Original Research Article

Aromatic Hydrocarbons Degradation and Plasmid Profile of Marine Bacteria Isolated from Contaminated Marine Environments of Niger Delta of Nigeria

ABSTRACT

Aims: To determine the aromatic hydrocarbons degradability and plasmid profile of the marine bacteria isolated from Rivers State contaminated marine environments of Niger Delta.
Study Design: Nine treatments and the controls designs were set up in triplicates containing 100 mL of sterile modified mineral basal medium in 500 mL conical flasks supplemented with 1 mg /L of xylene, anthracene and pyrene each; nine marine hydrocarbon degraders and incubated at 24 $^{\circ}$C for 24 days study. The nine treatments and control set ups designated as ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8, PYR9 and CTRL (Without hydrocarbons) were used to determine the aromatic hydrocarbons degradability and plasmid profile of the marine bacteria.
Place and Duration of Study: Biotechnology Laboratory, Institute for Science and Technology Education, University of South Africa, Pretoria, South Africa between September, 2015 to March, 2018.
Methodology: A laboratory scale study was carried on six composite samples of the sediment and water samples from the three studied areas using enrichment, screening, selection, molecular, degradation and plasmid assays.
Results: The findings revealed that the three sampling sites harbour a lot of efficient aromatic degrading bacterial strains belonging to the genera: *Providencia, Alcaligenes, Brevundimonas, Myroides, Serratia, and Bacillus* able to significantly (P = .05) tolerate and grow on the aromatic hydrocarbons. The bacterial strains especially *Serratia marcescens* XYL7 significantly (P = .05) removed 99.50 ± 0.05 % and 60.00 ± 0.02 % in weights of xylene and pyrene, respectively while *Alcaligenes faecalis* PYR5 significantly (P =.05) degraded 97.40 ± 0.01 % in weight of anthracene. The degradations of the respective hydrocarbons were found to be plasmid mediated with plasmid sizes between 200 bp - 1.2 kbp.
Conclusion: Thus, the excellent degradative abilities of these bacterial strains especially *Serratia marcescens* XYL7 could be exploited in bioremediation purposes in Nigeria.

Keywords: Aromatic hydrocarbons, marine bacteria, biodegradation, plasmid, Niger Delta.

Comment [F1]: Arrange alphabetically

1. INTRODUCTION

The Niger Delta ecosystem of Nigeria is subjected to man-induced changes and seriously threatened by increasing environmental deterioration. The aquatic ecosystem of the region faces increasing ecological and toxicological problems from the release of petroleum pollutants [1]. Besides this direct pollution, the occasionally pipeline leaks, transportation accidents, storage tank ruptures and refining petroleum is further intensifying the pollution of this area. Most of these compounds especially the aromatic hydrocarbons are

considered as carcinogenic, mutagenic and potent immunotoxicants and classified as priority environmental pollutant by the US Environmental Protection Agency [2].

Polycyclic Aromatic Hydrocarbons (PAHs) are found in considerable amounts in crude oil and oily effluents of petroleum refineries. Low molecular weight (LMW) PAHs are relatively volatile, high soluble in water and more degradable than high molecular weight (HMW) PAHs [3]. Aromatic with one, two or three aromatic rings (xylene and anthracene) are also efficiently biodegraded; however, those with four or more aromatic ring (pyrene) are quite resistant biodegradation [4]. Besides, the physical processes are often limited to aquatic environments only. The microorganisms should possess all the necessary enzymes needed to degrade PAHs [5].

Extensive studies have been done on the biodegradation of isolated bacteria from the natural environment leading to isolation of some bacteria which have the ability of using PAHs compounds as the sole carbon and energy source. Isolating the bacteria with necessary performance for degradation of organic pollutants such as xylene, anthracene and pyrene in soil and water ecosystems can be the perfect solution for improving the microbial population in areas contaminated by hydrocarbons [6]. Several species of bacterial genera *Pseudomonas, Serratia, Marinobacter, Providencia, Alcaligenes, Pseudomonas, Salmonella, Nocardia, Mycobacterium, Cunninghamella, Rhodococcus, Beijerinckia, Lysinibacillus, Corynebacterium, Diaphorobacter, Pseudoxanthomonas, Bacillus* and *Sphingomonas* have been found highly capable of degrading xylene, anthracene and pyrene hydrocarbons and well documented [3,7,8,9,10,11].

Plasmid or chromosomal mediated degradation can be useful and effective during the remediation of crude oil contaminated sites. Many bacterial strains have genetic determinants of resistance/degradative abilities to pollutants. These determinants are often found on plasmids, chromosomes and transposons. The plasmid profiles of strains of several bacterial genera: *Pseudomonas*, *Rhodococcus*, *Micrococcus, Citrobacter koseri, Serratia, Nitrosomona*s, *Nitrobacter Shewanella, Vibrio* and *Bacillus* isolated from petroleum-contaminated soils and sediments selected for their capacities to grow in the presence of petroleum and some aromatic hydrocarbons have been determined and the biodegradative abilities have proved to be plasmid related [1,8,12,13,14].

There are dearth of information regarding microbial degradation of aromatic hydrocarbons and plasmid profile of the aromatic bacteria degraders in crude oil - impacted Niger Delta ecosystem and hence necessitated and justifies this study. This study was undertaken to determine the aromatic hydrocarbon degradability and plasmid profile of the marine bacteria isolated from Rivers State contaminated marine environments of Niger Delta of Nigeria.

2. MATERIALS AND METHODS

2.1 Description of the Sampling Sites

The studied areas were Abonema Wharf Water Front (Figure 1) in Akuku-Toru Local Government Area, Nembe Water-side (Figure 2) in Port Harcourt Local Government Area and Onne Light Flow Terminal Seaport (Figure 3) located in Eleme Local Government Area of Rivers State. Abonema town is 53 km and Abonema Wharf Water Front is 3 - 5 km from Port Harcourt capital city; Nembe water side is located within Port Harcourt capital city of Rivers State, while Onne Light Flow Terminal is about 35 km east from Port Harcourt capital city of Rivers State and 7 km from Onne town. These sites were geo - referenced using Handheld Global Positioning System (GPS) GPSMAP 76 sc with the coordinates obtained from the sampling points or positions Abonema Wharf Water Front, Nembe Water-side and Onne Light Flow Terminal Seaport were located between latitude 4°46'15.82"N to latitude 4°46'38.01"N and longitude 7°0'0.54"E to longitude 7°0'34.82"E with average elevation of 4.1 m , latitude 4°45'8.72"N to latitude 4°45'26.42"N and longitude 7°1'11.37"E to longitude 7° 2'14.54"E with average elevation of 2.7 m and latitude 4°41'32.58"N and 4°41'58.18"N and longitude 7°9'26.34"E and 7°10'48.82"E with average elevation of 2.3 m, respectively. These water - ways are subjected to human - induced pressures resulting from urbanization, industrialization and intensive navigation. Abonema Wharf Water Front community is a popular and busy commercial but dangerous jetty area close to Portharcourt city inhabiting tens of thousands of different families living close to petroleum tank farms and tankers queue up daily to load refined petroleum products. Nembe Waterside is situated very close to Creek road market, Port Harcourt, Nigeria. It shares boundary with Bayelsa and links Port Harcourt city with Bonny Island where most of the oil installations in Rivers State are. It also links the Island directly with the Atlantic ocean through which crude oil is exported by massive oil tankers. Onne Light Flow Terminal Seaport (Plate 16) is a port of Nigeria and the largest oil and gas free zone in the world supporting exploration and production for Nigerian activities. It is situated on the Bonny River Estuary along Ogu creek and account for over 65 % of the export cargo through the Nigerian Sea Port. Anthropological survey revealed the presence of human activities such as transportation of petrochemical products through tankers, canoes, boats and ships to neighboring villages, towns, cities, states and nations due to the presence of multinational petrochemical and oil servicing industries such as Chevron Nigeria Limited, Cameron Offshore services, Exon Mobil Nigeria Limited, Socotherm Pipecoaters, Beker Hughes Oil Servicing Company, Aiteo Energy Resource, Sorelink Oil and Dozzy Oil and gas et cetra that generate the wastes that contaminate the sites above.

