World J Microbiol Biotechnol (2014) 30:335–341
DOI 10.1007/s11274-013-1437-7

SHORT COMMUNICATION

Biodegradation of anthracene by a novel actinomycete, *Microbacterium* sp. isolated from tropical hydrocarbon-contaminated soil

Lateef B. Salam · Oluwafemi S. Obayori · Nojeem O. Olatoye

Received: 11 March 2013 / Accepted: 15 July 2013 / Published online: 24 July 2013

Abstract A novel anthracene-degrading Gram-positive actinomycete, *Microbacterium* sp. strain SL10 was isolated from a hydrocarbon-contaminated soil at a mechanical engineering workshop in Lagos, Nigeria. The polluted soil had an unusually high total hydrocarbon content of 157 g/kg and presence of various heavy metals. The isolate tolerated salt concentration of more than 4 %. It resisted cefotaxime, streptomycin and ciprofloxacin, but susceptible to meropenem, linezolid and vancomycin. The isolate exhibited growth rate and doubling time of 0.82 $days^{-1}$ and 0.84 days, respectively on anthracene. It degraded 57.5 and 90.12 % of anthracene within 12 and 21 days, respectively while the rate of anthracene utilization by the isolate was 4.79 mg l^{-1} d^{-1}. To the best of our knowledge, this is the first report of isolation and characterization of anthracene-degrading *Microbacterium* sp.

Keywords Biodegradation · Anthracene · Hydrocarbon-contaminated soil · *Microbacterium*

Introduction

Anthracene is a low molecular weight polycyclic aromatic hydrocarbon. It is a regulated contaminant at sites polluted with petroleum. It also results from anthropogenic pyrolysis of petroleum products and incomplete combustion of organic matter (Moody et al. 2001). High concentrations of anthracene (up to 13,000 mg/kg) have been detected in agricultural soils in the oil-rich Niger Delta region of Nigeria (Oviasogie et al. 2006). Anthracene is highly recalcitrant, in part because of its low solubility in water (0.015 mg/l) accounted for by its high aromaticity compared with phenanthrene or pyrene (Cerniglia 1992; Garcia-Cruz and Martinez-Magadan 2007).

Although not known to exhibit any genotoxic or carcinogenic effects in humans, anthracene shares structure with known carcinogens such as benzo(a)anthracene and is useful in modeling the metabolism of such counter-part PAH (Cerniglia and Shuttleworth 2002). Anthracene is degraded in the environment mainly by bacteria and fungi. It has been demonstrated long ago that bacteria capable of degradation of anthracene can easily be isolated from soil (Evans et al. 1965 Cerniglia 1992). Degraders of anthracene reported in the literature span wide range of genera of both Gram-positive and Gram-negative bacteria, including *Alcaligenes*, *Arthrobacter, Beijerinckia*, *Micrococcus*, *Mycobacterium, Nocardia, Pseudomonas*, *Rhodococcus* and *Sphingomonas* (Kastner et al. 1994; Bastiaens et al. 2000; Ilori and Amund 2000; Dean-Ross et al. 2001; Mrozik et al. 2003). It is worthy of note that many of the early reports detailed degradation by *Pseudomonas* species such as *P. putida*, *P. cepacia*, *P. paucimobilis* and *P. fluorescence* (Foght and Westlake 1988; Mueller et al. 1990; Cerniglia 1992; Dagher et al. 1997).

However, in recent years, there has been increase in the number of reports of novel anthracene degraders, including actinomycetes, and even rhizosphere bacteria such as *Bacillus circulans* and *Kurthia* sp. SBA4 (Bisht et al. 2010). Others include *E. coli* and *Thiobacter subterraneus* (Abd-Elsalam et al. 2009).

Actinomycetes are a group of diverse bacteria that are Gram positive with high guanine plus cytosine in the DNA (>55 mol %). They are good candidates for bioremediation of polluted soils due to their production of extracellular

L. B. Salam (✉) · O. S. Obayori · N. O. Olatoye
Department of Microbiology, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria
e-mail: babssalaam@yahoo.com

enzymes that degrade a wide range of complex organic compounds, production of spores that are impervious to dessication and filamentous growth that favors colonization of soil particles (Ensign 1992). Particularly, actinomycetes are good candidates for degradation of hydrophobic compounds such as PAHs due to their surfactant-producing activity. The biosurfactant may be extracellular such as glycolipids (trehalose lipid and lipopeptide produced by *Rhodococcus* and *Arthrobacter* species) or cellular biosurfactants such as mycolic acid, which allow adherence of the microbial cells to hydrophobic phases in two-phase systems (Singer and Finnerty 1990; Morikawa et al. 1993; Neu 1996).

