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ORIGINAL RESEARCH

Degradation of crude oil (Escravos light) by *Pseudomonas* strains isolated from poultry droppings and cow dung.

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Abstract:

Introduction: Poultry manure and cow dung have been established as potential material for the bioremediation of petroleum polluted sites, with emphasis on nutrient addition.

Aim: Our aim was to isolate from poultry droppings and cow dung bacteria with ability to degrade petroleum hydrocarbons.

Materials and Methods: Bacteria were isolated from poultry droppings and cow dung by continuous enrichment and vapor transfer techniques. Petroleum utilization by each isolate was confirmed in carbon free medium containing Escravos crude oil (1%). The two best isolates were selected for further study. Isolates were identified by Analytical Profile Index (API). Antibiotic sensitivity was determined by multidisc (Abitek Multidisc, UK). Growth was assayed in broth culture by plate count. Residual oil was determined by Gas Chromatography equipped with Flame Ionisation detector (GC-FID).

Results: The isolates were putatively identified as Pseudomonas putida (MP2) and Pseudomonas sp. (MC4). Both isolates were susceptible to ciprofloxacin and chloramphenicol and tarivid. They resisted amoxicillin and gentamycin, augmentin, sparfloxacin and septrin. The growth rates were 0.17 and 0.23/day for strains MP2 and MC4 respectively, while the organisms degraded 88.39% and 89.06 % of crude oil respectively in 20 days. Aliphathic hydrocarbons in the range C11 to C22 were mostly reduced to less than 20%, while C22 – C26 disappeared completely within the same period in both cases.

Conclusion: Bacteria capable of extensive degradation of Escravos crude oil were isolated from poultry droppings and cow dung. Such isolates could be veritable candidates for bioaugmentation of hydrocarbon polluted environmental compartments.

Keywords: Biodegradation ; Bioremediation ; Poultry droppings ; Cow dung ; Crude oil ; Gas chromatography

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Petroleum pollution of environmental compartments as a result of exploration, transportation, refining, use and disposal of crude oil and its by-products remains a major problem in the world today. On small scales, farmlands and community holdings, particularly in the Third World countries, remain at great risk both from occasional acute accidental releases and chronic small-scale releases of hydrocarbons [1]. Mechanical and chemical methods for remediation of hydrocarbon-polluted environments are often expensive, technologically complex and lack public acceptance [2]. Bioremediation is, therefore, regarded as a cost effective and environmentally friendly approach to reclamation of such sites [3].

The use of poultry droppings, cow dung and other manures and agricultural wastes as potential materials for bioremediation of hydrocarbon-polluted soil has been well described in the literature [4,5,6]. However, emphasis has been on the stimulatory effect of the nutrient rich organic waste. The high moisture content, inorganic nutrient and organic components present in manures such as poultry droppings and cow dung make them highly veritable growth media for a wide variety of microorganisms. There is enough ground to believe that hydrocarbon degraders can occur in substantial number in these manures, as hydrocarbon degraders have been isolated from various sources [7,8]. Plants and animals synthesize these hydrocarbons as part of their cellular components, defense chemicals, sex pheromones and waste products excreted from the cells. Some hydrocarbon compounds are also present as storage material in plants [9]. Bacteria often isolated from hydrocarbon polluted sites belong to the genera *Pseudomonas, Sphingomonas, Acinetobacter, Alcaligenes, Flavobacterium, Bacillus, Rhodococcus, Mycobacterium, Actinobacter* among others [10,11]. However, in recent time, genera that are even more diverse such as *Microbacterium* and *Methylonomas* and have been reported, and from the most unlikely sources [12]. Previous reports have shown the presence of hydrocarbon degraders in both poultry droppings and cow dung These organisms spanned the following genera *Pseudomonas, Bacillus, Alcaligenes, Acinetobacter, Micrococcus* and *Serratia* [13,14].

