5°C/min up to 250°C for 30 min. Bacterial acid methyl esters mix (SUPELCO) was used as qualitative standard.

Determination of G+C content

G+C content of genomic DNA from the isolates was determined by the fluorimetric dye-binding method (Gerhardt et al. 1994) based on the differential binding of Hoechst 33258 and ethidium bromide to A+T and G+C regions of DNA, respectively. *Escherichia coli* DH5α (51%) *Agrobacterium tumefaciens* (60%) and *Pseudomonas aerugenosa* (67%) were used as standards.

Analyses of 16S rRNA gene sequences

For PCR amplification of 16S rRNA genes, genomic DNA isolated using the AquaPure Genomic DNA Isolation kit (BIO-RAD) served as template. At first, for all of the isolates, a short fragment (395 bp) of 16S rRNA genes was amplified with primers 984 forward, 5′-AACGCGAAGAACCTTAC-3′, and 1,378 reverse, 5′-CGGTGTGTACAAGGCCCGGGAAGG-3′, corresponding to the related positions of the *E. coli* 16S rRNA gene sequences. Additionally, for strain Ophe1 a larger, 1,451-bp fragment of 16S rRNA gene was also amplified with the primers 27 forward, 5′-AGAGTTTGATCMTGGCTCAG-3′, and 1,492 reverse, 5′-TACGGYTACCTTGTTACGACTT-3′, as described in the literature (Feng et al. 1997). The PCR mixtures were preheated at 95°C for 5 min prior to running the following 30 cycles: 94°C for 1 min, 53°C for 1 min, 72°C for 1 min. At the end of the final cycle, a chain-elongation step at 72°C for 10 min was programmed. The amplified fragments were cloned using a TA cloning kit (Invitrogen) according to the manufacturer's instructions, and sequenced by MWG Biotech (Germany).

Taxonomic analysis was conducted with the BLAST program on the NCBI website (http://www.ncbi.nlm.nih.gov). Phylogenetic trees were constructed using the MegAlign program (DNASTAR).

Sequence access numbers

The sequences obtained in this study were deposited in the GenBank database. The accession numbers for the three short fragments are AY325767 (strain Sphe1), AY325768 (strain Wphe1), and AY325769 (strain Ophe1). The accession number for the long 16S rDNA fragment of strain Ophe1 is AY325770.

Results and discussion

Isolation of PAH-degrading bacteria

A total of 18 bacterial strains were isolated from the enriched contaminated soil samples, essentially on the basis of the formation of clear zones on solid MM with sprayed phenanthrene as the sole carbon source. Monitoring of clear-zone formation provides a simple and effective first-hand evidence of the ability of bacterial colonies to degrade PAHs (Kiyohara et al. 1982). However eight strains lost their ability to degrade any PAH after 4 months preservation. Three strains, designated as Wphe1, Ophe1, and Sphe1, stably retained their ability to degrade various PAHs as well as a number of other carbon substrates and were the focus of this work. The remaining strains were not studied further.

Carbon-source utilization of the isolates

Strains Wphe1, Ophe1, and Sphe1 were tested for their ability to grow on a variety of carbon sources, including various low and high molecular weight PAHs, as well as other simple aromatic hydrocarbons and *n*-alkanes. These chemicals represent the most common organic pollutants and are the main components of crude oil (Harayama et al. 1999). Strain Wphe1 was able to degrade naphthalene, phenanthrene, and *m*-cresol. Additionally, strain Wphe1 formed blue indigo pigmentation in the presence of indole,

indicating naphthalene dioxygenase activity (Ensley et al. 1983). Strain Sphe1 degraded phenanthrene and all of the tested *n*-alkanes. Simultaneous utilization of both PAHs and aliphatic hydrocarbons by a bacterial strain has been rarely reported (Bogan et al. 2003). Strain Ophe1 exhibited a very broad substrate profile, being able to utilize anthracene, phenanthrene, fluorene, fluoranthene, chrysene, and pyrene as sole carbon and energy source, as well as cresol compounds and aliphatic hydrocarbons containing up to 28 carbon atoms (Table 1). In contrast, five-membered benzene ring PAHs or simple aromatic hydrocarbons, such as toluene or xylene, were not utilized as carbon sources by any of these strains. The possibility that these compounds were toxic to the cells was excluded because all of the strains grew on glucose-containing media in their presence.