The initial reactions in the aerobic degradation of anthracene are catalysed by a multi-component enzyme system and involve the incorporation of other atoms of oxygen molecules into the PAH nucleus to produce a dihydrodiol. The dihydrodiol is re-aromatised and the dihydroxylated product is cleaved to products, which are further processed through the pathways of naphthalene degradation (Moody et al. 2001; Mrozik et al. 2003; Van Herwijnen et al. 2003). In some of the anthracene degraders so far reported, the genes for anthracene metabolism have been found to be localized on plasmids (Sanseverino et al. 1993; Ilori and Amund 2000; Kumar et al. 2010).

In recent years, there has been a remarkable shift in focus from isolation to demonstration of organisms capable of degrading PAHs in soil and other environmental compartments using molecular methods and other non-culture dependent techniques (Stach and Burns 2002). However, this has not obfuscated the need for continuous search for culturable organisms. As noted by Hilyard et al. (2008), on the one hand, such isolates offer opportunity for examination in order to elucidate links between phylogeny and specific catabolic capability and could as well be sources of novel or highly divergent PAH degrading enzymes. Secondly, such new strains may be useful in bioaugmentation of PAH-contaminated sites for bioremediation. In this paper, we report for the first time, to the best of our knowledge, the isolation and characterization of a *Microbacterium* species strain able to use anthracene as sole source of carbon and energy.

Materials and methods

Sampling

Soil samples for this study were collected from a Mechanical engineering workshop at Okokomaiko, Lagos. The coordinates of the sampling site was latitude 6° 79′N, longitude 3° 31′E, respectively. The site has a long history of contamination with indiscriminately disposed spent oils spanning a period of more than 10 years. Soil samples were collected at a depth of 10–12 cm with sterile trowel after clearing debris from the soil surface. Samples for physico-chemical analyses were collected in polyethylene bags, while those for microbiological analyses were collected in sterile screw-capped bottles. Samples were analyzed immediately upon arrival in the laboratory. Leftover samples were refrigerated at 4 °C.

Physico–chemical analysis of soil samples

The pH of the soil samples was determined with a pH meter (Jenway 3051) in 1:1 soil solution in distilled water. The moisture content, organic content, total nitrogen content, potassium content and available phosphorous were determined as described previously (Bray and Kurtz 1945; Black 1965; Chopra and Kanwar 1998). Conductivity was determined using a conductivity cell (PW 9504 Philips) with a cell constant of 1.2 while the heavy metal content of the soils was determined using atomic absorption spectrophotometer (α 4, AAS) following mixed acid digestion and extraction of the soil samples. The total hydrocarbon content of the soil was extracted using n-hexane:dichloromethane solvent systems (1:1).

Enrichment and isolation of anthracene-degrading bacteria

Bacteria able to degrade anthracene were isolated on anthracene mineral salts medium (MSM) by continual enrichment method. The mineral salts medium described by Mills et al. (1978) and modified by Okpokwasili and Amanchukwu (1988) was used. The medium contained the following in g/l of distilled water: NaCl, 10.0; KCl, 0.29; $MgSO_4 \bullet 7H_2O$, 0.42; KH_2PO_4, 0.83; K_2HPO4, 1.25; $NaNO_3$, 0.42; and Na_2HPO_4, 1.25. The medium was supplemented with yeast extract (0.005 g/l) as source of growth factors. After adjusting the pH to 7.0, the medium was fortified with 50 μg/ml and 20 μg/ml of nystatin and nalidixic acid for selective isolation of actinomycetes.

Air-dried contaminated soil (5 g) was added to 45 ml of MSM containing 50 ppm of anthracene. Enrichment was carried out by incubation with shaking (180 rpm) at room temperature (29 ± 2 °C) in the dark for 4–5 weeks until there was turbidity. After five consecutive transfers, anthracene degraders were isolated by plating out dilutions from the final flasks on Luria–Bertani (LB) agar. The colonies that appeared were further purified by subculturing once onto LB agar. Ability to degrade anthracene was confirmed by inoculating washed LB broth grown culture in fresh MSM flask supplemented with 50 ppm anthracene as sole carbon source. One isolate out of the four screened was singled out for further study based on its extensive

degradative ability. The isolate was maintained at −20 °C in glycerol: LB broth medium (50:50).

Identification and characterization of isolate

Pure culture of bacterial isolate was identified based on their colonial morphology, cellular morphology and biochemical characteristics according to the identification scheme of Bergey's Manual of Determinative Bacteriology (Holt et al. 1994).