Petroleum is a complex mixture of aliphatic, aromatic, resin and asphaltene hydrocarbons. Reduction of the n-alkanes relative to the isoprenoid is a good measure of biodegradation, especially in the early stage of degradation by microorganisms [15]. Bacterial degradation of petroleum starts from linear alkanes and branched alkanes, while cycloalkanes and aromatics have the ability to resist biodegradation [16]. Bacteria capable of degrading all the aliphatic and aromatic components of crude oil are not very common. Often, complete degradation results from the activities of consortium of organisms. However, enrichment technique is a classical approach to thinning out the population of less versatile strains and boosting the population of degraders. This often results in the isolation of the few best degraders under the growth conditions.

Although there have been reports on hydrocarbon degrading organisms from poultry droppings and cow dung, not much is known about the versatility abilities of the microflora of the waste when exposed to hydrocarbon as sole sources of carbon and energy. Here we report the *in vitro* degradation of crude oil and common petroleum products including gasoline, diesel, engine oil, and kerosene by bacteria isolated from chicken droppings and cow dung from farms in Lagos, Nigeria.

2. MATERIALS AND METHODS

2.1. Sampling and isolation of hydrocarbon degraders

Poultry droppings were obtained from Ayedoto Farm Settlement, Ojo, Lagos State, Nigeria. Samples were collected in sterile screw-capped bottles and placed in ice packed cooler. Fresh cow dung was collected likewise from a livestock pen at Alaba Rago, Ojo, Lagos State, Nigeria. Sample analyses were carried out immediately upon arrival in the laboratory.

Bacteria able to degrade hydrocarbons were isolated on carbon-free mineral medium (CFMM) by continuous enrichment method. The carbon free medium described by Habe *et al*. [17] was used. The medium contained per liter of distilled water Na_2HPO_4 (2.20 g), KH_2PO_4 (0.8g), NH_4NO_3 (3.00 g), $MgSO_4.7H_20$ (0.2g), $FeCl_3.6H_2O$ (0.05g) and $CaCl_2.2H_20$ (0.01g). All chemical reagents were of analytical grade and products of Sigma-Aldrich Chemicals, England. After the pH was adjusted to 7.0 it was fortified with nystatin (50 µg/ml) to suppress fungal growth.

Poultry droppings (or cow dung; 1 g) were added to 49 mL of CFMM containing 0.5 ml of crude oil. Enrichment was carried out with shaking (150 rpm) at room temperature (27± 2°C) in the dark until there was growth (turbidity). After three consecutive transfers at shorter intervals (1 week), hydrocarbon degraders were isolated by plating out the final flask on Luria-Bertani (LB) agar. Several colonies that appeared were further purified by subculturing on LB agar. Ability of pure isolates to degrade crude oil was confirmed by inoculating LB broth-grown pure cultures (18 h) into fresh CFMM flask containing crude oil (Escravos light) at 1% (*v/v*), incubated was carried out in the dark with shaking at room temperature for two weeks. Growth and degradation were indexed by appearance of turbidity and disappearance of the oil slick.

2.2. Identification and characterization of isolates

Organisms were maintained in glycerol nutrient:broth (1:1, v/v). Colonies growing on LB agar were harvested with sterile inoculating loop, pooled and transferred to the medium. The mixture was shaken well to homogenize it while avoiding foaming and kept at –20°C. Isolates were identified on the basis of their

colonial morphology, cellular morphology and biochemical characteristics according to the scheme of Cowan and Steel's Manual [18]. Identification of the isolates was complemented by Analytical Profile Index (API) phenotypic typing using the API 20 NE V6.0 rapid test kit according to the manufacturer's specifications (Biomerieux Inc., Durham, NC, USA).

2.3 Antibiotic and substrate susceptibilities of isolates

Antibiotic sensitivities of isolates were determined using Gram negative bacteria multidiscs (Abtek Biologicals Limited, United Kingdom). Antibiotic susceptibility patterns were interpreted according to the clinical and Laboratory Standards Institute Guidelines [19]

The abilities of the isolates to grow on different cuts of petroleum were evaluated in liquid media 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 in section 2.1 for 20 days. Degradation was monitored by cell increases and observation for turbidity. The cell density of bacterial suspensions was determined by measuring the absorbance at 600 nm and). using a UV/Vis spectrophotometer (Beckman Coulter DU 720, Fullerton, CA). Blanks were inoculum-free MSM broths. relating the value to a calibration curve (10^9 cfu l^{-1} = 1 OD unit).

