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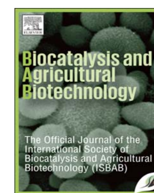

Biodegradation potentials of polyaromatic hydrocarbon (pyrene and phenanthrene) by *Proteus mirabilis* isolated from an animal charcoal polluted site

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ABSTRACT

Indiscriminate disposal of animal charcoal from skin and hides cottage industries often impact the environments with toxic hydrocarbon components and thus require eco-friendly remedial strategies. A bacterial strain isolated from a site polluted with animal charcoal was characterized, identified as *Proteus mirabilis* 10c, and studied for ability to degrade pyrene and phenanthrene. The bacterium resisted 30 µg chloramphenicol, 10 µg ampicillin, 30 µg amoxicillin and 10 µg perfloxacin; while it utilized a number of polycyclic aromatic hydrocarbons and cinnamic acid. Specific growth rate on pyrene and phenanthrene were 0.281 d^{-1} and 0.276 d^{-1}, respectively. Kinetics of degradation of pyrene was 87.92 $mg\,l^{-1}$ in 30 days at the rate of 2.93 $mg\,l^{-1}\,d^{-1}$, biodegradation constant at 0.073 d^{-1} and half-life of 9.50 d. The corresponding values for phenanthrene degradation kinetics by the bacterium were 90.12 $mg\,l^{-1}$, 3.02 $mg\,l^{-1}\,d^{-1}$, 0.079 d^{-1} and 8.77 d, respectively. Efficient degradation of crude oil (92.3%) in chemically defined medium was evident with near-disappearance of most aromatic spectra in 30 days. Considering its unique physiologies and broad specificities for aromatic and aliphatic hydrocarbons, the bacterium has potentials for decommissioning environments contaminated with toxic components of animal charcoal.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), also known as arenes, are large family of non-functionalised aromatic compounds containing either two or more fused benzene rings. They are derived from natural and anthropogenic sources, like combustion and pyrolysis, making them abundant in the environment. They are created when substances such as coal, oil, gas and organic matter/waste are burned incompletely. At high temperature, organic compounds are partially cracked to smaller unstable fragments, mostly radicals that recombine to give relatively stable PAHs. Also, aromatisation at lower temperatures (100–150 °C) requiring much more time produces large quantities of alkylated PAHs (Moret and Conte, 2000). The main sources of PAHs in economically less developed countries, as found in Sub-Saharan Africa, include domestic combustion of wood, coal, or straw, bush burning and steppe fires, or mundane burning-induced processes in cottage industries. PAHs enter the environment mainly through the air as the sooty part of smoke or ash, then adsorbed to particulate matters and get deposited into the lithosphere and hydrosphere. PAHs are characterized by low aqueous solubility and high hydrophobicity but readily bind to sediments, become suspended in water, and are as a result highly recalcitrant and persistent in the environment, with tendency to bioaccumulate and biomagnify in biological systems (Abdel-Shafy and Mansour, 2016). There is a positive correlation between increase in size of a PAH molecule and its angularity, and its hydrophobicity and electrochemical stability (Obayori et al., 2013). Human exposure to PAHs is via inhalation, ingestion, or direct dermal contact, portending high risks to public health through exhibition of various degrees of toxicity, mutagenicity, teratogenicity and carcinogenicity (Domingo and Nadal, 2015; Abdel-Shafy and Mansour, 2016).

Pyrene and phenanthrene are among the most widely transported PAHs in hydrosphere because they are highly associated with industrial process. Despite its higher molecular weight, aqueous solubility of pyrene (0.135 $mg\,l^{-1}$) is higher than anthracene (0.015 $mg\,l^{-1}$), but

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less soluble than naphthalene (30 mg l^{-1}) and phenanthrene (1–2 mg l^{-1}). (Obayori et al., 2013). Efficient, eco-friendly removal of PAHs from the environment has been depended upon bio-catalytic activities of various microorganisms leading to degradation of the compounds. Whereas, degraders of lower molecular weight PAH such as phenanthrene are readily isolated from polluted environments, mineralizers of higher molecular weight PAHs like pyrene are much more tedious to isolate. Beginning with the first isolation of pyrene degrader by Heitkamp et al. (1988), earlier reports showed that pyrene is degraded mainly by nocardioforme actinomycetes like *Mycobacterium* and *Rhodococcus* species. However, in the last 15 years there has been near explosion in the number of isolated non-actinomycetes degraders of pyrene spanning diverse genera such as *Pseudomonas, Burkholderia, Sphingomonas, Micrococcus, Bacillus, Cycloclasticus*, amongst others (Obayori et al., 2008; Kanaly and Harayama, 2010).