Antibiotic sensitivity of the isolate was carried out using multidiscs. Salt tolerance was tested in LB broth amended with NaCl ranging from 1 to 10 % (w/v). Incubation was carried out at room temperature (27 ± 2 °C) for one week with shaking and daily observation for growth.

Genomic DNA was isolated from the isolate using standard protocols (Ausubel et al. 1990). Using the purified genomic DNA as template, the gene coding for 16S rRNA was amplified using the universal primers 27f (5′-AGA GTTTGATC{A/C}TGGCTCAG-3′) and 1378r (5′-CGG TGTGTACAAGGCCCGGGAACG- 3′) (Heuer et al. 1997). The amplification reaction mixture contained 20 pmol each of universal primers, 10 µl of Ex *Taq* buffer (Mg^{2+} plus), 2.5 mm of each dNTPs, 2.5 U (0.5 µl) of Ex *Taq* polymerase (Takara) and 1.0 µl of purified genomic DNA in a total volume of 100 µl. Thermocycling conditions consisted of an initial denaturation step at 95 °C for 3 min, followed by 30 amplification cycles of 95 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min. The final elongation temperature was maintained at 72 °C for 7 min before cooling to 4 °C.

The amplicon was analyzed on 1 % agarose gel in 1xTAE, ran at 100 V for 30–35 min. The PCR product (~1,500 bp DNA fragment) was cloned into the plasmid vector pT7Blue® (Novagen, USA) after purification by centrifugation using Wizard® SV Gel and PCR Clean-up System (Promega, Madison, Wis.). The purified PCR product was transformed into *E coli* strain DH5α (Toyobo). The cloned fragment was partially sequenced using T7 (5′-TAATACGACTCACTATAGGG- 3′) (Sigma Genosys, Japan) and U-19mer (5′-GTTTTCCCAGTCACGACGT-3′) (Invitrogen) sequencing primers along with f2L' and R2L' primers (Sigma Genosys, Japan) that anneals with the inside of the insert.

Nucleotide sequencing was carried out by the chain termination method using the ABI Prism 3730xl DNA Sequencer (Applied Biosystems, UK) according to the manufacturer's instructions. The 16S rRNA nucleotide sequence obtained from both strands was aligned (CLUSTAL W) and the homology search for 16S rRNA were performed in the DDBJ/EMBL/GenBank database using the Basic Local Alignment Search Tool (BLAST) program (Altschul et al. 1990). Phylogenetic tree with retrieved sequences from NCBI database was constructed using neighbor joining algorithm within the program MEGA 5.1 (The Biodesign Institute) and bootstrapped with 100 repetitions.

Evaluation of anthracene biodegradation

Anthracene degradation potential of the pure isolate was assayed by inoculating 250-ml replicate flasks containing 50 ml of MSM supplemented with 50 ppm of anthracene as sole source of carbon. Flasks were inoculated with 0.5 ml of MSM-washed 18–24 h LB agar-grown cells and subsequently incubated at 180 rpm in the dark for 21 days at room temperature (29 ± 2 °C).

Controls consisted of flasks supplied with anthracene as carbon and energy source as described above but inoculated with heat-killed cells. Samples were withdrawn from each flask at 3 days interval and aliquots of appropriate dilutions were plated (in triplicates) onto nutrient agar for total viable counts

Extraction of residual anthracene

Residual anthracene was extracted by liquid–liquid extraction. Briefly, broth culture (20 ml) was extracted twice with an equal volume of hexane. After removing the aqueous phase with separating funnel, the organic fraction was concentrated to 1 ml and the residual anthracene concentration was determined by gas chromatography. Control flasks were also extracted similarly.

Analytical method

Hexane extracts (1.0 µl) of residual anthracene were analyzed with Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector (FID) and 30 m long HP-5 column (internal diameter, 0.25 mm; film thickness, 0.25 µm). The carrier gas was nitrogen. The injector and detector temperatures were maintained at 300 and 320 °C respectively. The column temperature was programmed from 60 to 500 °C for 27 min. The gas chromatograph column was programmed at an initial temperature of 60 °C; this was held for 2 min, and then ramped at 12 °C/min to 205 °C and held for 16 min. Nitrogen column pressure was 37 psi, the hydrogen pressure was 9 psi and compressed air pressure was 13 psi. The software was Chem Station. Rev. A. 05. 01.