2.4. Time course of growth of isolate on crude oil

Replicate flasks containing 50 mL of CFMM with 0.5 mL (1%, v/v) of crude oil were prepared. Thus, for each isolate, there were 10 triplicate sets, making 30 flasks per isolate. Isolates were inoculated to achieve an initial concentration of the total viable count (TVC) of about 2.0 x 10^7 cfu/mL and incubated with shaking at room temperature (27 ± 2.0°C) for a period of 20 days. Flasks containing CFMM and crude oil were inoculated with heat-killed cells as control. At each time point, randomly selected triplicates were sacrificed. Total viable counts were determined at 2-day interval by plating out appropriate dilutions of the cultures onto nutrient agar and counting the colonies after 24 hrs. Mean generation times and specific growth rates were calculated using nonlinear regression of growth curves for the period when growth rates were maximal (Graphpad software, San Diego, CA, USA).

2.5. Extraction of residual oil

Residual oil was extracted by liquid-liquid extraction [1]. In this procedure, 10 mL of hexane was added to 20 mL of broth culture in flask and shaken thoroughly. After removing the aqueous phase by separating funnel, the organic phase was reconstituted to 5 mL and the residual oil concentration was determined by gas chromatography.

2.6. Analytical method

Hexane extracts (1.0 µL) of residual oil were analyzed with Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector (FID). 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 degradation rate constant was determined by fitting the residual oil data to the kinetics model [20]

$y = ae^{-kt}$ where *y* is the residual crude oil in culture (mg/l), *a* is the initial crude oil in culture (mg/l), k is the degradation constant (day^{-1}), and t is the time (day). Half-life ($t_{1/2}$) was then calculated as

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

3. RESULTS

3.1. Isolation, Identification and characterization of isolates

Continuous enrichment of poultry droppings sample resulted in the isolation of three morphologically distinct isolates. on the other hand, five morphologically distinct pure bacterial isolates were obtained by continuous enrichment of cow dung sample, but the most promising one from each manure in terms of being the dominant population on plate and turbidity in crude oil supplemented CFFM broth were selected for further study. The morphological and biochemical characterisics of the two isolates selected for further study are shown Table I. Isolate MP2, from poultry droppings, was Gram-negative motile rod. It was positive for catalase and oxidase. It produced slightly raised round, smooth edged colonies without pigment. It was negative for urease, eschulin ferric citrate and gelatin but positive for glucose, mannose, mannitol, potassium gluconate, capric acid, citrate, malic acid, and phenyl acetic acid. It was identified as *Pseudomonas putida*.

Isolate MC4, from cow dung, was Gram-negative motile rods, positive for catalase, oxidase, nitrate, mannitol, gelatin and malic acid. It was negative for indole, urease. esculin, lactose, arabinose, mannose, N-acetyl glucosamine, adipic acid and phenyl acetic acid.

3.2. Antibiotic and substrate susceptibilities of isolates

The antibiotic susceptibilities of the isolates are shown in Table II. Streptomycin (30 µg) showed activity

against *Pseudomonas* species strain MC4. Both isolates were susceptible to Ciprofloxacin (10 µg), Chloramphenicol (30 µg) and tarivid (10 µg). They resisted Amoxicillin (30 µg) and Gentamycin (10 µg), Augmentin (30 µg), Sparfloxacin (10 µg) and Septrin (30 µg). MP2 was susceptible to Pefloxacin (30 µg).