The length and breadth of Nigeria is dotted by tens of thousands of cottage industries involved in the production of animal skin-based delicacy, 'Ponmo'. This process is characterized by removing furs of animal hides and skin through burning of wood, tyre and petroleum products in low oxygen conditions. Thereby, leading to high level and indiscriminate disposal of animal charcoals and ashes, this are inadvertently transported through runoffs into hydrosphere and pose environmental hazard due to toxic compounds such as PAHs, dioxins, furans and benzene present in animal charcoals and ashes (Nguyen et al., 2013). There is currently dearth of information on the physicochemistry and bacterial community diversities of sites polluted with animal charcoal with reference to biodegradative potentials of the autochthonous bacterial populations that would eventually become biotechnological tools for decommissioning the polluted environment. The present study seeks to determine the pollution level of a site around a "Ponmo" cottage industry, which is exposed to huge animal charcoal and ashes. We also aim at isolating autochthonous bacteria strain(s) exhibiting efficient degradation of pyrene and phenanthrene that would be used as bioremediation tools to mitigate systems polluted with animal charcoals and ashes.

2. Materials and methods

2.1. Sampling

Composite runoff sediments were collected from a site chronically polluted with animal charcoal and ashes of a cottage industry where animal hide and skin was burnt to remove the furs for production of "*Ponmo*" (a local Nigerian delicacy). The coordinates of the site are 6° 28′ 5″ N, 3° 11′ 5″ E. Samples for both physico-chemical and microbiological analyses were collected in sterile bottles. Samples were treated immediately upon arrival in the laboratory, and leftovers kept at 4 °C.

2.2. Determination of physicochemistry and microbial community of sediment

The pH of the sediment was determined with a pH meter (Jenway 3510, Jenway, Staffordshire, England) in 1:2.5 sediment solutions in distilled water. The moisture content, organic content, total nitrogen content, potassium content and available phosphorous were determined according to standard methods (Bray and Kurtz, 1945; Black, 1965; Chopra and Kanwar, 1998). The total hydrocarbon content of the sediment was extracted using n-hexane: dichloromethane solvent systems (1:1) and determined by gas chromatography (HP 6890, Agilent Technologies, Santa Clara, U.S.A.). The total heterotrophic bacterial and fungal counts were enumerated by plating aliquots (0.1 ml of appropriate dilution) of sediment on nutrient agar (Oxoid Limited, Basingstoke, Hampshire, England) and potato dextrose agar (LabM Limited, Lancashire, United Kingdom), respectively. Starch casein nitrate agar containing (per litre) Soluble starch, 10.0 g; Casein, 0.30 g; KNO_3, 2.0 g; NaCl, 2.0 g; KH_2PO_4, 2,0 g; $CaCO_3$, 0.02 g; $MgSO_4·7H_2O$, 0.05 g; $FeSO_4·7H_2O$, 0.01 g; mycostatin, 50 mg; nalidixic acid, 50 mg and bacteriological agar, 18 g; was used to enumerate actinomycetes. The population of hydrocarbon-utilizers was estimated on mineral salts medium (MSM) (Kastner et al., 1994) that contained (per litre): Na_2HPO_4, 2.13 g; KH_2PO_4, 1.30 g; NH_4Cl, 0.50 g and $MgSO_4·7H_2O$, 0.20 g. The medium was supplemented with yeast extract (0.005 gl^{-1}) as source of growth factors, pH adjusted to 7.2, sterilised and sterile trace elements solution (1 mll^{-1}) as described by Bauchop and Elsden (1960) was added aseptically to the sterilised medium afterwards. For hydrocarbon utilizing bacteria, the MSM was fortified with filter-sterilised nystatin (50 µg ml^{-1}) to suppress fungi, while filter-sterilised streptomycin (10 µm ml^{-1}) was incorporated into the MSM to bacteria for the isolation of hydrocarbon utilizing fungi. The crude oil (Escravos light crude oil) used in this study was obtained from Chevron Nigeria Limited, dispensed in screw-capped bottle and sterilised separately by autoclaving (121 °C, 15 Ib, 15 min). Sterile crude petroleum served as the sole carbon and energy source and was made available to the cultures through vapour-phase transfer (Raymond et al., 1976). The plates counted after 5–7 days of incubation at room temperature.

2.3. Isolation of PAH degrader by continuous enrichment

Pyrene degrading bacteria were isolated on mineral salts medium (MSM) amended with pyrene (100 mg l^{-1}) by continual enrichment method. It was equally fortified with 50 µg ml^{-1} of nystatin to suppress fungi. The pyrene in the medium was added as solution in acetone and the solvent allowed to vent off in a fume chamber overnight. Air-dried contaminated sediment (10 g) was added to 90 ml of MSM containing 100 ppm of pyrene. Enrichment was carried out by incubation with shaking (150 rpm) at room temperature (27 ± 2 °C) in the dark until there was turbidity in 30 d. After 5 consecutive transfers, pyrene degraders were isolated by plating out dilutions from the final flasks on Luria–Bertani (LB) agar. The colonies that appeared were further purified by sub-culturing once onto LB agar. Ability to grow on pyrene was checked by plating out on pyrene coated MSM agar plate and observed for clearing zones around the colonies upon incubation (Kiyohara et al., 1982). The most promising pyrene degrader based on size of cleared zone was selected for further study and maintained in glycerol:nutrient broth (1:1, v/v) at −40 °C.