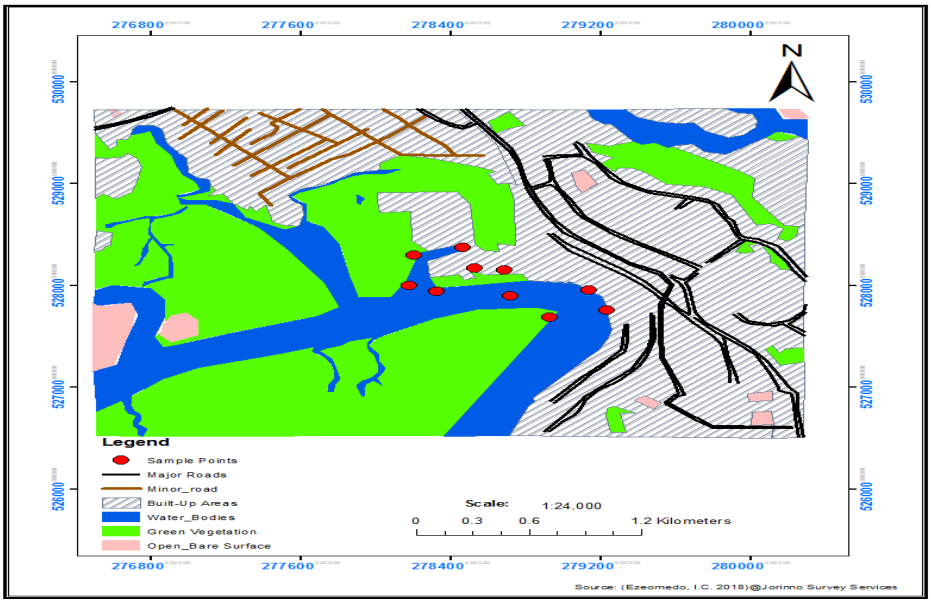
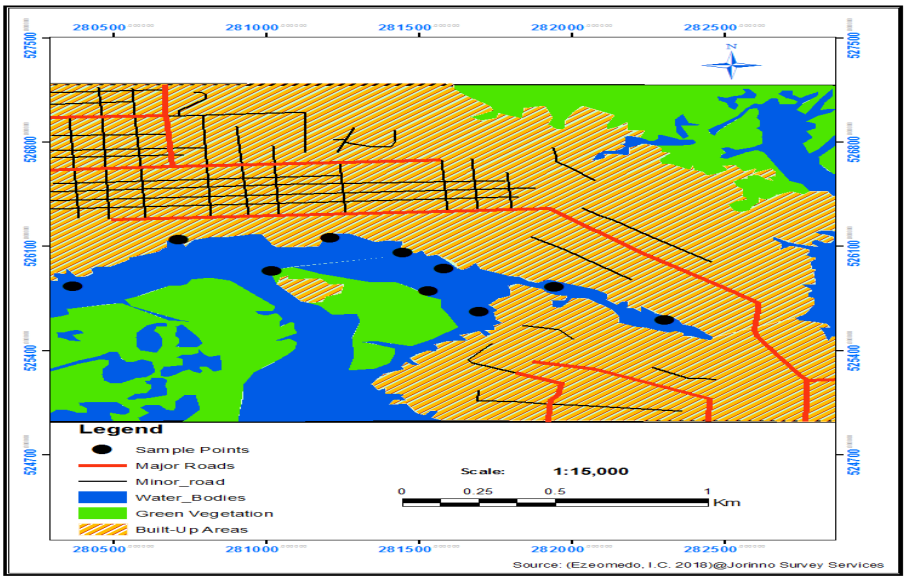


Fig. 1. Thematic map indicating the locations of sample points and its land cover in Abonema study area



3.2

Fig.2. Thematic map indicating the locations of sample points and its land cover in Nembe study area

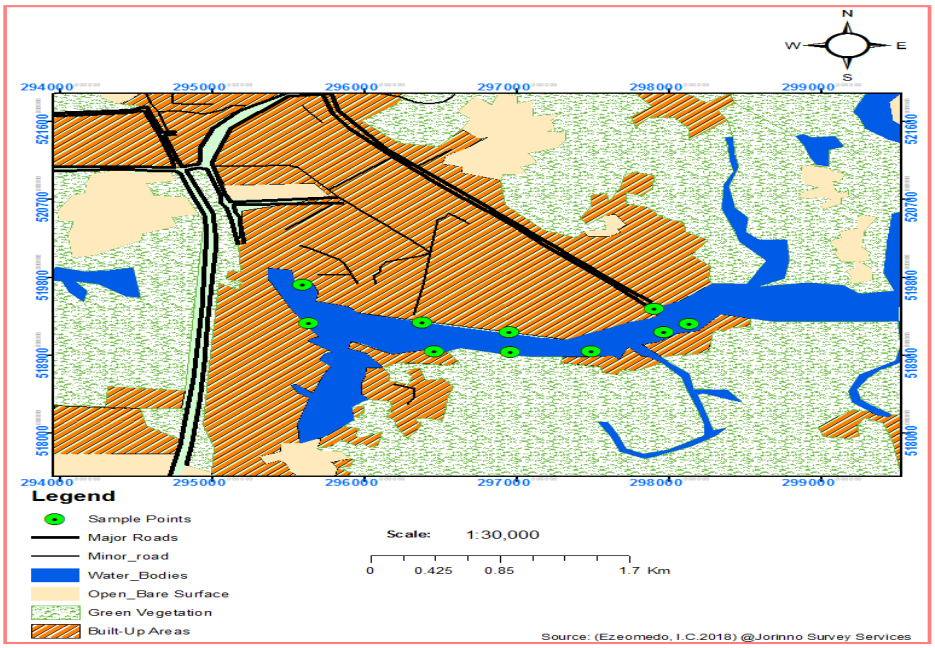


Fig. 3.Thematic map indicating the locations of sample points and its land cover in Onne study area

2.2 Sample Collection and Processing

Ten samples were collected randomly at each designated points in the three particular sampling sites (Figures 1, 2 and 3) and mixed together after which a total of six representative sediment and water samples were taken for the analysis. The surface aerobic sediment samples were collected with a 95 % ethanol - sanitized plastic spatula at 5 cm depth inside 95 % ethanol - sanitized wide mouthed plastic containers. The water samples were collected at the air-water interface by hand dipping the 95 % ethanol - sanitized cylindrical shaped 2 L plastic containers. The containers were rinsed with the sediment and water samples before collecting the samples. All the composite or representative sediment and water samples containers were placed into a sterile polythene bag and then transported to the laboratory for microbiological analyses [15,16, 17].