Statistical analysis

Mean generation times (T_d) and growth rate (K) of the isolate on anthracene was calculated using non-linear regression of growth curves for the period when growth

rates were maximal using Prism version 5.0 (Graphpad software, San Diego, CA).

Results

Physico-chemical properties of the soil

Physico-chemical analysis of the soil sample used for enrichment revealed a weakly acidic pH (6.10) with a moisture content of 6.85 %. It also showed an unusually high total hydrocarbon content (157 g/kg). The detection of various heavy metals such as lead, zinc, iron, manganese and nickel in significant concentration further suggest heavy contamination of the sampling site (Table 1).

Characterization and identification of anthracene-degrading isolate

Anthracene-degrading strain SL10 is a Gram-positive bacterium with circular, smooth, translucent, bright yellow-pigmented colonies. The organism was catalase positive but negative for oxidase, methyl red, Voges-Proskauer, indole and H_2S production. It showed positive reaction to starch hydrolysis and utilized sugars such as arabinose, cellobiose, fructose, galactose, glucose, mannose, maltose, sucrose and trehalose. It also produced acids from the fermentation of xylose, rhamnose and sucrose. The isolate tolerated salt concentration of more than 4 %. It resisted cefotaxime, ciprofloxacin and streptomycin, but was susceptible to meropenem, linezolid and vancomycin (Table 2).

Comparison of the 16S rDNA partial fragments of strain SL10 (1376 bp) with the nucleotide sequences in the DDBJ/EMBL/GenBank database indicates significant alignments of the strain with *Microbacterium* species. Strain SL10 exhibited 99 % homology with the nucleotide sequences of almost all *Microbacterium* spp. in the database. Worthy of note is the 99 % clustering of the nucleotide sequence of strain SL10 with the sequences of hydrocarbon-degrading *Microbacterium* spp. such as *Microbacterium* sp. PHD-5 (DQ227343.1), *Microbacterium* sp. CQ0110Y (DQ852355.1) and *Microbacterium* sp. ADAT-G (JF834172), respectively (Fig. 1). The nucleotide sequence of *Microbacterium* sp. strain SL10 was deposited at the DDBJ/EMBL/GenBank database and assigned the accession number AB646581.2.

Kinetics of anthracene biodegradation

The growth kinetics of strain SL10 on anthracene is depicted in Fig. 2 and Table 3. The strain exhibited a slight lag phase followed by consistent increase in cell density with concomitant decrease in anthracene concentration. Strain SL10 grew exponentially from an initial cell density of 4.2×10^5 cfu/ml to 8.2×10^9 cfu/ml in 12 days. It thereafter maintained a decreasing trend. During the exponential growth of the isolate on anthracene, it exhibited a growth rate and doubling time of 0.82/day and 0.84 days respectively.

Gas chromatographic analysis of residual anthracene showed that strain SL10 degraded 57.5 % of the anthracene in 12 days. At the end of the 21-day cultivation period, 90.12 % of the anthracene was consumed by strain SL10. In the heat-killed control flasks, no apparent decrease of the substrate was observed, thus affirming that anthracene depletion from the MSM was due to biodegradation by strain SL10 rather than to non-specific abiotic losses. It is however noteworthy that in spite of decline in growth observed from day 15; anthracene biodegradation persists throughout the experimental period.

Table 1 Physicochemical properties of hydrocarbon-contaminated sampling site

Parameter	Sampling site
pH	6.10
Moisture (%)	6.85
Conductivity (μs/cm)	318
Total organic carbon (%)	1.93
Total hydrocarbon content (mg/kg)	157067.9
Potassium (mg/kg)	2.10
Nitrogen (%)	0.10
Phosphorus (mg/kg)	1.34
Iron (mg/kg)	2.27
Lead (mg/kg)	0.11
Zinc (mg/kg)	3.31
Manganese (mg/kg)	1.83
Nickel (mg/kg)	4.34

Table 2 Antibiotic sensitivity pattern of anthracene-degrading strain SL10

Antibiotic	Isolate (SL10)
Streptomycin (30 μg)	R
Erythromycin (15 μg)	S
Vancomycin (30 μg)	S
Doxycycline (30 μg)	S
Meropenem (10 μg)	S
Linezolid (30 μg)	S
Cefotaxime (30 μg)	R
Ciprofloxacin (5 μg)	R
Amoxycilin (30 μg)	R
Pefloxacin (30 μg)	S