Table I: Biochemical reactions of *Pseudomonas putida* MP2 and *Pseudomonas* sp. MC4

Test	MP2	MC4
Nitrate	-	+
Tryptophan	-	-
Glucose fermentation	+	-
L-arginine	+	+
Urease	-	-
Eschulin	-	-
Gelatin	-	+
4-nitrophenyl-βD-galactopyranoside	+	-
Glucose assimilation	-	-
Arabinose	-	-
Mannose	+	-
Mannitol	+	+
N-acetyl glucosamine	-	-
Maltose	-	-
Potassium gluconate	+	+
Capric acid	+	+
Adipic acid	-	-
Malic acid	-	+
Citrate	+	-
Phenyl acetic acid	+	-
Oxidase	+	+
Catalase	+	+

+: Positive response; - : negative response

Table II: Antibiotic susceptibility patterns of bacteria

Antibiotic	*Pseudomonas putida* MP2	*Pseudomonas* sp. MC4
Streptomycin (30 µg)	R	S
Ciprofloxacin (10 µg)	S	S
Amoxicillin (30 µg)	R	R
Gentamycin (10 µg)	R	R
Pefloxacin (10 µg)	S	R
Chloramphenicol (30 µg)	S	S
Augmentin (30 µg)	R	R
Sparfloxacin (10 µg)	R	R
Tarivid (10 µg)	S	S
Septrin (30 µg)	R	R

isolated from poultry droppings and cow dung

R: resistant; S: susceptible; ND: not determined

The susceptibilities of isolates to various hydrocarbon substrates are shown in Table III. Both isolates grew at least moderately on crude oil, engine oil and diesel oil. Kerosene and gasoline did not appreciably support the growth of the isolates. Also, none of the isolates grew on anthracene or pyrene.

Table III: Substrate specificities of bacteria isolated from poultry droppings and cow dung

Parameter	*Pseudomonas putida* MP2	*Pseudomonas* sp. MC4
Crude oil	+++	++
Engine oil	++	+++
Diesel	+++	++
Kerosene	-	-
Gasoline	+	+
Anthracene	-	-
Pyrene	-	-

+++: luxuriant growth; ++: moderate growth; +: poor growth; - : no growth

3.3. Growth profiles and kinetics of degradation of crude oil by isolates

The time courses of growth of isolates on CFMM supplemented with crude oil as sole source of carbon and energy are shown in Figure 1. *Pseudomonas putida* MP2 grew exponentially before attaining its highest concentration of 1.52 x10^7 cfu/ml on day 10. Similarly, *Pseudomonas* sp. MC4 attained highest concentration on day 10. The growth rate measured for *P. aeruginosa* MP2 was 0.17 /d, while for *Pseudomonas* sp. MC4 it was 0.23 /d.

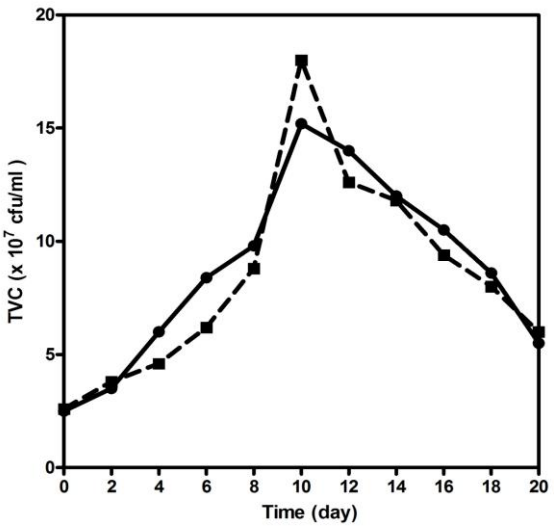

Fig. 1 Growth profile of isolates in carbon free mineral medium (CFMM) supplemented with Escravos light crude oil (1% V/V) as sole carbon and energy source. *Pseudomonas putida* MP2 (●); *Pseudomonas* sp. MC4 (■).

The kinetics of degradation of crude oil indicated that the two isolates had similar degradation rates (Table IV). In the first ten days *P. putida* MP2 degraded 60.70 % of the oil at the rate of 217.15 mg/l/d, while overall 88.39 % was degraded at the end of 20 days but with a lower degradation rate of 99.30 mg/l/d in the last ten days. *Pseudomonas* sp. MC4 on the other hand initially degraded 61.43% at the rate of 214.0 mg/l/day in the first ten days after which the rate fell to 117.6 mg/l/d with a total of 89.06% degraded at the end of 20 days. The degradation rate constant and half-life were 0.108 /d and 6.40 d for *P. putida* MP2 respectively, while for *Pseudomonas* sp. MC4 the corresponding values were 0.111 /d and 6.27 d.