Evidence for the relevant pathways was obtained by testing the degradation of intermediate compounds. All three strains were able to degrade salicylic and phthalic acid, both intermediates of phenanthrene degradation by two pathways (Kiyohara and Nagao 1978). Furthermore, strain Ophe1 could utilize as a carbon source 9-hydroxyfluorene and 2-carboxybenzaldehyde, which are intermediates of fluoranthene and phenanthrene, respectively (Kastner 2000). Based on the utilization of intermediate metabolites (Table 1) and the specific activities of some PAH-pathway-related enzymes estimated in crude extracts of cells grown on phenanthrene as sole carbon source (Table 2), we propose that Sphe1 and Ophe1 degrade phenanthrene via both the phthalate and salicylate pathways (Kiyohara and Nagao 1978). Strain Wphe1 apparently utilizes naphthalene and phenanthrene via the salicylate pathway because it grows on salicylate as sole carbon source and possesses activities of naphthalene dioxygenase, 1-hydroxy-2-naphthoate hydroxylase and salicylate hydroxylase (Table 2). Additionally it was found that Wphe1 exhibits also catechol 2,3-dioxygenase activity. Although Wphe1 metabolizes phthalate as well as salicylate, we believe that the phthalate pathway is not functional in this strain, because of its inability to grow on 2-carboxybenzaldehyde. In parallel with this result, strain Wphe1 lacks 2-carboxybenzaldehyde dehydrogenase activity (Table 2). The detection of both pathways, especially in strain Ophe1, may account for the ability of these strains to degrade a wide range of PAHs. However, more work is required to elucidate the biochemistry of PAH utilization by these bacteria.

Table 2 Specific activities of PAH catabolic-pathway-related enzymes. *I* 1-Hydroxy-2-naphthoate hydroxylase, *II* salicylate hydroxylase, *III* 2-Carboxybenzaldehyde dehydrogenase. Specific activities are duplicates of three independent repeats with a standard error less than 5%. *ND* Activity not detectable

Strains	Specificactivity (μmol/min mg protein)		
	I	II	III
Wphe1	0.14	0.11	ND
Sphe1	0.10	0.07	0.04
Ophe1	0.12	0.12	0.02

Degradation assays

The results of GC analyses demonstrated that strain Wphe1 completely degraded naphthalene and phenanthrene within 32 h and 8 days, respectively; strain Sphe1 completely degraded phenanthrene within 6 days; and strain Ophe1 completely degraded phenanthrene, fluoranthene, chrysene and pyrene within 3, 20, 16 and 12 days, respectively. The degradation rates for each of the above PAH substrates were estimated (Table 3). As shown in Fig. 1 for one substrate for each bacterium, the biomass increased as the remaining concentration of the substrate decreased (data for the other substrates and strains not shown for clarity). The ability of Ophe1 to degrade mixtures of all PAH substrates of its capacity was also tested in order to investigate the potential presence of inhibitors or inducers of degradation in the mixture. The same degradation rates were estimated for all PAHs in the mixtures as shown in Table 2, except for chrysene for which the rate was increased from 0.013 to 0.025 mg/day. Therefore, the degradation of chrysene by Ophe1 is facilitated almost twofold when other PAHs are present, implying the induction of some specific pathway.

Taxonomic identification of the isolates

Strain Wphe1 was found to be a gram-negative rod, motile, and resistant to ampicillin at a concentration of 100 μg/ml. Its growth temperature on naphthalene or phenanthrene ranged from 4 to 37°C with an optimum at 30°C. The bacterium produced fluorescent pigmentation on King's A and King's B media (Bano and Musarrat 2003), was decarboxylase, dextrose and catalase positive, and grew on citrate, gelatin and nitrate. The major phospholipids were phosphatidylethanolamine (PE) (84.1%) and phosphatidylglycerol (PG) (7.1%). The main fatty acids were 16:0 (38.0%), $16{:}1^{9}$ (34.7%) and $18{:}1^{9}$ (*cis*-) (25.1%). G+C content of Wphe1 genomic DNA was 60%. Analysis of 16S rRNA gene sequence

Table 3 Degradation rates of the strains Wphe1, Sphe1 and Ophe1 for various PAHs when provided as sole carbon sources. Values of rates are given as milligrams of PAH degraded per day. –: Not degradable.