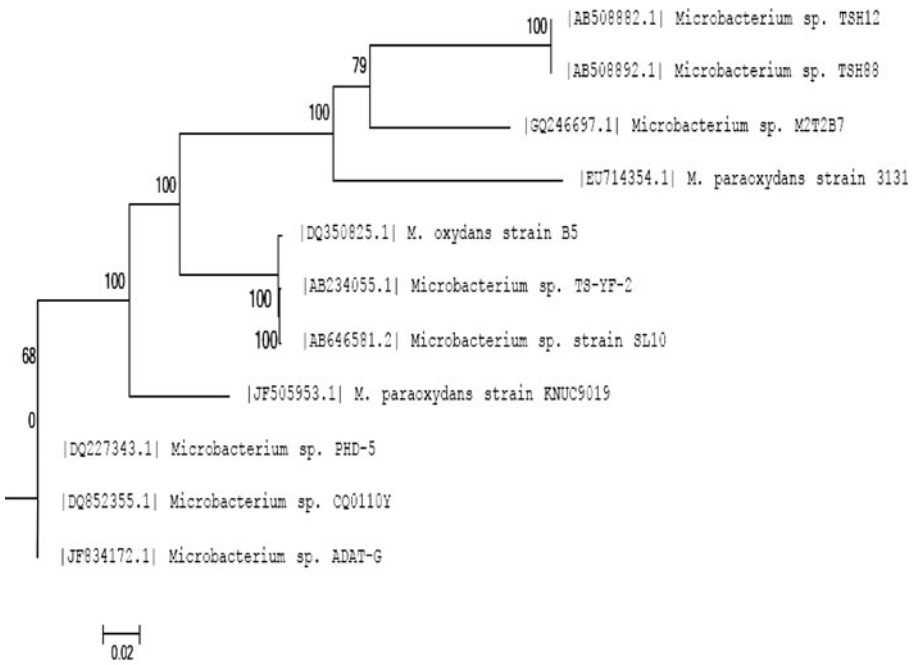

Fig. 1 Phylogenetic tree resulting from neighbour-joining (NJ) analysis of 16S rRNA showing the phylogenetic positions of anthracene-degrading *Microbacterium* sp. strain SL10 (AB646581.2) and related species of *Microbacterium* retrieved from NCBI GenBank. Accession number of each microorganism used in the analysis is shown before the species name. The *scale bar* indicates the numbers of nucleotide substitutions per position. Bootstrap values (expressed as percentage of 100 replicons) are shown at the branch

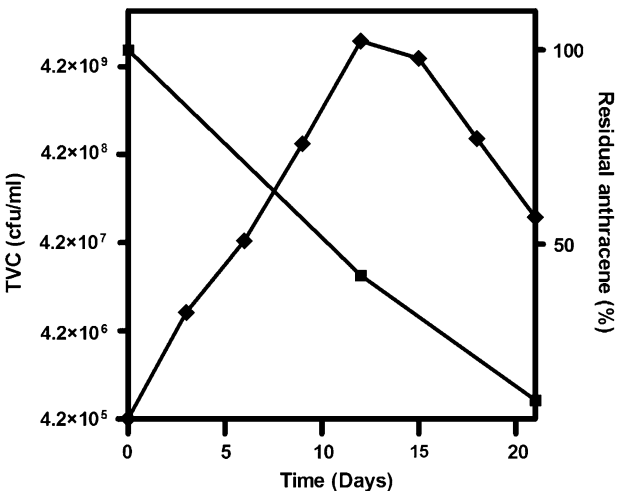

Fig. 2 Growth dynamic of *Microbacterium* sp. strain SL10 in minimal medium supplemented with 50 mg l^{-1} anthracene showing total viable count, TVC (*diamond*) and residual anthracene (*square*). Data points represent the mean of three replicate flasks. In the case of population counts, *error bars* that represent standard deviation were removed for clarity. Residual anthracene were determined with reference to anthracene recovered from heat-killed controls

Discussion

PAHs are ubiquitous environmental contaminants with deleterious biological effects including acute toxicity, mutagenicity and carcinogenicity (Cerniglia 1992). Their low bioavailability, hydrophobicity, and bioaccumulation potential are premised on their high degree of conjugation and aromaticity (Nkansah et al. 2011).

In this study, anthracene, a tricyclic PAH was degraded by a Gram-positive, high G + C actinomycete, *Microbacterium* sp. strain SL10, isolated from a heavily polluted hydrocarbon contaminated site. Previously, Ilori and Amund (2000) isolated four anthracene-degrading bacteria namely *P. aeruginosa*, *Alcaligenes eutrophus*, *Bacillus subtilis* and *Micrococcus luteus* from crude oil polluted soil in Lagos, Nigeria.