2.3 Enrichment, Culturing and Isolation of Aromatic Hydrocarbon Bacterial Strains

The hydrocarbon degraders were isolated from sediment and water samples of the three sampling sites using modified mineral basal agar (4 g K_2HPO_4, 1.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g $FeSO_4$,

0.1 g NaCl, 0.2 g $CaCl_2$, 15 g Agar agar and distilled water 1,000 mL at pH 7.00 ± 0.20) enriched with xylene, anthracene and pyrene as sole carbon and energy source. The medium was sterilized by autoclaving at 121 $^{\circ}$C and 15 psi[2] for 15 minutes. Thereafter, 0.2 mL acetone solution containing 0.1 % w/v of the selected hydrocarbons (xylene, anthracene and pyrene) were aseptically pipetted and uniformly spreaded on the agar surface of the pre - dried Petri dish plates. The acetone was allowed to evaporate under sterile condition and 0.1 mL aliquots of the 10^{-3} dilutions were spread plated on the surfaces of the solidified media with the aid of a glass spreader. The spreader was sterilized after each successive spreading by dipping it in 70 % ethanol and then passing it through flame of a Bunsen burner. The inoculated plates were sealed using adhesive tape and foil to prevent contamination and photolysis and later placed in black polythene bags, and then incubated in the dark at 28.00 ± 0.20 °C for 14 days [1,17].

2.4 Purification and Maintenance of Cultures

Colonies that developed on hydrocarbon - coated plates were replicated onto fresh hydrocarbon - coated agar plates and incubated for 14 days. Isolates that grew on these plates were selected as xylene, anthracene and pyrene degraders and sub - cultured on Bjou bottles where they are preserved at 4 °C in refrigerator [17].

2.5 Screening and Selection Test

In order to screen and select the best and strongest degrading strains, growth of the different organisms were tested by growing 5 mL of each desired isolates in large test tubes containing 25 mL of the modified mineral basal medium with 100 mg /L of xylene, anthracene and pyrene hydrocarbons which were dissolved in acetone and added to each tube after autoclaving. Thereafter, the test tubes were incubated at room temperature (28.00 ± 2.00 °C) for five days. Bacteria that started growing fast with high turbidity in the vicinity of the medium containing aromatic compounds measured at 600 nm using a UV - VIS spectrophotometer (Astell, UV - Vis Grating, 752 W) were selected as the candidate of xylene, anthracene and pyrene degrading bacteria [6,17,18].

2.6 Characterization and identification of selected hydrocarbon utilizing bacterial isolates

2.6.1 Morphological characteristics

2.6.1.1 Colonial morphology

After sub - culturing and incubation, culturing morphological properties such as shape, elevation, margin, optic, texture, colour, size and surface characteristics of the selected bacterial strains were observed and noted [19].

2.6.1.2 Microscopic morphology

The standard methods of Gram staining and endospore staining were carried out on the selected bacterial strains as described in Cheesbrough [20] and Health Protection Agency [21].

2.6.2 Biochemical characteristics

The standard methods of testing for catalase, indole, motility, methyl red – Voges Proskauer, citrate, urease, starch hydrolysis, gelatin, nitrate reduction, coagulase, Hydrogen sulphide production, Sugar fermentation, Oxidase and Casein hydrolysis tests were carried out as described in Willey *et al*. [19] and Cheesbrough [20].

2.7 Degradation assay

Following the methods of Bennet *et al*. [21] and John and Okpokwasili [1] as modified in this study, the degradation rates of bacterial isolates were determined using hydrocarbon supplemented modified mineral basal medium (4 g K_2HPO_4, 1.0 g $(NH_4)2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g $FeSO_4$, 0.1 g NaCl, 0.2 g $CaCl_2$, 15 g Agar agar and distilled water 1,000 mL at pH 7.00 ± 0.20). Precisely, 1 ml of 48 hrs old cultures of each bacteria was introduced into 28 sterile 200 ml capacity conical flasks (4 sets of 7 flasks) in triplicates containing 100 ml of sterile modified mineral basal medium supplemented with 100 mg /L of xylene, anthracene and pyrene hydrocarbons respectively as source of carbon at 24 $^{\circ}$C for 24 days. During incubation, representative samples from the four sets of flasks were withdrawn at intervals of 0, 4, 8, 12, 16, 20 and 24 days and the residual hydrocarbons were determined spectrophotometrically using ethyl acetate as the extraction solvent. For each sample, 5 ml ethyl acetate was added and vigorously shaken manually. The organic and aqueous layers from the media were separated by centrifugation at 5000 rpm for 20 mins. The aqueous layers were discarded while the organic layers were analyzed with UV - VIS spectrophotometer at 240 nm wavelength (Astell UV - Vis Grating, 752 W). The percentages of biodegradation of the hydrocarbons were determined as follows:

$$\% \text{ degradation } = \frac{a - b}{a} \times \frac{100}{1}$$

Where a = the absorbance of the medium before incubation; b is the maximum absorbance of the medium after each 4th day of the incubation period.

2.8 Plasmid Analysis

2.8.1 Plasmid curing experiment

Plasmid analysis was performed to show whether the hydrocarbon degrading genes are plasmid encoded or chromosomal encoded by adopting the method of John and Okpokwasili [1] and Kumar *et al.* [23]. Zero point one millilitre of the culture of the modified mineral basal medium (MBM) (4 g K_2HPO_4, 1.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g $FeSO_4$, 0.1 g NaCl, 0.2 g $CaCl_2$, 15 g Agar agar and distilled water 1,000 mL at pH 7.00 ± 0.20) were added to nine flasks containing 100 mL of nutrient broth containing ethidium bromide (450 µg / mL) for curing experiment. The set ups were incubated at 30 °C for 24 hrs and thereafter, the broth was agitated to homogenize the content and loopful of the broth medium was subcultured on utrient agar plate and also on MBM agar containing the respective hydrocarbons. The plates were incubated at 37 °C for 24 hrs and the colonies counted. Colonies that failed to grow on MBM agar plates were considered cured.