2.4. Characterization and identification of isolate

The selected isolate was identified on the basis of its colonial morphology, cellular morphology and biochemical characteristics according to the scheme of Cowan and Steel's Manual (Barrow and Feltham, 1995) and further complemented by Analytical Profile Index (API) phenotypic typing using the API 20E V6.0 rapid test kit according to the manufacturer's specifications (Biomerieux Inc., Durham, NC, USA). Antibiotic sensitivities of the isolate were determined using multidiscs. The ability of the isolate to grow on different cuts of petroleum was evaluated in MSM amended with respective hydrocarbons (1% v/v) as a sole carbon and energy source. The cuts tested include diesel, kerosene, engine oil and gasoline. Incubation was carried out under similar condition as described above for 20 days. Utilization of hydrocarbon substrates for growth was monitored by cell population increases using viable cell count and visual turbidity of culture. The ability of the isolate to dihydroxylate aromatic ring was tested according to Cerniglia and Shuttleworth (2002). The isolate was pregrown on Luria-Bertani broth and after 24 h, indole crystals were placed in the lid of the inverted plates. Appearance of blue pigments on the colonies indicated positive reaction or aromatic ring hydroxylating dioxygenase activity.

Colonies of overnight culture of selected bacterium were suspended in 100 µl of Tris-EDTA (TE) buffer (pH 8.0), vortexed, and DNA was extracted with equal volume of phenol: chloroform (1:1, v/v) upon vortex,

and centrifugation at 13,000 × g for 5 min. The DNA was precipitated from the aqueous phase by addition of 1:20 vol of 5.0 mol l^{-1} NaCl and 2.5 vol of ethanol, centrifuged for 10 min at 13,000 × g, and the supernatant was discarded. It was washed with 300 µl of 70% ethanol, centrifuged for 5 min at 13,000 × g, the supernatant discarded completely, and DNA pellet was air-dried and re-suspended in TE buffer. The 16 S rRNA gene in the purified genomic DNA was amplified by using Ex-Taq polymerase (TaKaRa, Ohtsu, Japan), and domain bacteria-specific PCR primer sets of 8 f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGHTACCTTGTTACGACTT-3′) (Weisburg et al., 1991), according to the manufacturer's instructions. The PCR condition was: 5 min initial denaturation at 94 °C; followed by 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 50 °C, and 2 min extension at 72 °C; and final extension at 72 °C for 7 min. A 16 S rRNA gene amplicon of the expected size (approximately 1500 nt) was confirmed by electrophoresis and purified using QIAquick Spin gel extraction kit (QIAGEN, Hilden, Germany). The 16 S rDNA amplicons was partially sequenced using the Applied Biosystems 3130xl genetic analyser, upon cycle sequencing reaction with BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster, CA, USA). The sequences were compared to those present in the database using BLAST(N) and aligned using the Muscle 3.7 program. Regions with significant variations were automatically removed using the Gblocks software. Phylogenetic tree with retrieved sequences from NCBI database was constructed using neighbour joining algorithm within the program MEGA 6.06 (The Biodesign Institute) and bootstrapped with 100 repetitions. The 16 S rRNA gene sequence of strain 10c was deposited in the DDBJ/EMBL/GenBank databases under GenBank accession number LC063859.

2.5. Time course of growth of isolate on PAHs

Resuscitation of bacteria (stored at −40 °C in nutrient broth: glycerol, 1:1) was achieved by harvesting colonies on LB agar, pooling the several clumps and transferring this to screw-capped bottles containing 5 ml of sterile physiological saline (0.9% NaCl). The inoculum was also pre-cultured on LB broth in Erlenmeyer flask for 24 h at room temperature (27 ± 2 °C) and 150 rpm. The biomass was harvested (7000 g; 10 min), washed thrice with phosphate buffer (50 mM KH_2PO_4, pH 7.2) and suspended in the same buffer as inoculum stock (approx., 10^6 cfu/ml). Replicate flasks containing 50 ml of MSM containing 100 ppm of pyrene or phenanthrene were prepared. Thus, for each substrate, there were 10 triplicate sets, making 30 flasks per substrate. The bacterium was inoculated to achieve an initial optical density OD_{560} of approximately 0.03 and incubated at room temperature (27 ± 2.0 °C) for a period of 25 days. Flasks containing MSM and PAH were inoculated with heat-killed cells as control. At each time point, randomly selected triplicates were sacrificed. Optical densities at 560 nm absorbance (OD_{560}) were determined at 5-day interval using a UV/Vis spectrophotometer (Beckman Coulter DU 720, Fullerton, CA). Blanks were inoculum-free MSM broths. Growth rates (µ) were determined from: $\mu = \frac{InOD_f - InOD_0}{t}$ where OD_f is optical density at the end of exponential growth phase, OD_0 is initial optical density, and t is time interval between beginning and end of exponential growth (day).