Table IV: Kinetics of crude oil degradation by bacteria isolated from poultry droppings and cow dung

Parameter	*Pseudomonas putida* MP2	*Pseudomonas* sp. MC4
K(/d)	0.17	0.23
T (d)	4.13	2.95
PD_{10} (%)	60.70	61.43
PD_{20} (%)	88.39	89.06
DR_{10} (mg/l/d)	217.5	214.0
DR_{20} (mg/l/d)	99.3	117.6
k_d (/d)	0.108	0.111
$t_{1/2}$(d)	6.40	6.27

K: growth rate; T: mean generation time; PD_{10}: percentage degraded between day 0 and day 10; PD_{20}: percentage degraded between day 0 and day 20; DR_{10}: rate of degradation between day 0 and day 10; DR_{20}: rate of degradation between day 10 and day 20. K_d: degradation rate constant; $T_{1/2}$: half-life. Percent degradation values represent the net decrease (FID area counts) calculated with reference to the amount recovered from heat-killed control tubes.

Chromatographic fingerprints of n-hexane extracted cultures of *P. putida* MP2 and *Pseudomonas* sp. MC4 supplied with crude oil as sole sources of carbon and energy showed significant reduction in peak of n-alkane fraction (Figures 2 and 3). The amounts of residual hydrocarbon fractions recovered from CFMM inoculated with *P. putida* MP2 and *Pseudomonas* sp.MC4 are shown in Figure 4. In the case of *P. putida* MP2 (upper panel), after 10 days of incubation, most fractions between C_9 (nonane) and C_{20} (icosane) had been reduced to between 18.68% (C_{20}) and 59.22% (C_{14}). Higher fractions, however, ranged from 67.30% to 99.10%, except C_{21} (Heincosane), which was no longer detectable. After 20 days of growth, C_9, C_{17} (heptadecane), C_{24} (tetracosane), C_{25} (pentacosane) and C_{26} (hexacosane) had completely disappeared from the cultures, while others ranged from 0.03 to 16.40%, except C_{10} (decane) and C_{11} (undecane), which remained high at 67.42 and 20.32% respectively. *Pseudomonas* sp. MC4 also showed the same pattern of degradation (lower panel). However, uniquely, C_9 almost disappeared on day 10, just as C_{20} and C_{21} had completely disappeared. C_{10} to C_{19} had residuals ranging from 22.09 (C_{12}) to 58.85 (C_{14}). The higher fractions, C_{22} (docosane)-C_{26}, remained very high on Day 10 (64.78% - 99.10%) but completely disappeared on day 20. Similar to what was observed for *P. putida* MP2, for *Pseudomonas* sp. MC4, C_{11} to C_{22} were reduced averagely to less than 10% after 20 days but with the highest value being C_{11} (25.76%) and lowest being C_{17} (0.0%).