2.8.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility of bacterial isolates were performed on modified mineral basal agar (MBA) plates using a disc diffusion method in order to verify the curing experiment. In this case, 0.1 mL of the 24 - 48 hrs cultures of the test isolates were pippeted, inoculated and spreaded in nine Petri dishes (90 x 15 mm) containing MBA agar and allowed to stand for 30 mins to enable the inoculated test bacteria to pre-diffuse. Thereafter, commercial discs containing 15 µg each of levofloxacin, ampicillin, amoxicillin and cefadroxil; 25 µg each of tetracycline; 30 µg each of chloramphenicol, erythromycin and azithromycin (Anatech, South Africa) were aseptically placed on the surfaces of the sensitivity agar plates using sterile forceps and incubated at 37 °C for 24 hrs. After incubation, zones of inhibitions were observed against the entire antibiotics and recorded. The presence of zone of inhibition (sensitive colony) is indicative of chromosome - mediated resistance (plasmid not cured) while absence of zone of inhibition (resistant colony) was indicative of plasmid - mediated resistance (plasmid cured) [1,23].

2.8.3 Plasmid isolation and agarose gel electrophoresis

Plasmid DNAs were extracted by hot alkaline method for all plasmid sizes and bacteria with slight modifications both on the cured and uncured isolates. Two to three millilitres (2 - 3 mL) of the cultures were centrifuged, pellet resuspended in 1 mL of solution containing 0.04 M Tris-acetate, pH 8.00 (adjust pH with glacial acetic acid) and 2 mM EDTA. Two millilitres (2 mL) of lysis buffer (0.05 M Tris, 3 % SDS, pH 12.50, adjusted with 2 N NaOH) were added and mixed. The mixtures were incubated at 60 °C for 45 mins. Four hundred microlitres (400 µL) hot samples of phenol/chloroform (1:1) were added and mixed gently to complete emulsification. Phases were separated by centrifugation at 13, 400 x g for 15 mins. at room temperature and the upper aqueous phases were transferred carefully (avoid interphase which contains debris) to new tube containing 600 µL of chloroform. The supernatants were mixed and centrifuged again for separation of phases. The aqueous phases were recovered and used directly for agarose gel. After electrophoresis on a 0.7 % horizontal agarose gel at 50 V for 3 hrs, the gels were stained with ethidium bromide and bands were visualized with a UV transilluminator. Molecular -sized plasmids were determined by comparison with the known 1 kbp weight supercoiled DNA ladder (Inqaba Biotech, SA) as a control [1,14,23,24].

2.9 Data Analysis

The data were analyzed using Graph-Pad Prism statistical software version 7.00 (GraphPad software Inc. San Diego, California). All values were expressed as mean ± standard deviation. Ordinary one-way and two-way analyses of variance (ANOVA) followed by post Tukey's, multiple comparison of treatment with that of control. The results were considered statistically significant if the probability is less than 0.05 (P = .05) [6,12].

3. RESULTS

3.1 Isolation and Selection Test

The result of the growth performance (OD_{600} nm) of the aromatic hydrocarbon-degraders isolated from Abonema sampled location is presented in Table 1. From the result, 13 isolates were obtained with strains XYL2, ANT4 and PYR3 having the highest significant *(P* = .05) absorbance values of 0.952 ± 0.004, 0.775 ± 0.007 and 1.041 ± 0.008 on xylene, anthracene and pyrene hydrocarbons. The result of the growth performance (OD_{600} nm) of the aromatic hydrocarbon-degraders isolated from Nembe sampled location is presented in Table 2. From the result, 17 isolates were obtained with strains XYL7, ANT1 and PYR5 having

the highest significant (*P* = .05) absorbance values of 1.055 ± 0.002, 0.816 ± 0.007 and 0.933 ± 0.007 on xylene, anthracene and pyrene hydrocarbons. The result of the growth performance (OD_{600} nm) of the aromatic hydrocarbon-degraders isolated from Onne sampled location is presented in Table 3. From the result, 18 isolates were obtained with strains XYL8, ANT6 and PYR9 having the highest significant (*P* = .05) absorbance values of 0.741 ± 0.007, 1.433 ± 0.013 and 0.871 ± 0.001 on xylene, anthracene and pyrene hydrocarbons. On the basis of these results, strains ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 were selected as the best and strongest degraders of xylene, anthracene and pyrene hydrocarbons.

3.2 Morphological and Biochemical Characteristics of Bacterial Isolates

The result of the colonial morphology of aromatic hydrocarbon degrading bacteria is presented in Table 4. From the result, most colonies were circular and irregular in shape, flat in elevation, undulate in margin, translucent in optic, smooth in texture, creamy in colour, 4 mm in size and glistering in surface description.

The result of the morphological and biochemical properties of the aromatic hydrocarbon degrading bacterial isolates is presented in Table 5. From the result, most bacterial isolates were Gram negative in Gram reaction, rod shaped arranged in single or pair, negative to spore, indole, methyl red, Voges Proskauer, urease, gelatin, nitrate reduction, coagulase, hydrogen sulphide production, xylose, lactose, arabinose, maltose and casein hydrolysis tests while positive to catalase, motility, citrate, starch hydrolysis, mannitol, glucose, sucrose, saccharose and oxidase tests.

3.3 Degradation Assay

The results of the weight losses from xylene, anthracene and pyrene resulting from the growth of marine bacterial isolates are presented in Tables 6, 7 and 8. From the Tables 6 - 8 results, *Serratia marcescens* XYL7 exhibited the highest capabilities to degrade the aromatic hydrocarbons with 99.50 ± 0.05 % and 60.00 ± 0.02 % in weights of xylene and pyrene respectively while *Alcaligenes faecalis* PYR5 degraded anthracene with 97.40 ± 0.01 % reduction in weight after 24 days biodegradation study. There were extreme significant (*P* =.05) degradation among the treatment group of degraders and controls as well as very strongly significant positive correlation (r = 0.897 – 0.996) between incubation days and the percentage degradation of hydrocarbons by the degraders

3.4 Plasmid Analysis

The result of the zones of inhibition of different antibiotics against the marine bacterial isolates is presented in Table 9. From the result, zones of inhibition were observed against tetracycline (10.00 ± 0.20 – 18.00 ± 0.10 mm), levofloxacin (14.00 ± 0.10 – 21.10 ± 0.00 mm), cefradoxil (11.30 ± 0.00 – 22.90 ± 0.20 mm), chloramphenicol (9.00 ± 0.10 – 17.30 ± 0.15 mm), erythromycin (8.00 ± 0.40 – 16.00 ± 0.00 mm) and azithromycin (9.50 ± 0.25 – 18.00 ± 0.30 mm) antibiotics while ampicillin (0.00 ± 0.00 mm) and amoxicillin (0.00 ± 0.00 mm) antibiotics showed no zone of inhibition. The result of the electrophoretic separation profile of plasmid DNAs from cured and non-cured strains of the marine bacteria is shown in Plate 1. The result showed that seven of the nine selected isolates, had multiple plasmids each (except PYR3 and PYR5 had single plasmid).The two isolates were *Brevundimonas diminuta* PYR3 and *Alcaligenes faecalis* PYR5. The plasmids as revealed in Plate 1 below were of different sizes. The smallest is 200 bp while the largest was found to be above 1.2 kbp. The results also showed that the plasmids were partially cured hence the incomplete disappearance of all the bands after plasmid curing in the seven isolates as shown in the plate 1 (except PYR3 and PYR5).