2.6. Extraction of residual PAH

Residual PAHs were extracted by liquid-liquid extraction according to the method described by Sarma et al. (2004). Culture (20 ml) was extracted once with an equal volume of toluene and then twice with an equal volume of chloroform. The two extracts were pooled and the solvents evaporated in a fume hood. After the solvents were vented off, the residual pyrene was dissolved in hexane, and concentrated to 2 ml. For crude oil degradation culture, residual crude oil extraction was done as described earlier but equal volume of hexane as solvent was used.

2.7. Analytical method

Hexane extracts (1.0 µl) of residual PAH were analysed with Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector (FID) (Agilent Technologies, Santa Clara, U.S.A.). The column was OV-3 with length of 60 m. The carrier gas was nitrogen. The injector and detector temperatures were maintained at 220 °C and 270 °C, respectively. The column temperature was programmed at an initial temperature of 50 °C; this was held for 2 min, and then ramped at 5 °C/min to 250 °C and held for 5 min. Air flow rate was 450 ml/min, hydrogen 45 ml/min and nitrogen 22 ml/min. The GC runs were carried out on the sample at day 0, day 10 and day 20. The PAH data were fitted to the first order kinetics model of Yeung et al. (1997)

$$y = ae^{-kt}$$

Where y is the residual PAH in culture (mg l^{-1}), a is the initial PAH in culture (mg l^{-1}), k is the degradation constant (day^{-1}), and t is time (day). Half-life ($t_{1/2}$) was thereafter calculated as:

$$Half - life = \frac{In(2)}{k}$$

3. Results

3.1. Physico-chemical properties of soil sample

The physico-chemical and microbiological properties of the sediment from which strain 10c was isolated are shown in Table 1. The sediment was slightly alkaline (pH, 7.5), total organic carbon was 8.6%, and total hydrocarbon content was very high (2057 mg/kg) when compared with the 50 mg/kg recommended limit set by Department of Petroleum Resources (DPR) in Nigeria. Nutrients such as potassium, nitrogen and phosphorus were low. It was equally high in water content (24.02%). Total heterotrophic bacteria and hydrocarbon degrading bacteria in the sediment were 2.25 × 10^8 cfu/g and 3.01 × 10^7 cfu/g, respectively.

3.2. Identification and characterization of pyrene degrader

The selected bacterial strain with widest zone of clearance on PAH-coated agar plate was catalase-positive, oxidase-negative, Gram-negative motile rod. It utilized citrate as sole carbon source, decarboxylated ornithine and showed urease activities but failed to produce indole, hydrogen sulphide, acetoin, and unable to utilize most sugars tested (Table 2). Comparison of 16 S rDNA sequence of strain 10c with those

Table 1
Physico-chemical and microbiological characteristics of soil sample.

Parameter	Amount
pH	7.5 ± 0.50
Moisture content (%)	24.02 ± 1.15
Total organic carbon (%)	8.65 ± 1.40
Nitrogen content (%)	2.07 ± 1.56
Total hydrocarbon (mg/kg)	2057.55 ± 5.67
Potassium (mg/kg)	1.25 ± 0.09
Phosphorus (mg/kg)	0.95 ± 0.40
THB (× 10^8cfu/g)	2.26 ± 0.0.78
THUB (× 10^7cfu/g)	3.01 ± 0.19
THF (× 10^4 cfu/g)	2.41 ± 0.50
THUF (× 10^3 cfu/g)	3.06 ± 1.50
Actinomycetes (× 10^5 cfu/g)	2.41 ± 0.00

THB – total heterotrophic bacteria.
THUB – total hydrocarbon utilizing bacteria.
THF – total heterotrophic fungi.
THUF – total hydrocarbon utilizing fungi.

Table 2
Antibiotic susceptibility patterns and biochemical reactions of strain 10c.

Activities of strain 10c		Response
Antibiotic susceptibility		
	Streptomycin (30 µg)	S
	Ciprofloxacin (10 µg)	S
	Amoxicillin (30 µg)	R
	Rifampicin (10 µg)	S
	Pefloxacin (10 µg)	R
	Chloramphenicol (30 µg)	R
	Ofloxacin (5 µg)	S
	Sparfloxacin (10 µg)	S
	Ampicillin (10 µg)	R
	Septrin (30 µg)	S
Biochemical reaction		
	Catalase	+
	Oxidase	–
	Beta-galactosidase	–
	Arginine dihydrolase	–
	Lysine decarboxylase	–
	Ornithine decarboxylase	+
	Citrate utilization	+
	H_2S production	–
	Urea hydrolysis	+
	Tryptophan deaminase	–
	Indole production	–
	Acetoin production	–
	Gelatinase	–
	Glucose	+
	Mannitol	–
	Inositol	–
	Sorbitol	–
	Rhamnose	–
	Sucrose	–
	Melibiose	–
	Amygdalin	–
	Arabinose	–