Substrates	Wphe1	Sphe1	Ophe1
Naphthalene	5.333	–	–
Anthracene	–	–	0.500
Phenanthrene	0.125	0.166	0.333
Fluorene	–	–	0.100
Fluoranthene	–	–	0.050
Pyrene	–	–	0.083
Chrysene	–	–	0.013

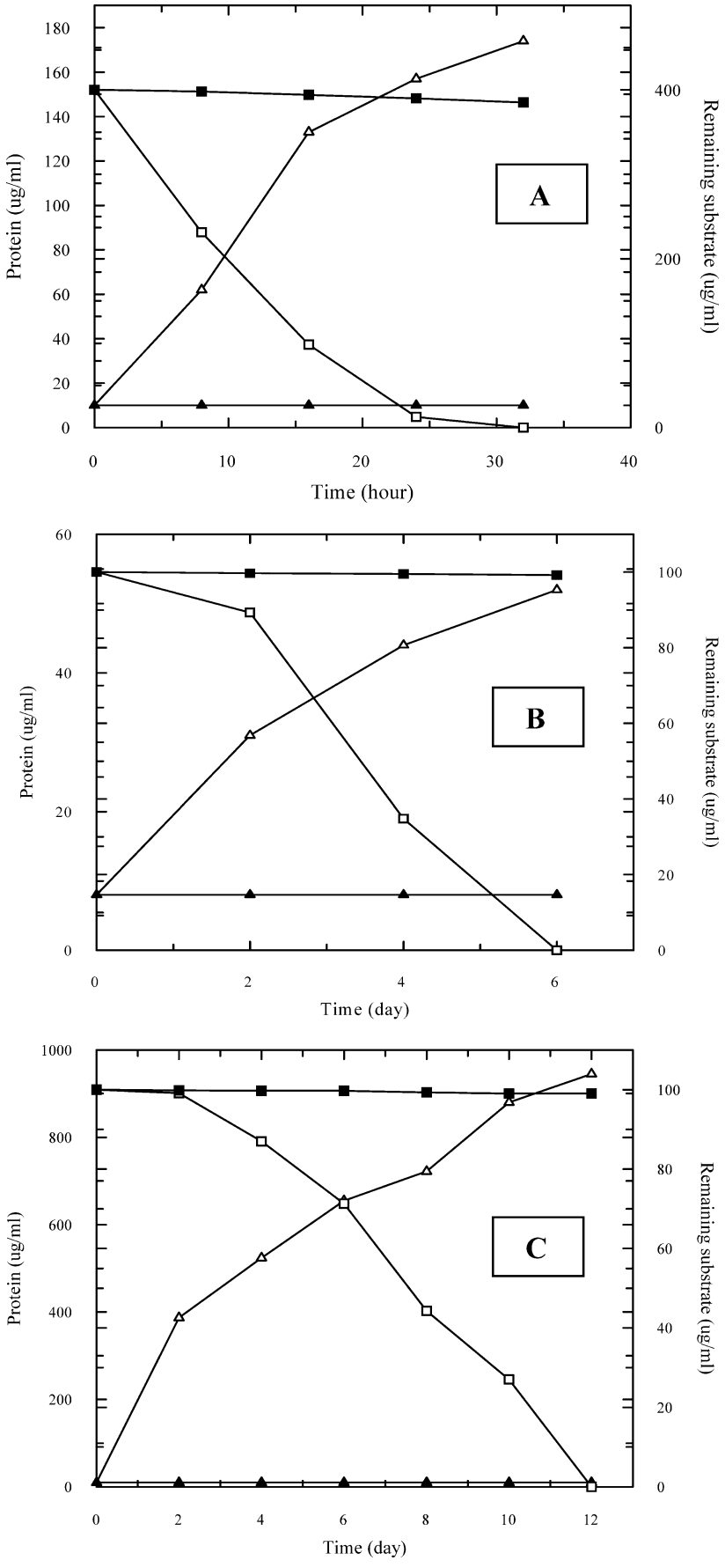

Fig. 1 PAH degradation and biomass production by strains Wphe1, Sphe1 and Ophe1 in liquid MM cultures in the presence of polycyclic aromatic hydrocarbons (PAHs). **a** Strain Wphe1 grown on 0.04% (w/v) naphthalene, **b** strain Sphe1 grown on 0.01% (w/v) phenanthrene, **c** strain Ophe1 grown on 0.01% (w/v) pyrene. *Triangles* Biomass production as total protein (μg/ml), *squares* concentration of remaining substrate (μg/ml), *open symbols* cultures inoculated with living cells, *solid symbols* cultures inoculated with dead cells. Each value represents the mean of four repeats with a standard error <5%