4. DISCUSSION

It has been established by many studies that bioremediation i.e. the exploitation of microorganisms for detoxifications of heavy metal ions, aromatic hydrocarbons, petroleum products, pesticides and other toxic organic molecules is the method of choice owing to fewer secondary hazards and generally low cost. Studies showed that hydrocarbon degrading bacteria are ubiquitously distributed in soil and aquatic environments. However, their populations constitute less than 1 % of total microbial communities. Many of the microorganisms proposed for biodegradation and bioremediation have been isolated from contaminated soils and waters [25].

In this study, a total of nine (9) isolates ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 out of the 48 isolates (9/48) representing 18.75 % of the isolates were screened and selected as best and strongest degraders of xylene, anthracene and pyrene hydrocarbons which they significantly (P = .05) utilize as source of carbon and energy and is indicated by absorbance values below of each isolates (Tables 1, 2 and 3). This study agrees with the explanation of Mao *et al*. [26] that the most important aspect of microbial degradation of PAH is enrichment and isolation of indigenous PAH degraders because the

indigenous PAH degrading bacteria are already adapted to utilizing PAH. Pathak and Bhatnagar [27], argued that enrichment culturing is important for the success of hydrocarbon bioremediation because the process leads to selection of microorganism accustomed to hydrocarbon degradation. Esedafe *et al*. [28] reported that an occurence of 3/41 representing 7.32 % isolates from refinery effluent were capable of utilizing phenanthrene and anthracene as sole carbon and energy sources. It also indicates that only these isolates had the physiological capabilities to metabolize the aromatic hydrocarbons.

The result in Table 4 showed that most colonies were circular and irregular in shape, flat in elevation, undulate in margin, translucent in optic, smooth in texture, creamy in colour, 4 mm in size and glistering in surface description. The results of this research agree with the research carried out by Al -Thani *et al.* [5] who reported that a diverse microbial population can be isolated from hydrocarbon contaminated samples. In agreement with research carried out by Arulazhagan *et al*. [29] and Akinbankole *et al*. [11] who reported that bacteria isolated from pyrene and anthracene enriched medium are known to utilize pyrene and anthracene as their sole carbon source for growth and energy. The result in Table 5 showed that most of the marine bacterial isolates were Gram negative in Gram reaction, rod shaped arranged in single or pair, with variable reactions to different biochemical tests. The marine bacteria were identified as *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain XYL2, *Brevundimonas diminuta* strain PYR3, *Alcaligenes faecalis* strain ANT4, *Alcaligenes faecalis* strain PYR5, *Myroides odoratus* strain ANT6, *Serratia marcescens* strain XYL7, *Providencia* sp. strain XYL8 and *Bacillus cereus* strain PYR9 using Bergey's Manual for Determinative Bacteriology by Holt *et al.* [30]. These findings agree with the reports of Mrozik *et al*. [31], Okerentugba and Ezeronye [32], Chikere *et al*. [33] John *et al.* [17), Irshaid and Jacob [34], Wanjohi *et al*. [35], Isiodu *et al*. [12] and Fagbemi and Sanusi [8] that two - third of most petroleum hydrocarbon degraders are Gram negatives with one - third being Gram positives but contradict the findings of Akinbankole *et al*. [11], Kafilzadeh *et al*. [36] and kafilzadeh and Pour [6], who reported that more of Gram positive bacteria were isolated than Gram negative bacteria. A lot of rod - shaped bacteria have also been implicated in hydrocarbon degradation studies (Okoh [37], Perfumo *et al*. [38], Chikere *et al*. [33], Alfreda and Ekene [39]; John *et al.*, [17], Akinbankole *et al*. [11], Irshaid and Jacob [34], Wanjohi *et al*. [35], Isiodu *et al.* [12] and Fagbemi and Sanusi [8] and similar result was obtained in this study.

The results of the weight losses from xylene, anthracene and pyrene resulting from the growth of marine bacterial isolates are presented in Tables 6, 7 and 8. From the results, the degree of weight loss was observed to increase with increase in incubation period but varied with different microbial species tested. *Serratia marcescens* XYL7 significantly exhibited the highest capabilities to degrade the aromatic hydrocarbons with 99.50 ± 0.05 % and 60.00 ± 0.02 % in weights of xylene and pyrene respectively while *Alcaligenes faecalis* PYR5 significant degraded anthracene with 97.40 ± 0.01 % reduction in weight after 24 days biodegradation study with evidence of increasely low optical density ($OD_{600\ nm}$) against their controls. However, the level of xylene, anthracene and pyrene degradations also included 5.20 ± 0.03 %, 4.60 ± 0.01 %, 11.10 ± 0.06 % degradation by abiotic factor as observed in controls with no bacterial inocula. After deduction of pyrene degradation by abiotic factor, 94.30 ± 0.02 % and 49.90 ± 0.06 % of xylene and pyrene degradation was in fact contributed by *Serratia marcescens* XYL7 and 92.80 ± 0.02 % by *Alcaligenes faecalis* PYR5 during this period. Differences in xylene, anthracene and pyrene degradation abilities of bacterial strains were clearly reflected even after 4 days of incubation which was further magnified during incubation periods (4 – 24 days) with very strongly significant positive correlation (r = 0.897 – 0.996). Thus, xylene, anthracene and pyrene were degraded by all nine bacterial strains, but they differed widely in their inherent abilities. The differences might be due to high molecular weight PAHs that are more recalcitrant and hard to microbial attack and the sequence of degradation is xylene > anthracene > pyrene. Biodegradation of PAHs is depends on their chemical structure and corresponding physiochemical properties and low molecular weight PAHs degrade rapidly than high molecular weight PAHs [40]. Similar results are obtained in the present study. Singh *et al.* [41] reported that *Rhodococcus pyridinvorans* NJ2 was the highest degrader (60 %) of pyrene, followed by *Pseudomonas* sp. BP10 (44 %) and the least was *Ochrobactrum intermedium* P2 (42 %) in MSM with pyrene (50 µg/ml) in 8 days. Swaathy *et al.* [10], reported that the marine isolate, *Bacillus licheniformis* MTCC 5514 degraded > 95 % of 300 ppm anthracene in an aqueous medium within 22 days and the degradation percentage reduced significantly when the concentration of anthracene increased to above 500 ppm. Akinbankole *et al.* [11] reported that pyrene and anthracene utilizing bacteria (*Salmonella enterica* and *Bacillus toyonesis*) were isolated from water and used engine oil contaminated soil degraded 99 % of the PAH within seven days. Arulazhagan *et al*. [29] in their report in which bacteria they isolated from contaminated sites also degraded over 99 % of flourene and anthracene in seven days. Poornachander *et al*. [42] reported that *Bacillus cereus* CPOU13 degraded phenanthrene about to 73.46 % and its initial concentration declined from 216.32 µg to 56.57 µg;

anthracene to 85.76 % and its initial concentration reduced from 209.20 µg to 32.63 µg and pyrene to 47.88 % and its initial concentration reduced from 230.14 µg to 119.95 µg. Qi *et al*. [43] reported that *Gordonia* sp. nov. Q8 could remove 73.8 % and 53.4 % of anthracene and pyrene hydrocarbons in 7 days. Lily *et al*. [9] reported that *Brachybacterium paraconglomeratum* strain BMIT637C was the efficient degrader of anthracene being capable of degrading 70.32 % of anthracene within 10 days showing 2 x 10^{45}- fold increase in the CFU number that indicated anthracene utilization as a sole source of carbon and energy. Akpe *et al.* [13] reported that the lower the optical density value, the higher the percentage degraded. Pandey *et al.* [44] reported that PAH (phenanthrene, flourene, anthracene, pyrene) dissipation level ranged between 38.7 to 99.7 % with highest depletion recorded in phenanthrene within seven days. The degradation rate of 3 - ring PAH was higher as compared to 4 - ring PAH (pyrene) by *Serratia marcescens* L - 11. The findings of these authors supported the results of this study.