The ability of *Microbacterium* sp. strain SL10 to grow at salt concentration higher than 4 % could favour their use for bioaugmentation purpose as salinity has been regarded as a very important factor that determine the survival of bacterial inoculum seeded to soils (Kastner et al. 1998; Obayori et al. 2008). Microorganisms produce antibiotics as a survival strategy and for competitive edge in a nutrient limiting, highly compacted and diverse contaminated soil environment. As such, knowledge of sensitivity and resistance patterns of organisms with potentials for use as seeds for bioremediation is important. Resistance of strain SL10 to streptomycin, cefotaxime and ciprofloxacin may be attributed to acquisition of resistant genes to these antibiotics through gene transfer, as soil environments are replete with these antibiotics, which could allow evolution of resistance by indigenous strains (Obayori et al. 2008).

Strain SL10 degraded 90.12 % of the initial concentration of anthracene (50 ppm) in 21 days with a rate of degradation of 4.79 mg l^{-1} day^{-1} and growth rate and doubling time of 0.82 day^{-1} respectively. This degradation rate is lower than 92 % reported for *Mycobacterium* sp strain PYR-1 after 14 days of incubation (Moody et al. 2001). However, the degradation rate and rate of

Table 3 Growth kinetics of strain SL10 on anthracene

Isolate	Growth rate, K (day^{-1})	Mean generation time, ΔT_d (day)	% Degradation[1] (day 12)	% Degradation[1] (day 21)	Degradation rate (%/day)	Rate of degradation ($mg\ l^{-1}d^{-1}$)
Microbacterium sp. strain SL10	0.82	0.84	57.5	90.12	4.29	4.79

[1] Percent degradation values represent the net decrease (FID area counts) calculated with reference to the amount recovered from heat-killed control tubes

degradation of this strain is higher than 56 and 71 % (3.90 mg l^{-1} day^{-1}) after 48 days reported for two anthracene-degrading *Pseudomonas* strains isolated from a petrochemical sludge landfarming site (Jacques et al. 2005). It is equally higher than the 87.5 and 86.6 % reported for rhizosphere bacteria *Bacillus circulans* SBA12 and *Kurthia* sp. SBA4 respectively by Bisht et al. (2010).

The ability of *Microbacterium* sp. strain SL10 to degrade anthracene may be attributed to the fact that the strain was isolated from a site where spent oils with its attendant PAHs and heavy metals are indiscriminately dumped and disposed allowing the organism to adapt and evolve necessary gene battery to degrade pollutants and also resist the presence of heavy metals. This underscores the role of vertical (point mutation, DNA rearrangement by IS elements) and horizontal evolution (plasmid transfer, conjugative transposons, integrons and genomic islands) in the emergence of degradative pathways by microorganisms (van der Meer et al. 1992; Top and Springael 2003; Obayori and Salam 2010).

The phylum Actinobacteria encompasses bacteria genera such as *Mycobacterium*, *Rhodococcus*, and *Gordonia* with unrivalled capability to degrade recalcitrant pollutants due to their metabolic versatility, genetic plasticity and ability to survive in harsh environments (Larkin et al. 2005; Mutnuri et al. 2005; Kanaly and Harayama 2010). In fact, Dean-Ross et al. (2001) reported anthracene degradation by a *Rhodococcus* sp. that utilized 53 % of 3 μg/ml within 24 h with concomitant production of two ring fission products as metabolites.

Information on biodegradative abilities of *Microbacterium* spp is relatively new as the genus is a known human opportunistic pathogen. However, two crude oil degrading *Microbacterium* spp. identified as *M. oleivorans* and *M. hydrocarbonoxydans* were isolated by Schippers et al. (2005) from oil storage cavern 126 and oil contaminated soil in Germany. Similarly, Manickam et al. (2006) reported a hexachorocyclohexane-degrading *Microbacterium* sp. ITRCI capable of degrading four major isomers of the toxic compound. Furthermore, four bioemulsifier-producing *Microbacterium* strains were isolated from oil-contaminated mangrove with heavy metals removal abilities (Aniszewski et al. 2010). In this study, phylogenetic analysis of strain SL10 shows 99 % homology with a phenol-degrading *Microbacterium* PHD-5 strain (DQ227343.1), a di-2-ethylhexylphthalate-degrading *Microbacterium* CQ0110Y strain (DQ852355.1), and acenaphthene-degrading *Microbacterium* ADAT-G strain (JF834172), respectively. This shows the evolutionary relationship between strain SL10 and other hydrocarbon-degrading *Microbacterium* strains isolated elsewhere.