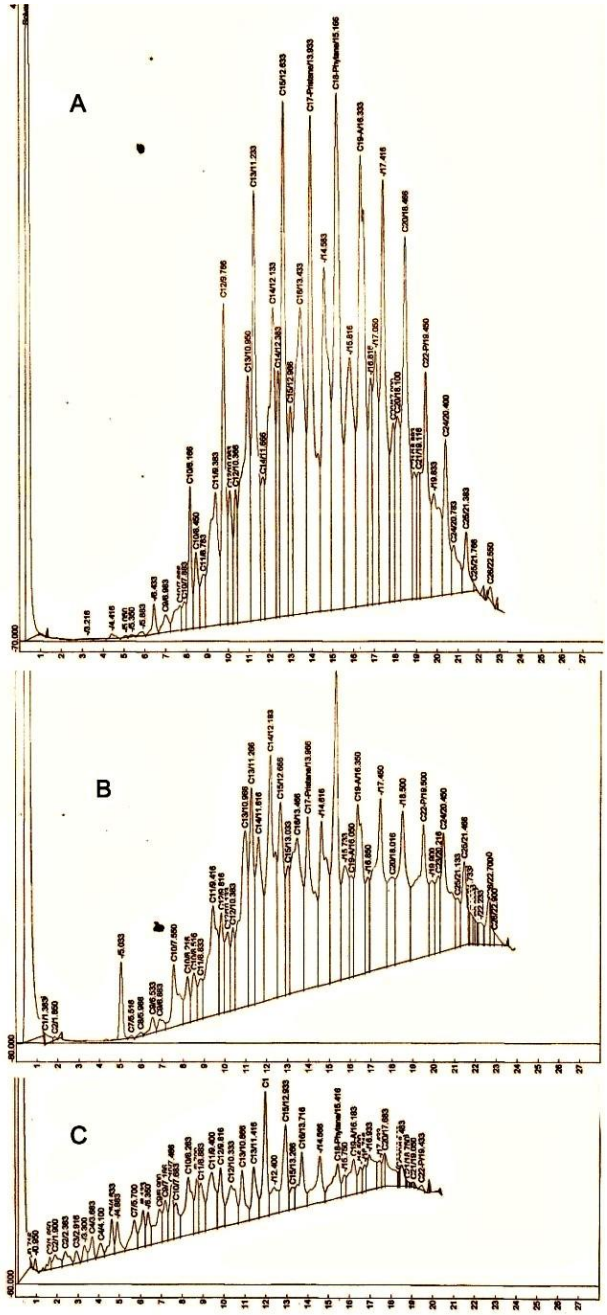

Fig. 2. Gas chromatographic traces of n-hexane extract of recovered crude oil (Escravos light) from Day 0 (A) Day 10 (B) and Day 20 (C) experimental flasks inoculated with *Pseudomonas putida* MP2. The aliphatic fractions were nearly completely utilised in panel (C).