indicated that strain Wphe1 belongs to the genus *Pseudomonas* (99% identity) with nearest type strain *P. mediterranea* CFBP5447 (Fig. 2a). Thus far, *Pseudomonas*, first discovered by Migula in 1895 (Nakazawa et al. 1996), is now the most well studied PAH-degrading genus. Normally, *Pseudomonas* has been reported to utilize single low-molecular-weight PAH like naphthalene or phenanthrene and rarely both (Kang et al. 2003). Our results demonstrate that Wphe1 can degrade naphthalene and phenanthrene over a wide range of temperatures. This ability makes the strain attractive for field bioremediation applications.

Strain Sphe1 was found to be a gram-positive rod, with optimum growth on phenanthrene at 30°C. It possessed catalase activity and grew on dextrose, sucrose and nitrate, and was sensitive to all of the test antibiotics. The Major phospholipids were: PG (76.0%) and PE (16.0%). Fatty acids mainly contained α-15:0 (59.0%), 17:0$^{\Delta}$ (12.3%), and i-16:0 (10.0%). The G+C content of strain Sphe1 genomic DNA is 65%. Analysis of 16S rRNA gene sequence identified this strain as *Microbacterium* sp.

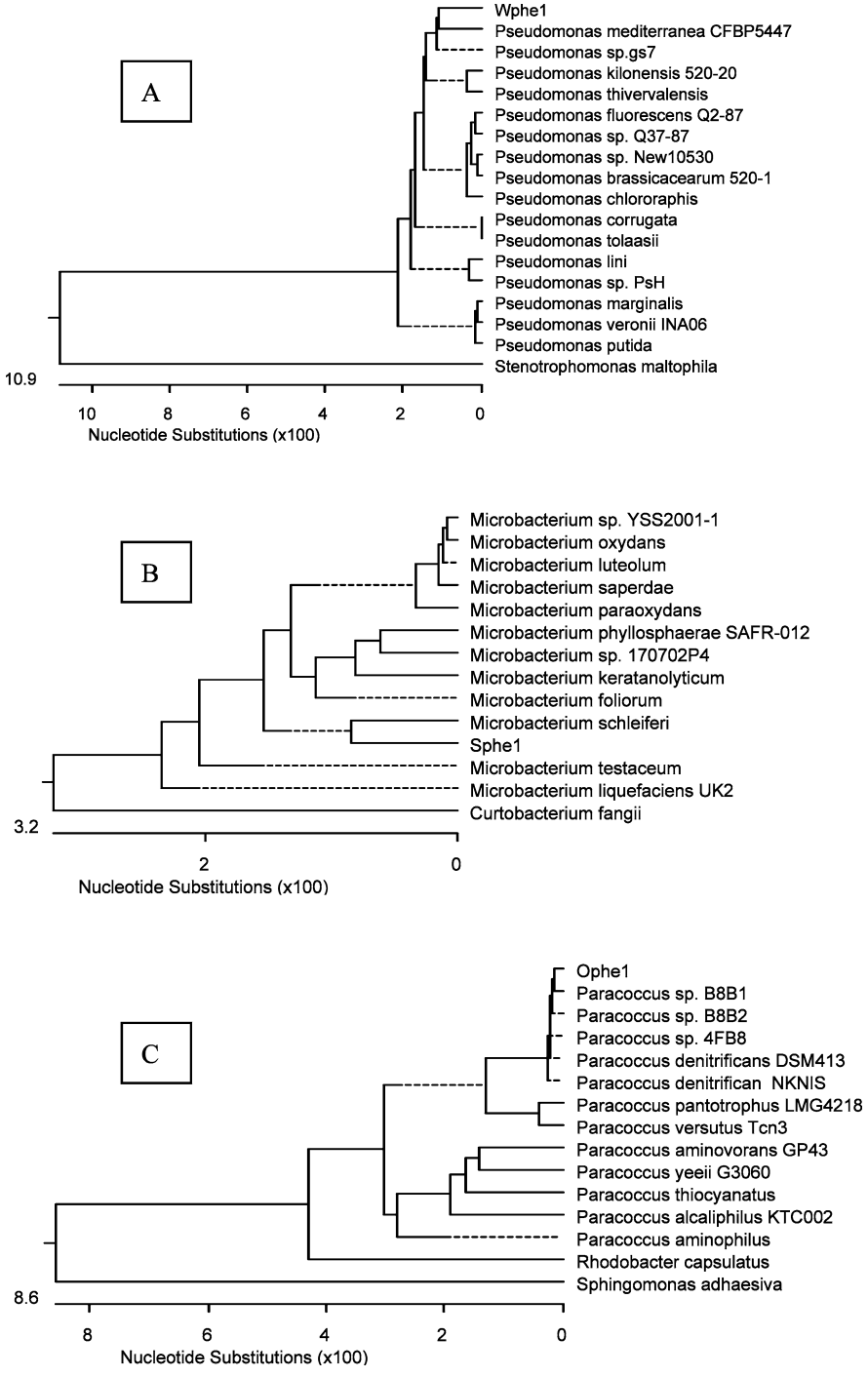

Fig. 2 Phylogenetic trees for the taxonomic location of strains **a** Wphe1, **b** Sphe1 and **c** Ophe1. This dendrogram was produced by the MegAlign software program of DNASTAR

(closest species *M. schleiferi*, 99% identity, Fig. 2b) supporting the recently reported results that representatives of this genus might also be PAH-degrading (Gauthier et al. 2003).

Strain Ophe1 was found to be a gram-negative coccus, resistant to streptomycin (100 μg/ml) and kanamycin (50 μg/ml), but sensitive to ampicillin (100 μg/ml), tetracycline (20 μg/ml), chloramphenicol (50 μg/ml) and rifampicin (20 μg/ml). This strain grew well on its PAH substrates from 25 to 37°C with an optimum at 30°C. Catalase, dextrose, and nitrate tests were positive. The major phospholipids were: PE (52.7%) and PG (23.4%). FAME analysis showed that $18{:}1^{9}$ (*cis*-) (74.1%) and 16:0 (19.6%) were the main components of fatty acids. The G +C content of strain Ophe1 genomic DNA was 64%. Taxonomical identification based on both