Catabolic pathways which encoded different aromatic hydrocarbon degradation routes, are frequently located on plasmids, although degradative genes can be located on either chromosome or plasmid [45]. The curing experiment was verified by carrying out antimicrobial susceptibility of the isolates and the result of the zones of inhibition of different antibiotics against the marine bacterial isolates is presented in Table 35. This suggests that the marine bacterial strains may possess the resistance genes for these two antibiotics (ampicillin and amoxicillin) and hence, these antibiotics were used as marker for the screening of plasmid cured bacterial colonies. Similar observation was obtained by Kumar *et al*. [23] who tested several antibiotics for their resistances against *Pseudomonas* sp. strain E and observed that strain E was resistant against cefadroxil and ampicillin antibiotics. John and Okpokwasili [1] reported that *Nitrosomonas* and *Nitrobacter* species were resistant against cefadroxil and ampicillin antibiotics. The result in Plate 1 showed that the plasmids were partially cured hence the incomplete disappearance of all the bands after plasmid curing in the seven isolates as shown in the plate 1 (except PYR3 and PYR5). Although there was partially curing, it further revealed that catabolic genes responsible for xylene, anthracene and pyrene degradation were located on the plasmids. Plasmid cured colonies of marine bacterial strains were not able to grow on MSM with xylene, anthracene and pyrene as sole carbon sources. It is assumed that this may be because of the removal/inactivation of gene (s) responsible for aromatic hydrocarbon degradation from the bacterial strains. It can be deduced that their presence in polluted substrate encourage the development of adaptive features such as plasmid which support hydrocarbon cometabolism [1]. The presence of multiple plasmids

in hydrocarbon degrading bacteria were already reported in *A. chroococcum* [46], *Klebsiella pneumoniae* and *Serratia marscencens* [13] *Pseudomonas* species [47] and *Enterobacter cloacae* [48]. Rasool *et al.* [49] reported that most of the cured isolates had lost the potential *in toto* to degrade xylene and octane while majority of the cured isolates were only partially cured to degrade naphthalene. Infact, a complete or partial curing is the function of the copy number of plasmids plus the scattered distribution of these copies (the ones residing away from the periphery are not affected by the curing agents). Similar kind of observation was reported by Fagbemi and Sanusi [8] who observed that the genetic factor responsible for crude oil degradation in *Bacillus coagulans* and *Citrobacter koresi* were chromosomal mediated while that of *Serratia ficaria* was plasmid mediated.

Table 1. Growth performance of the aromatic hydrocarbon - degraders isolated from Abonema sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
AB1	0.657 ± 0.008	0.657 ± 0.001	0.580 ± 0.003
ANT4*	0.676 ± 0.004	0.775 ± 0.007	0.822 ± 0.002
AB3	0.701 ± 0.021	0.467 ± 0.029	0.666 ± 0.003
AB4	0.715 ± 0.004	0.598 ± 0.024	0.841 ± 0.001
PYR3*	0.598 ± 0.005	0.511 ± 0.003	1.041 ± 0.008
AB6	0.641 ± 0.001	0.494 ± 0.002	0.653 ± 0.001
AB7	0.687 ± 0.004	0.690 ± 0.002	0.803 ± 0.016
AB8	0.618 ± 0.001	0.638 ± 0.007	0.782 ± 0.003
AB9	0.457 ± 0.002	0.475 ± 0.001	0.573 ± 0.004
XYL2*	0.952 ± 0.004	0.312 ± 0.002	0.838 ± 0.021
AB11	0.793 ± 0.014	0.495 ± 0.002	0.970 ± 0.003
AB12	0.647 ± 0.002	0.446 ± 0.001	0.621 ± 0.005
AB13	0.328 ± 0.001	0.415 ± 0.001	0.451 ± 0.001

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 2. Growth performance of the aromatic hydrocarbon - degraders isolated from Nembe sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
NW1	0.885 ± 0.003	0.236 ± 0.005	0.708 ± 0.008
PYR5*	0.710 ± 0.003	0.216 ± 0.005	0.933 ± 0.007
NW3	0.466 ± 0.007	0.201 ± 0.000	0.806 ± 0.004
NW4	0.893 ± 0.002	0.356 ± 0.008	0.827 ± 0.008
NW5	0.750 ± 0.004	0.132 ± 0.005	0.767 ± 0.008
NW6	0.644 ± 0.004	0.246 ± 0.004	0.724 ± 0.008
NW7	0.561 ± 0.003	0.193 ± 0.005	0.808 ± 0.001
NW8	0.628 ± 0.008	0.472 ± 0.001	0.826 ± 0.008
XYL7*	1.055 ± 0.002	0.588 ± 0.005	0.927 ± 0.001
NW10	0.809 ± 0.002	0.785 ± 0.002	0.881 ± 0.004
NW11	0.826 ± 0.001	0.444 ± 0.002	0.891 ± 0.001
NW12	0.625 ± 0.005	0.563 ± 0.001	0.728 ± 0.006
NW13	0.374 ± 0.008	0.775 ± 0.001	0.760 ± 0.001
NW14	0.701 ± 0.001	0.622 ± 0.003	0.788 ± 0.007
NW15	0.705 ± 0.008	0.529 ± 0.004	0.830 ± 0.002
NW16	0.769 ± 0.002	0.380 ± 0.001	0.822 ± 0.001
ANT1*	0.804 ± 0.003	0.816 ± 0.007	0.583 ± 0.001