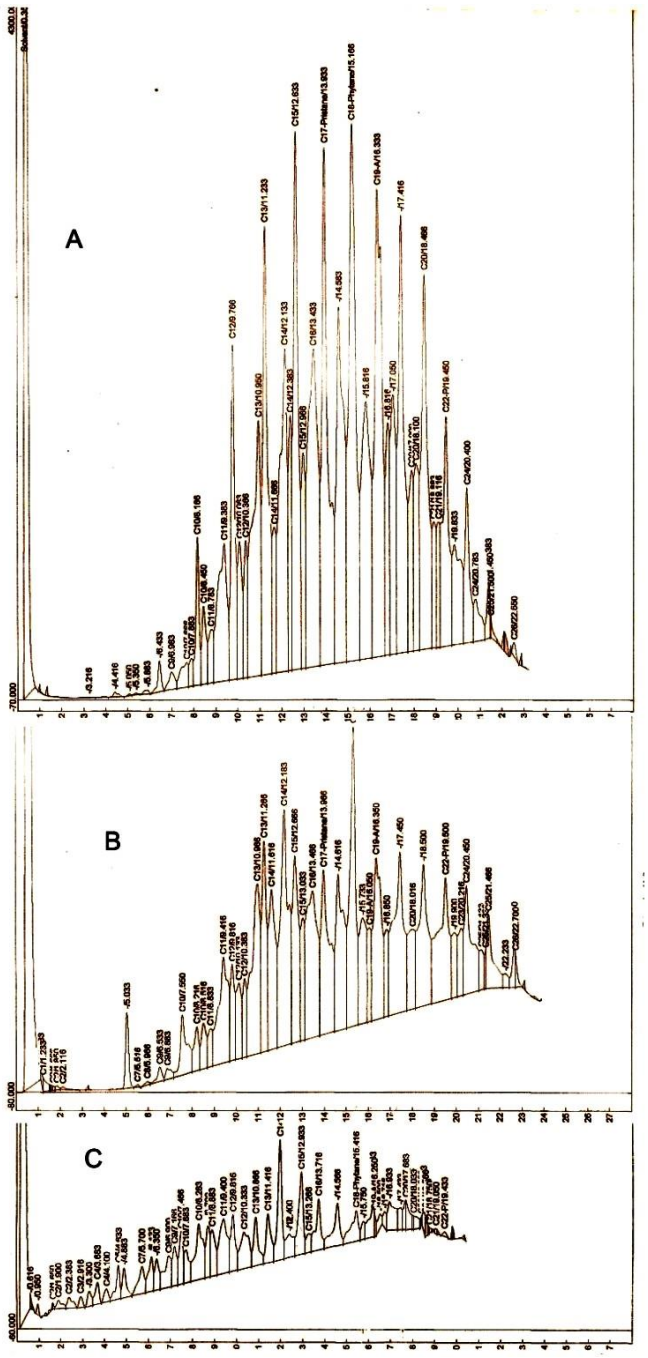

Fig. 3. Gas chromatographic traces of n-hexane extract of recovered crude oil (Escravos light) from Day 0 (A) Day 10 (B) and Day 20 (C) experimental flasks inoculated with *Pseudomonas* sp. MC4. The aliphatic fractions were nearly completely utilised in panel (C).

4. DISCUSSION

The use of autochthonous microorganisms inhabiting hydrocarbon-polluted niches for biodegradation and bioremediation has been widely accepted as a formidable approach due to the avalanche of successes recorded by various researchers [1]. Adaptation of autochthonous microbial communities, which implies series of physiological and genetic changes that confer

degradative ability on the autochthonous organisms have been regarded as a key factor [21]. However, increasing evidences have demonstrated the propensity for hydrocarbon degradation by microorganisms isolated from non-hydrocarbon polluted sources [22,23]. *Pseudomonas* strains isolated in this study are well-known hydrocarbon degraders [24].

Fig. 4. Percentage hydrocarbon remaining after 10 and 20 days of incubation of isolates with crude oil (Escravos light) in carbon free mineral medium (CFMM).

Microorganisms synthesize antibiotics as a survival strategy and for competitive edge. As such, knowledge of sensitivity and resistance patterns of organisms with potentials as seed for bioremediation is important. Resistance to amoxicillin and gentamycin by *P.putida* MP2 and *Pseudomonas* sp. MC4, and augmentin *Pseudomonas* sp. MC4 may be attributed to acquisition of resistance genes to these antibiotics through prolonged exposure, selective pressure, adaptation and gene transfer. In addition, poultry and livestock feeds are replete with these antibiotics, which could allow evolution of resistance by indigenous strains. The broad range of resistance can provide advantage for survival over natural microfloral [25].

Substrate spectrum analysis of hydrocarbon degraders isolated in this study on various hydrocarbon cuts and aromatic substrates revealed different utilization patterns. Both isolates failed to grow on pyrene and anthracene but shared specificity for crude oil, engine oil and diesel by all the isolates. These observations indicate either the absence of catabolic genes for the degradation of these aromatic hydrocarbons in the isolates or lack of ability to express such traits under prevailing conditions. The low to non-specificity for kerosene and gasoline exhibited by the isolates may be attributed to the narrow nutrient options of these two carbon sources compared to crude oil, as they mainly contain short chain hydrocarbons of 5-14 carbon atoms in length. Crude oil, a mixture of different hydrocarbon components, favourably supports growth of microorganisms due to its diverse nutrient options as source of carbon and energy [26].

The genus *Pseudomonas* encompasses arguably the most diverse and ecologically significant group of bacteria due to their remarkable degree of physiological and genetic adaptability [27]. *Pseudomonas* is reputed to possess broad substrate affinity for different classes of hydrocarbons such as alicyclics, heterocyclics, and aromatics [28, 29]. In this study, the *Pseudomonas* species isolated from poultry manure exhibited specific growth rates and doubling times *P. putida*. lower than the 0.304/d and 2.28 d reported by Obayori *et al*. [30] for a hydrocarbon-degrading *Pseudomonas* sp. LP1. These were however higher than 0.21 /d and 3.31 d reported by Salam *et al*. [27] for *P. aeruginosa* strain K1 isolated from hydrocarbon-contaminated soil.