R: resistant; S: susceptible; +: positive; –: negative.

in the DBBJ/EMBL/GenBank database indicated significant alignments with *Proteus mirabilis* strains (100% similarity). Therefore, the bacterium was identified as *Proteus mirabilis* 10c strain. A phylogenetic tree constructed based on neighbour joining algorithm of strain 10c and related *Proteus mirabilis* strains retrieved from NCBI database is shown in Fig. 1. Antibiotic susceptibility pattern of strain 10c is equally shown in Table 2. The bacterium resisted chloramphenicol, ampicillin, amoxicillin and perfloxacillin, but was susceptible to other antibiotics. The bacterium exhibited luxuriant growth in chemically defined medium containing DBT, fluoranthene, phenanthrene, pyrene and crude oil. On the contrary, strain 10c grew poorly in medium where acenaphthylene and fluorine serve as sources of carbon and energy, whilst biphenyl, cinnamic acid, dibenzofuran, 1-naphthol and succinic acid did not support the growth of the bacterium. Colonies of the isolate showed blue pigmentation when grown in the presence of indole, indicating production of ring hydroxylating dioxygenase.

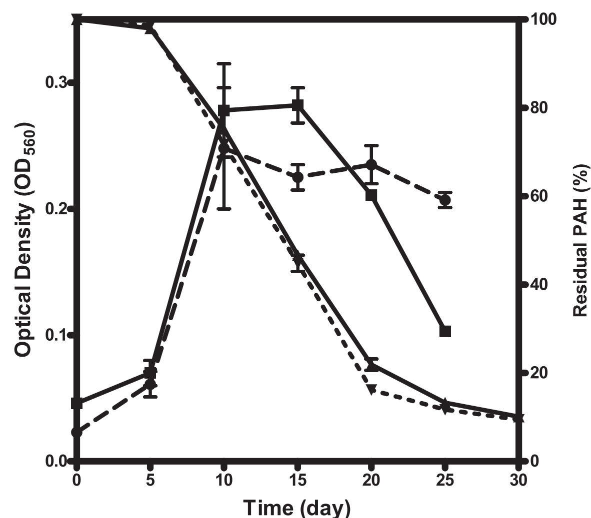

Fig. 2. Time course of growth of strain 10c on pyrene (•) and phenanthrene (■) as sole sources of carbon and energy and residual PAH reduction dynamics for pyrene (▲) and phenanthrene (▼). Incubation was carried out with shaking (150 rpm) at room temperature (27 ± 2 °C) for 30 days.

3.3. Growth profiles of isolate along degradation of pyrene and phenanthrene

The growth kinetics of isolate 10c in medium where pyrene and phenanthrene are sole sources of carbon and energy are shown in Fig. 2. On pyrene, the isolate showed initial slow growth between Day 0 and 5, but grew exponentially from OD_{560} of 0.061 to peak at 0.248 on Day 10. Similar growth pattern was observed on phenanthrene with OD_{560} values of 0.070 and 0.278 on Day 5 and 10 respectively. Growth rates were 0.281 d^{-1} and 0.276 d^{-1} on pyrene and phenanthrene, respectively (Table 3). Degradation kinetics of pyrene by *Proteus mirabilis* 10c (Table 3) showed that 87.92 mg l^{-1} was degraded in 30 days at the rate of 2.93 mg l^{-1} d^{-1}. The biodegradation constant (k) and half-life ($t_{1/2}$) were 0.073 d^{-1} and 9.495 d, respectively. Pyrene degradation was