Though reports on hydrocarbon degrading abilities of *Microbacterium* spp. are gaining momentum, to the best of our knowledge, this is the first report highlighting anthracene degradative ability of a *Microbacterium* strain from tropical hydrocarbon-contaminated soil. Further research works will focus on the metabolic pathway employed by strain SL10 for anthracene degradation and the degradative genes involved in the process.

Acknowledgments The author expresses his sincere gratitude to Prof. Hideaki Nojiri of Biotechnology Research Centre, University of Tokyo for the opportunity given to conduct the Phylogenetic characterization of strain SL10.

References

Abd-Elsalam HE, Hafez EE, Hussain AA, Ali AG, El-Hanafy AA (2009) Isolation and identification of three rings polyaromatic hydrocarbons (anthracene and phenanthrene) degrading bacteria. Am Eurasian J Agric Environ Sci 5:31–38

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410

Aniszewski E, Peixoto RS, Mota FF, Leite SGF, Rosado AS (2010) Bioemulsifier production by *Microbacterium* sp. strains isolated from mangrove and their application to remove cadmium and zinc from hazardous industrial residue. Brazilian J Microbiol 41:235–245

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1990) Current protocol in molecular biology, 3rd edn. Green Publishing Associates and Wiley-Interscience, New York

Bastiaens L, Springael D, Wattiau P, Harms H, de Watcher 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

Bisht S, Pandey P, Sood A, Sharma S, Bisht NS (2010) Biodegradation of naphthalene and anthracene by chemotactically active rhizobacteria of *Populus detoides*. Brazilian J Microbiol 41:922–930

Black CA (1965) Methods of Soil Analysis No. 9, Part 2. American Society of Agronomy, Madison, Wisconsin

Bray RH, Kurtz LT (1945) Determination of total organic and available forms of phosphorus in soils. Soil Sci 59:39–45

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

Cerniglia CE, Shuttleworth KL (2002) Methods for isolation of polycyclic aromatic hydrocarbons (pahs)–degrading microorganism and procedures for determination of biodegradation intermediates and environmental monitoring of pahs. In: Hurst CJ, Crawford RL, Knudson GR, MClnerny J, Stetzenbach LD (eds) Manual of Environmental Microbiology, 2nd edition edn. ASM Press, Washington D.C, pp 972–986

Chopra SL, Kanwar JS (1998) Analytical agricultural chemistry. MacMillian Press, London

Dagher F, Déziel E, Lirette P, Paquette G, Bisaillon JG, Villemur R (1997) Comparative study of five polycyclic aromatic hydrocarbon degrading bacterial strains isolated from contaminated soils. Can J Microbiol 43:368–377

Dean-Ross D, Moody JD, Freeman JP, Deorge DR, Cerniglia CE (2001) Metabobism of anthracene by *Rhodococcus* species. FEMS Microbiol Lett 204:205–211

Ensign JC (1992) Introduction to the actinomycetes. In: the Prokaryotes. a handbook on the biology of bacteria, ecophysiology, isolation, identification, application (A. Ballows, HG Truper, M Dworkin, W Harder, KH Schleifer, eds). Springer-Verlag, New York pp. 811–815

Evans WC, Fernley HN, Griffiths F (1965) Oxidative metabolism of phenanthrene and anthracene by soil pseudomonads: the ring fission mechanism. Biochem J 95:819–831

Foght JM, Westlake DWS (1988) Degradation of polycyclic aromatic hydrocarbons and aromatic heterocycles by *Pseudomonas* species. Can J Microbiol 34:1135–1141

Garcia-Cruz I, Martinez-Magadan JM (2007) DFT molecular dynamics study of pyrene radical species. Petrol Sci Technol 25:67–80

Heuer H, Kresk M, Baker P, Smalla K, Wellington EM (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63:3233–3241

Hilyard EJ, Jones-Meehan JM, Spargo BJ, Hill RT (2008) Enrichment, isolation, and phylogenetic identification of polycyclic aromatic hydrocarbon-degrading bacteria from Elizabeth River Sediments. Appl Environ Microbiol 74:1176–1182

Holt JG, Krieg NR, Sneath PHA (1994) Bergey's manual of determinative bacteriology. William and Wilkins, Baltimore

Ilori MON, Amund OO (2000) Degradation of anthracene by bacteria isolated from oil polluted tropical soils. Z Naturforsch C 55: 890–897

Jacques RJS, Santos EC, Bento FM, Peralba MCR, Selbach PA, Sá ELS, Camargo FAO (2005) Anthracene biodegradation by *Pseudomonas* sp. isolated from a petrochemical sludge landfarming site. Int Biodet Biodegrad 5:143–150