** = Isolates with highest degradability; values are mean ± Standard deviation of triplicate determination.*

Table 3. Growth performance of the aromatic hydrocarbon - degraders isolated from Onne sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
ON1	0.721 ± 0.001	0.884 ± 0.007	0.500 ± 0.001
ON2	0.204 ± 0.001	0.660 ± 0.011	0.454 ± 0.001
ON3	0.473 ± 0.003	0.476 ± 0.036	0.561 ± 0.013
ON4	0.207 ± 0.001	0.766 ± 0.001	0.565 ± 0.033
ON5	0.477 ± 0.002	0.457 ± 0.001	0.378 ± 0.005
ON6	0.409 ± 0.005	0.489 ± 0.100	0.562 ± 0.021
ON7	0.251 ± 0.003	0.428 ± 0.014	0.728 ± 0.001
ON8	0.111 ± 0.005	0.429 ± 0.014	0.425 ± 0.021
ON9	0.463 ± 0.008	0.357 ± 0.011	0.281 ± 0.006
PYR9*	0.106 ± 0.001	0.335 ± 0.001	0.871 ± 0.001
ON11	0.700 ± 0.001	0.901 ± 0.005	0.417 ± 0.002
ANT6*	0.511 ± 0.006	1.433 ± 0.013	0.568 ± 0.009
ON13	0.273 ± 0.002	0.386 ± 0.002	0.527 ± 0.001
ON14	0.278 ± 0.005	0.553 ± 0.022	0.684 ± 0.003
ON15	0.291 ± 0.003	0.748 ± 0.009	0.522 ± 0.010
ON16	0.662 ± 0.001	0.919 ± 0.002	0.494 ± 0.002
XYL8*	0.741 ± 0.007	0.510 ± 0.013	0.602 ± 0.004
ON18	0.354 ± 0.002	1.004 ± 0.001	0.478 ± 0.001

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 4. Colonial morphology of the aromatic hydrocarbon degrading bacterial isolates

Isolate	Colonial description							
	Shape	Elevation	Margin	Optics	Texture	Colour	Size	Surface
ANT1	Circular	Flat	Undulate	Translucent	Smooth	Creamy	4.0 mm	Dull
XYL2	Irregular	Raised	Erose	Translucent	Smooth	Creamy	3.0 mm	Dull
PYR3	Circular	Flat	Undulate	Translucent	Smooth	Creamy	2.0 mm	Glistering
ANT4	Circular	Flat	Erose	Translucent	Smooth	Creamy	5.2 mm	Glistering
PYR5	Circular	Flat	Undulate	Translucent	Smooth	Creamy	4.0 mm	Dull
ANT6	Rhizoid	Flat	Lobate	Translucent	Rough	Yellow	4.2 mm	Glistering
XYL7	Irregular	Flat	Undulate	Translucent	Rough	Red	4.0 mm	Glistering
XYL8	Irregular	Flat	Undulate	Translucent	Smooth	Creamy	4.2 mm	Glistering
PYR9	Irregular	Raised	Undulate	Translucent	Smooth	Creamy	4.0 mm	Glistering

Table 5. Morphological and biochemical properties of the aromatic hydrocarbon degrading bacterial isolates

Property	Isolate								
	ANT1	XYL2	PYR3	ANT4	PYR5	ANT6	XYL7	XYL8	PYR9
Gram reaction	–	–	–	–	–	–	–	–	+
Cellular morphology	Paired short rods	Singled long Rods	Singled long rods	Singled short rods	Singled long rods	Single longer rods and long chains	Paired short rods	Single paired long Rods	Singled short rods
Spore test	–	–	–	–	–	–	–	–	+
Catalase	+	+	+	+	+	+	+	+	+
Indole	+	–	–	–	–	–	–	+	+
Motility	+	+	+	+	+	–	+	+	+
Methyl red	–	–	–	–	–	–	–	–	+
Voges-Proskauer	–	–	–	–	–	–	+	–	–
Citrate	–	+	–	+	+	+	+	–	+
Urease	+	–	–	–	–	–	–	+	+
Starch hydrolysis	+	+	–	+	–	+	–	+	+
Gelatin hydrolysis	–	–	–	–	–	–	+	–	–
NO_3 reduction	+	–	–	–	–	–	+	+	+
Coagulase test	+	–	–	–	–	–	+	+	–
H_2S production	+	–	–	–	–	+	–	+	+
Mannitol	+	–	–	–	–	+	+	+	+
Glucose	+	+	–	+	+	+	+	+	+

Xylose	_	_	_	_	_	_	+	_	+
Lactose	_	_	_	_	_	_	_	_	+
Sucrose	_	+	_	+	+	_	+	_	+
Arabinose	+	_	_	_	_	+	+	_	_
Maltose	_	_	+/_	_	_	_	_	_	+
Saccharose	+	+	_	+	+	_	+	+	+
Oxidase	_	+	+	+	+	+	+	_	+
Casein hydrolysis	_	_	_	_	_	_	+	_	_

NO_3 = Nitrate; H_2S = Hydrogen sulphide; _ = Negative result; + = Positive result

	Days of incubation						
Isolate code	0	4	8	12	16	20	24
ANT1	0.248	0.026	0.024	0.021	0.018	0.016	0.015
		89.50 ± 0.02 %	90.30 ± 0.02 %	91.50 ± 0.01 %	92.70 ± 0.08 %	93.50 ± 0.01%	93.90 ± 0.06 %
XYL2	0.248	0.031	0.028	0.026	0.024	0.020	0.018
		87.50 ± 0.06 %	88.70 ± 0.01 %	89.50 ± 0.01 %	90.30 ± 0.02 %	91.90 ± 0.02 %	92.70 ± 0.06 %
PYR3	0.248	0.070	0.068	0.065	0.062	0.060	0.057
		71.80 ± 0.01 %	72.60 ± 0.03 %	73.80 ± 0.02 %	75.00 ± 0.08 %	75.80 ± 0.01 %	77.00 ± 0.01 %
ANT4	0.248	0.057	0.055	0.053	0.051	0.044	0.040
		77.00 ± 0.02 %	77.80 ± 0.01 %	78.60 ± 0.02 %	79.40 ± 0.06 %	82.20 ± 0.01 %	83.90 ± 0.02 %
PYR5	0.248	0.075	0.072	0.070	0.067	0.064	0.060
		69.80 ± 0.01 %	70.90 ± 0.07 %	71.70 ± 0.04 %	73.00 ± 0.08 %	74.10 ± 0.01%	75.80 ± 0.01 %
ANT6	0.248	0.061	0.059	0.057	0.054	0.052	0.050
		75.40 ± 0.02 %	76.20 ± 0.05 %	77.00 ± 0.06 %	78.00 ± 0.01 %	79.00 ± 0.08 %	79.80 ± 0.02 %
XYL7	0.248	0.019	0.018	0.017	0.015	0.010	0.001

		92.30 ± 0.02 %	92.70 ± 0.05 %	93.10 ± 0.02 %	93.90 ± 0.03 %	96.00 ± 0.02 %	99.50 ± 0.05 %
XYL8	0.248	0.046	0.044	0.042	0.040	0.038	0.035
		81.50 ± 0.04 %	82.30 ± 0.07 %	83.10 ± 0.31 %	83.90 ± 0.05 %	84.70 ± 0.06 %	85.90 ± 0.08 %
PYR9	0.248	0.057	0.053	0.050	0.048	0.046	0.043
		77.00 ± 0.02 %	78.60 ± 0.01 %	79.80 ± 0.05 %	80.70 ± 0.03 %	81.20 ± 0.01 %	83.90 ± 0.01 %
Control	0.248	0.246	0.243	0.241	0.240	0.237	0.235
		0.80 ± 0.02 %	2.00 ± 0.01 %	2.80 ± 0.05 %	3.20 ± 0.02 %	4.40 ± 0.05 %	5.20 ± 0.03 %

Table 6. Weight loss of xylene resulting from the growth of marine bacterial isolates