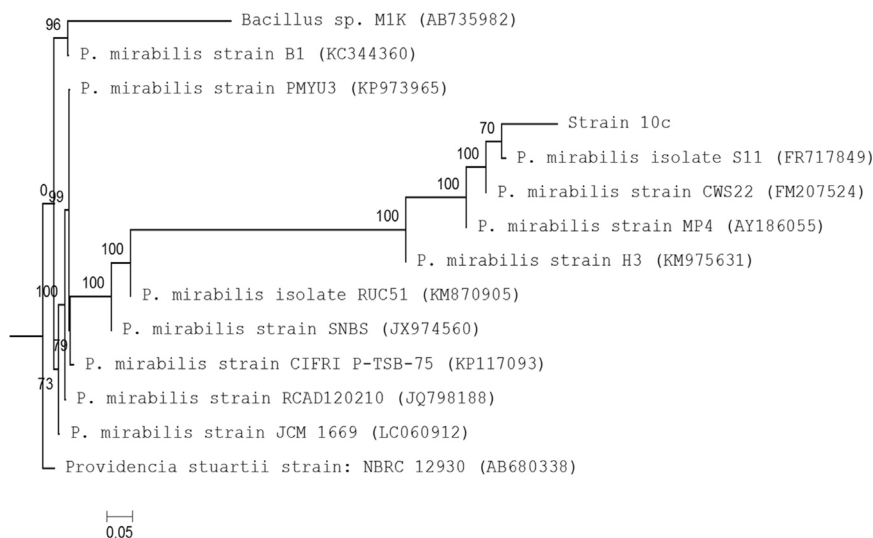

Fig. 1. Phylogenetic tree resulting from neighbour joining (NJ) analysis of 16 S rRNA showing the phylogenetic position of PAHs-degrading *Proteus mirabilis* strain 10c (LC063859) and related species of *Proteus mirabilis* retrieved from NCBI GenBank. Accession number of each organism used in the analysis is shown in parentheses. *Bacillus* sp M1K (AB735982) and *Providencia stuartii* NBRC 129 (AB680338) were used as out-groups for the tree rooting. The scale bar indicates the numbers of nucleotide substitutions per position. Bootstrap values (expressed as percentage of 100 replicons) are shown at the branch.

Table 3
Kinetics of biodegradation of pyrene and other hydrocarbons by *Proteus mirabilis* 10c.

Substrate	Specific growth rate μ, (d^{-1})	*Amount degraded ($mg\ l^{-1}$)	Rate of degradation ($mg\ l^{-1}\ d^{-1}$)	Degradation rate constant, k (d^{-1})	Half life, $t_{1/2}$ (day)
Pyrene	0.281	87.92	2.93	0.073	9.50
Phenanthrene	0.276	90.52	3.02	0.079	8.77
Fluoranthene	ND	93.00	3.10	0.089	7.78
DBT**	ND	97.25	3.24	0.126	5.50
Crude oil	ND	1893.88	63.13	0.085	8.12

* Values are means of triplicate readings obtained from difference between amounts of residual pyrene on Day 0 of incubation of bacteria and Day 30.
** DBT - Dibenzothiophene.

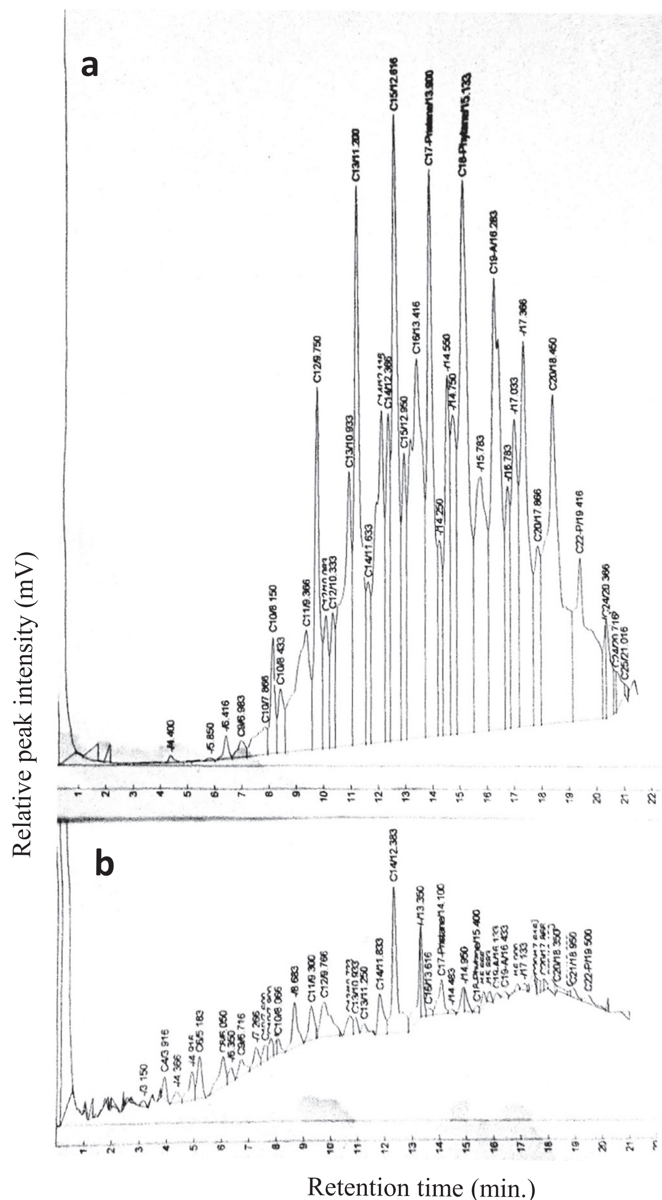

Fig. 3. Gas chromatographic traces of n-hexane extract of recovered crude oil from Day 0 (A) 30 (B) experimental flasks inoculated with *Proteus mirabilis* 10c and incubated at room temperature (27 ± 2 °C). The aliphatic fractions were nearly completely utilized in panel (B). The oil components were separated on a 60 m long OV- 3 column.

better than the other PAHs studied with degradation ranging from 90.56 mg l^{-1} (phenanthrene) to 97.25 mg l^{-1} (Dibenzothiophene), and half-life 8.774 d (phenanthrene), 7.788 d (fluoranthene) and 5.501 d (Dibenzothiophene). When supplied with crude oil (Escravos light) as sole source of carbon and energy, 1893.88 mg l^{-1} (92.26%) was degraded in 30 days. The corresponding degradation rate, degradation rate constant and half-life values were 63.13 mg l^{-1} d^{-1}, 0.0852 d^{-1} and 8.12 d, respectively. Chromatographic fingerprints of n-hexane extract of the culture (Fig. 3) showed that most of the aliphatic hydrocarbon fractions were degraded to less than 10%. The only exception, however, was C_{14} which remained quite high at more than 25%.