Kanaly RA, Harayama S (2010) Advances in the field of high molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. Microbiol Biotechnol 3(2):132–164

Kastner M, Breuer-Jammali M, Mahro B (1994) Enumeration and characterisation of the soil microflora from hydrocarbon-contaminated soil sites able to mineralise polycyclic aromatic hydrocarbons. Appl Microbiol Biotechnol 41:267–273

Kastner M, Breuer-Jammali M, Mahro B (1998) Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Appl Environ Microbiol 64: 359–362

Kumar G, Singla R, Kumar R (2010) Plasmid associated anthracene degradation by *Pseudomonas* sp. isolated from filling station site. Nature Sci 8(4):89–94

Larkin MJ, Kulakov LA, Allen CC (2005) Biodegradation and *Rhodococcus*- masters of catabolic versatility. Curr Opin Biotechnol 16:282–290

Manickam N, Mau M, Schlömann M (2006) Characterization of the novel HCH-degrading *Microbacterium* sp. ITRC1. Appl Microbiol Biotechnol 69:580–588

Mills A, Brenil LC, Colwell RR (1978) Enumeration of petroleum degrading marine and estuarine microorganisms by the most probable number method. Can J Microbiol 24:552–557

Moody JD, Freeman JP, Doerge DR, Cerniglia CE (2001) Degradation of phenanthrene and anthracene by cell suspensions of *Mycobacterium* sp. strain PYR-1. Appl Environ Microbiol 67:1476–1483

Morikawa M, Daido H, Takao T, Murata S, Shimonishi Y, Imanaka T (1993) A new lipopeptide biosurfactant produced by Arthrobacter sp. strain MIS38. J Bacteriol 175:6459–6466

Mrozik A, Piotrowska-Seget Z, Labuzek S (2003) Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. Polish J Environ Stud 12(1):15–25

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

Mutnuri S, Vasudevan N, Kaestner M (2005) Degradation of anthracene and pyrene supplied by microcrystals and non-aqueous-phase liquids. Appl Microbiol Biotechnol 67:569–576

Neu TR (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol Rev 60: 151–166

Nkansah MA, Christy AA, Barth T (2011) The use of anthracene as a model compound in a comparative study of hydrous pyrolysis methods for industrial waste remediation. Chemosphere 84(4):403–408

Obayori OS, Salam LB (2010) Degradation of polycyclic aromatic hydrocarbons: role of plasmids. Sci Res Essays 5(25):4093–4106

Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Amund OO (2008) Pyrene-degradation potentials of Pseudomonas species isolated from polluted tropical soils. World J Microbiol Biotechnol 24:2639–2646

Okpokwasili GC, Amanchukwu SC (1988) Petroleum hydrocarbon degradation by *Candida* species. Environ Int 14:243–247

Oviasogie PO, Ukpebpor EE, Umoti U (2006) Distribution of polycyclic aromatic hydrocarbons in rural agricultural wetland soils of the Niger Delta Region. Afr J Biotechnol 5(15): 1415–1421

Sanseverino J, Applegate BM, King JMH, Sayler GS (1993) Plasmid-mediated mineralization of naphthalene, phenanthrene, and anthracene. Appl Environ Microbiol 59:1931–1937

Schippers A, Bosecker K, Spröer C, Schumann P (2005) *Microbacterium oleivorans* sp. nov. and *Microbacterium hydrocarbonoxydans* sp. nov., novel crude-oil-degrading Gram-positive bacteria. Int J Syst Evol Microbiol 55:655–660

Singer ME, Finnerty WR (1990) Physiology of biosurfactant synthesis by *Rhodococcus* sp H13-A. Can J Microbiol 36:741–745

Stach JEM, Burns RG (2002) Enrichment versus biofilm culture: a functional and phylogenetic comparison of polycyclic aromatic hydrocarbon-degrading microbial communities. Environ Microbiol 4(3):159–182

Top EM, Springael D (2003) The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. Curr Opin Biotechnol 14:262–269

van der Meer JR, DE VOS WM, Harayama S, Zehnder AJB (1992) Molecular mechanisms of genetic adaptation to xenobiotic compounds. Microbiol Rev 56(4):677–694

Van Herwijnen R, Spingael D, Slot P, Govers AJ, Parsons JR (2003) Degradation of anthracene by *Mycobacterium* sp. strain LB50 IT proceeds via a novel pathway, through O-phthalic acid. Appl Environ Microbiol 69:186–190