short and long 16S rRNA gene sequence analyses indicated that strain Ophe1 belongs to the genus *Paracoccus*, with closest type strain *Paracoccus* sp. B8B1 (99% identity). Our data on FAME analysis parallel those reported before for this genus (Baj 2000). The position of strain Ophe1 in the phylogenetic tree is presented in Fig. 2c. The first species of this genus, which belongs to the α-3 subgroup of the Proteobacteria, was isolated by Beijerinck and Minkman in 1910, designated as *Micrococcus denitrificans* and renamed later as *Paracoccus denitrificans* by Davis et al. (1969). It has been reported that *Paracoccus* spp. metabolize a variety of simple organic compounds, including ethanol (Chanprateep et al. 2001) and methanol (Ras et al. 1995). Our studies extend the substrate range of *Paracoccus* sp. from the simple compounds to the utilization of *n*-alkanes and PAHs as sole carbon sources as well. Moreover, up to now, almost all pyrene and chrysene degraders encountered in natural environments have been gram-positive bacteria (Gauthier et al. 2003; Walter et al. 1991). Recently, gram-negative strains have also been reported (Juhasz et al. 2000), but they only co-metabolized pyrene and chrysene upon addition of other organic chemicals (Ye et al. 1996) and did not degrade them as a sole carbon source, as described here for strain Ophe1.

Conclusions

Following the enrichment procedure described above, several soil bacteria capable of degrading PAHs were isolated. Strains Wphe1, Sphe1 and Ophe1, which were found to belong to *Pseudomonas* sp., *Microbacterium* sp. and *Paracoccus* sp., respectively, are of particular interest because of their broad substrate profile and growth temperature range. In particular, strain Ophe1, the first representative of the genus *Paracoccus* able to utilize low as well as high molecular weight PAHs as sole carbon source, could also degrade cresol compounds and *n*-alkanes. All three bacterial strains, and especially strain Ophe1, may prove to be promising microorganisms for bioremediation to remove PAH-containing pollutants from contaminated sites.

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