4. Discussion

PAHs are a diverse group of more than 100 organic compounds containing two or more fused benzene and/or pentacyclic-ring structures that are arranged in linear, angular and cluster conformations. Clean-up of environments polluted with PAHs is important due to their acute toxicity, low bioavailability, hydrophobicity, mutagenicity and carcinogenicity (Nkansah et al., 2011).

Physicochemistry of the soil sample used in this study indicated low concentrations of macronutrients such as nitrogen, potassium and phosphorus. This may be attributed to high demand of these inorganic nutrients by microorganisms for sugar phosphorylation, synthesis of amino acids, nucleic acids and other cellular processes (Andrew and Jackson, 1996; Salam et al., 2014). In a bioremediation sense, presence of these macronutrients in low concentration in soil sample may be an impediment for pollutant degradation (Vidali, 2001). The baseline limit set for total petroleum hydrocarbons by the petroleum regulatory body in Nigeria is 50 mg/kg (DPR, 1991), which is far lower than 2057 mg/kg contained in the sediments polluted with animal charcoal and ashes. It is equally noteworthy that the limit for residential area, set by EPA in USA, is 50 mg/kg while that of industrial area is 500 mg/kg. Furthermore, our value is similar to the average value recently recorded in oil spill site in the oil-producing Niger-Delta region of Nigeria, which shows that hydrocarbon pollution of soil and sediments in cottage industries which are very close to residential areas could be as bad if not worse than found in spill sites (Alinnor et al., 2014).

Environmental isolates primed as seed for bioremediation must be able to compete favourably with autochthonous community in a nutrient limiting, highly compacted and diverse contaminated soil environment. As such, ability to resist antibiotics freely produced by many soil microorganisms as a survival strategy and for competitive edge will be of immense advantage for the allochthonous bacterial strains. Resistance of strain 10c to amoxicillin, perfloxacin, chloramphenicol and ampicillin may be attributed to acquisition of resistant genes to these antibiotics through horizontal gene transfer from closely as well as distantly-related indigenous bacterial strains in the polluted soil (Obayori et al., 2008; Salam et al., 2014).

Two model PAHs, pyrene and phenanthrene, were degraded by *Proteus mirabilis* strain 10c isolated from an animal charcoal polluted site in Lagos, Nigeria. Preponderance of PAHs degraders from polluted sites in Lagos have been reported by many researchers (Ilori and Amund, 2000; Obayori et al., 2008; Salam et al., 2014), however, globally, this is the first report detailing metabolism of two model PAHs by a *Proteus* species. Furthermore, previous reports have shown that pyrene degraders to have phenanthrene catabolic capability, ostensibly on account of shared pathways (Kanaly and Harayama, 2010).

Although the growth rate of strain 10c on pyrene (0.0117 h^{-1}) and phenanthrene (0.0115 h^{-1}) is low compared to those reported in previous findings (Boldrin et al., 1993; Obayori et al., 2008), pyrene utilization rate of the isolate (0.12 mg l^{-1} h^{-1}) is, however, higher than 0.111, 0.082 and 0.067 mg l^{-1} h^{-1} reported by Obayori et al. (2008) for *Pseudomonas* species strains LP1, LP5, and LP6, respectively. Earlier reports have indicated that growth and utilization rates of isolates on substrates are not only predicated on the intrinsic properties of the

isolates but also culture conditions such as crystal size of substrates and variations in physicochemical parameters (Boldrin et al., 1993). It is not unlikely that the organism has special mechanism for uptake of the hydrocarbon substrate as microorganisms are generally known to take up the hydrocarbons dissolved in the aqueous phase and such mode may become limiting at low aqueous solubility (Maier et al., 2009). Several mechanisms have been adduced for utilization of substrates at such low solubility, namely production of biosurfactants, uptake systems with high substrate affinity, which efficiently reduce concentration of substrate thereby increasing diffusive rate, and reduction of the distance between cells and substrates by means of cell surface structures which promote adhesion to hydrophobic surfaces (Bastiaens et al., 2000)

In this study, strain 10c degraded 87.92% of the initial concentration of pyrene (100 mg l^{-1}) in 30 days. This degradation rate is lower than 72% (500 mg l^{-1}) in two weeks and 99% (100 mg l^{-1}) in 16 days reported for *Rhodococcus* sp. *UW1* and *Diaphorobacter*, respectively (Walter et al., 1991; Klankeo et al., 2009). It is, however, higher than 61.5% reported for *Leclercia adecarboxylata* (Sarma et al., 2004). It is also higher than 65.8% and 33.7% (50 µg ml^{-1}) within 21 days reported for *Bacillus cereus* Py5 and *Bacillus megaterium* Py6 as well as 68%, 67% and 47% within 30 days reported for *Pseudomonas* spp. strains LP1, LP5 and LP6, respectively (Lin and Cai, 2008; Obayori et al., 2008).