Remarkably, the degradation rates of strain *P. putida* MP2 and *Pseudomonas* sp. MC4 compares favourably with those of *Pseudomonas* species isolated from hydrocarbon-contaminated niches. As revealed by gas chromatographic analyses of residual oil, after 10 days of incubation,*P. putida* MP2and *Pseudomonas* sp. MC4 *degraded* higher percentage of crude oil than the 70, 54, and 83.5% reported by Obayori *et al.* [31] for three *Pseudomonas* species strains P11, BB3 and WL2 isolated from oil- and asphalt-contaminated soils in Lagos, Nigeria. They were also relatively close to 90.89 to 92.05% Bonny light crude oil degradation rates reported by Salam *et al.* [29] for pure culture strains of *Pseudomonas* species strains G1 and K1 isolated from hydrocarbon-contaminated soil.

The decline in rates of degradation between Day 10 and 20 as compared to that observed between Day 0 and 10 may be attributed to accumulation of toxic metabolites. This inevitably led to decline in growth and a drop in pollutant degradation. This fact is accentuated by the growth profile of the two isolates. *P. putida* MP2 reaches its peak on Day 14 while Pseudomonas sp. MC4 peaked on Day 10 (Figure 1). In essence, degradation of Escravos crude oil by the two isolates was growth associated.

The aliphatic fractions consisting of straight chain, branched chain and cyclic chain carbon moieties are the major constituent of crude oil [32] and are the most readily degraded hydrocarbon compounds [33,34]. In this study, significant reduction in peaks of the aliphatic hydrocarbon components between Day 0 and Day 10 concomitant with exponential growth of strains *P. putida* MP2 and *Pseudomonas* sp. MC4 indicate that the isolates use the hydrocarbon as carbon and energy source. In P. putida MP2, the 41 to 79% reduction in peaks of C_9-C_{20} fractions in 10 days as well as about 90% reduction in peaks of C_{11}-C_{22} fractions after 20 days may be attributed to contaminant structure. Mid-size straight chain aliphatics (C_{10}-C_{18}) are known to be utilized more readily than alkanes with either shorter or longer chains [35]. In addition, the complete disappearance of hydrocarbon fractions C_{17}, C_{24}, C_{25} and C_{26} after 20 days' incubation suggests that the fractions may be saturated alkanes, which are amenable to complete degradation.

In *Pseudomonas* sp. MC4, the complete degradation of C_9 fraction after 10 days is unique as shorter chain n-alkane is difficult to degrade when compared to mid-size straight chain aliphatics [35]. However, in contrast to strain MP2, the complete disappearance of C_{20} and C_{21} after 10 days of incubation indicates the catabolic versatility of this strain as an efficient hydrocarbon degrader than strain *P. putida* MP2. This perhaps explains the higher percentage degradation observed in this strain at Day 10 and Day 20. The complete disappearance of C_{22}-C_{26} fractions after 20 days suggests that the fractions are saturated alkanes, which are easily degraded by microorganisms and are amenable to complete degradation [36].

5. CONCLUSION

While previous studies majorly focused on the use of these organic wastes (poultry droppings and cow dung) as nutritional stimulants to enhance the biodegradation of pollutants, this study brings to the fore the ubiquity of hydrocarbon degrading bacteria from non-hydrocarbon contaminated sources. Our results have shown that these isolates may as well act on varieties of environmental hydrocarbon mixtures such as crude oil, engine oil, diesel and polycyclic aromatic hydrocarbon such as anthracene. Furthermore, we have shown through gas chromatographic analyses, the biodegradative abilities of *Pseudomonas* strains MP2 and MC4 on different components of Escravos light crude oil.

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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.

COMPETING INTERESTS

The authors have no competing interests to declare,

AUTHORS' CONTRIBUTIONS

OSO participated in experimental design, collation and interpretation of data, manuscript preparation, and overseeing execution of experimentation; LBS participated in collation of data, experimentation and manuscript preparation; OOO participated in sample collection, experimentation and data collation; MOA participated in sample collection, experimentation and data collation. All authors read and approved the final manuscript.

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