The isolate also degraded 93% of the initial concentration of fluoranthene (100 mg l^{-1}) in 30 days. This value is higher than 35% (100 mg l^{-1}) in 24 days reported by Klankeo et al. (2009) for *Pseudoxanthomonas* sp. It is also higher than 24% and 46% (20 mg l^{-1}) reported for *Pasteurella* sp. IFA (B-2) and *Mycobacterium* sp. PYR-1 (AM) (Sepic et al., 1998). It is, however, lower than 99% (100 mg l^{-1}) in 24 days reported for *Diaphorobacter* sp. (Klankeo et al., 2009). Degradation of fluoranthene by strain 10c is not surprising as the isolate is recovered from pyrene enrichment. Fluoranthene, a non-alternant PAH, is a structural isomer of the alternant PAH pyrene and this may partly explain the shared specificity of strain 10c on fluoranthene and pyrene. Degradation of fluoranthene by the isolate is additional advantage in view of it being one of the 16 priority pollutant PAHs and being listed by IARC as Group 3 Carcinogen with carcinogenic properties on newborn mice (Busby et al., 1984).

In this study, strain 10c degraded 97.25% (100 mg l^{-1}) of dibenzothiophene in 30 days. This degradation rate is higher than 82% (40 mg l^{-1}) in 14 days and 100% (40 mg l^{-1}) in 11 days reported for *Arthrobacter* sp. P1-1 and *Mycobacterium aromativorans* strain JS19b1 respectively (Seo, 2006, 2012). Dibenzothiophene, a sulfur containing heterocycle consisting of two benzene rings on either side of a thiophene ring is a persistent organic pollutant and is a component of crude oil, creosote and often coexist with PAHs and other aromatic compounds in the environment.

Oftentimes, polluted soil environment such as animal charcoal site used in this study are predominantly inundated with complex hydrocarbons such as crude oil, spent engine oil and diesel with their aliphatic, alicyclic and aromatic components. As such, there is a need to assay the specificity pattern of PAHs degraders on complex hydrocarbon such as crude oil. The degradation of the aliphatic fractions of the crude oil to < 10% of their initial concentration give credence to the assertions that aliphatic components are the most readily degraded fraction of crude oil (Jain et al., 2005). Further, while organisms with broad specificity for PAHs and aliphatic hydrocarbons are not readily isolated from the environment, a number of remarkable isolates with such capabilities have been reported (Kanaly and Harayama, 2010).

Information on PAH degradation ability of *Proteus* species is relatively scarce. Globally, only one report exists detailing pyrene degradation by a *Proteus vulgaris* strain isolated from biofilm in water of a petrochemical industry plant in Turkey (Ceyhan, 2012). Although involvement of *Proteus* species in the degradation of pesticides, herbicides, crude oil, used engine oil, phenolic compounds, methyl-tert-butyl ether and detoxification of heavy metals have been reported (Olajire and Ogbeifun, 2010), globally, to the best of our knowledge, based on available information in literature, this is the first report detailing the biodegradation potentials of a *Proteus mirabilis* strain on PAHs (pyrene, phenanthrene, and fluoranthene), heterocyclic aromatics (dibenzothiophene) and aliphatic hydrocarbons.

This study describes, for the first time PAHs (pyrene, phenanthrene, fluoranthene) biodegradation potentials of a *Proteus mirabilis* strain 10c isolated from an animal charcoal polluted soil. It also shows the degradation ability of the isolate of heterocyclic aromatic and complex hydrocarbon mixtures (crude oil). The ability of the isolate to resist some antibiotics revealed its bioremediation potential for PAHs impacted soils. Further research works will focus on the metabolic pathway employed by strain 10c for pyrene and phenanthrene degradation and the degradative genes involved in the process.

Compliance with ethical standards

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Conflict of interest

Each of the Authors has no conflict of interest.

Ethical approval

Neither animal nor human was used in part or whole during this study, as regards the procedures performed during the present investigation. Therefore, this article does not contain any studies with human participants or animals performed by any of the authors.

Authors' contribution

OOS participated in experimental design, collation of data, manuscript preparation, and overseeing execution of experimentation; SLB participated in collation of data, experimentation and manuscript preparation; OGO participated in data collation and interpretation, experimentation and manuscript preparation; IM participated in sample collection, experimentation and data collation; AOO participated in experimental design, over seeing experimentation and manuscript preparation.

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