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 7: Weight loss of anthracene resulting from the growth of marine bacterial isolates

		Days of incubation					
Isolate	0	4	8	12	16	20	24
ANT1	0.386	0.089	0.088	0.086	0.085	0.085	0.084
		76.90 ± 0.05 %	77.20 ± 0.02 %	77.70 ± 0.01 %	77.90 ± 0.01 %	78.00 ± 0.01 %	78.20 ± 0.02 %
XYL2	0.386	0.098	0.090	0.084	0.075	0.060	0.031
		74.60 ± 0.08 %	76.70 ± 0.07 %	78.20 ± 0.08 %	80.60 ± 0.09 %	84.40 ± 0.02 %	91.20 ± 0.01 %
PYR3	0.386	0.098	0.096	0.094	0.093	0.092	0.091
		74.60 ± 0.02 %	75.10 ± 0.05 %	75.60 ± 0.06 %	75.90 ± 0.08 %	76.10 ± 0.02 %	76.40 ± 0.02 %
ANT4	0.386	0.105	0.104	0.103	0.102	0.101	0.100

		72.70 ± 0.05 %		73.00 ± 0.02 %		73.30 ± 0.06 %		73.60 ± 0.07 %		73.80 ± 0.03 %		74.00 ± 0.07 %	
PYR5*	0.386	0.060		0.050		0.040		0.030		0.020		0.010	
		84.50 ± 0.03 %		87.00 ± 0.06 %		89.60 ± 0.07 %		92.20 ± 0.00 %		94.80 ± 0.02 %		97.40 ± 0.01 %	
ANT6	0.386	0.070		0.066		0.054		0.050		0.043		0.046	
		81.80 ± 0.02 %		82.90 ± 0.06 %		86.00 ± 0.09 %		87.00 ± 0.08 %		87.60 ± 0.04 %		88.00 0.06 %	
XYL7	0.386	0.124		0.122		0.120		0.116		0.111		0.109	
		67.90 ± 0.08 %		68.40 ± 0.08 %		68.90 ± 0.04 %		69.90 ± 0.01 %		71.20 ± 0.04 %		71.80 ± 0.07 %	
XYL8	0.386	0.055		0.050		0.045		0.040		0.035		0.030	
		85.80 ± 0.02 %		87.00 ± 0.06 %		88.30 ± 0.09 %		89.60 ± 0.01 %		90.90 ± 0.01 %		92.20 ± 0.01 %	
PYR9	0.386	0.033		0.031		0.027		0.024		0.020		0.016	
		91.50 ± 0.01 %		92.00 ± 0.04 %		93.00 ± 0.01 %		93.80 ± 0.08 %		94.80 ± 0.07 %		95.90 ± 0.01%	
Control	0.386	0.383		0.380		0.376		0.374		0.370		0.368	
		0.70 ± 0.023 %		1.50 ± 0.04 %		2.60 ± 0.06 %		3.10 ± 0.08 %		4.10 ± 0.07 %		4.60 ± 0.01 %	

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 8. Weight loss from pyrene resulting from the growth of marine bacterial isolates

		Days of incubation					
Isolate	0	4	8	12	16	20	24
ANT1	0.117	0.078	0.075	0.073	0.071	0.069	0.068
		33.00 ± 0.08 %	35.90 ± 0.23 %	37.60 ± 0.07 %	39.30 ± 0.21 %	41.00 ± 0.06 %	41.90 ± 0.04 %
XYL2	0.117	0.098	0.086	0.083	0.081	0.079	0.077
		16.20 ± 0.03 %	26.50 ± 0.02 %	29.00 ± 0.06 %	30.80 ± 0.07 %	32.50 ± 0.08 %	34.20 ± 0.02 %
PYR3	0.117	0.103	0.100	0.099	0.097	0.094	0.090

		12.0	±	14.5	±	15.3	±	17.0	±	19.7	±	23.0	±
		0.07 %		0.09 %		0.03 %		0.02 %		0.08 %		0.03 %	
ANT4	0.117	0.097		0.090		0.089		0.086		0.084		0.080	
		17.00	±	23.00	±	23.90	±	26.50	±	28.20	±	31.60	±
		0.05 %		0.02 %		0.01 %		0.01 %		0.05 %		0.02 %	
PYR5	0.117	0.067		0.064		0.062		0.060		0.057		0.053	
		40.00	±	45.30	±	47.00		48.70	±	51.30	±	54.70	±
		0.06 %		0.03 %		0.01 %		0.04 %		0.03 %		0.03 %	
ANT6	0.117	0.091		0.087		0.084		0.080		0.078		0.075	
		22.00	±	25.60	±	28.00	±	31.60	±	33.30	±	35.90	±
		0.06 %		0.06 %		0.06 %		0.09 %		0.02 %		0.02 %	
XYL7*	0.117	0.064		0.061		0.059		0.057		0.054		0.052	
		45.30	±	47.90	±	49.60	±	51.30	±	53.80	±	60.00	±
		0.01 %		0.05 %		0.05 %		0.09 %		0.04 %		0.02 %	
XYL8	0.117	0.090		0.087		0.085		0.082		0.080		0.078	
		23.00	±	25.60	±	27.40	±	29.90	±	31.60	±	33.30	±
		0.01 %		0.05 %		0.03 %		0.01 %		0.06 %		0.06 %	
PYR9	0.117	0.087		0.081		0.079		0.077		0.075		0.070	
		25.60	±	30.80	±	32.50	±	34.20	±	35.90	±	40.00	±
		0.02 %		0.07 %		0.02 %		0.03 %		0.05 %		0.05 %	
	0.117	0.115		0.113		0.111		0.109		0.107		0.104	
		1.70	±	3.40	±	5.10	±	7.70	±	8.60	±	11.10	±
Control		0.03 %		0.02 %		0.02 %		0.06 %		0.02 %		0.06 %	

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 9. Zones of inhibition (mm) of different antibiotics against the marine bacterial isolates

Isolate	Antibiotics																			
	AMP		TE		LEV		CF		AMO		C		E		AZM					
ANT1	0.00	± 0.00	11.00	± 0.20	20.00	± 0.10	22.00	± 0.20	0.00	± 0.00	10.00	± 0.00	11.00	± 0.20	13.00	± 0.00				
XYL2	0.00	± 0.00	11.70	± 0.15	19.90	± 0.21	18.80	± 0.20	0.00	± 0.00	10.70	± 0.11	11.90	± 0.21	14.70	± 0.15				
PYR3	0.00	± 0.00	13.80	± 0.30	14.00	± 0.00	19.80	± 0.20	0.00	± 0.00	10.00	± 0.00	16.00	± ± 0.00	12.50	± 0.15				
ANT4	0.00	± 0.00	10.00	± 0.20	21.00	± 0.00	20.00	± 0.01	0.00	± 0.00	9.00	± 0.00	12.50	± 0.00	12.00	± 0.10				
PYR5	0.00	± 0.00	11.40	± 0.50	19.40	± 0.10	22.90	± 0.20	0.00	± 0.00	5.00	± 0.40	11.30	± 0.20	10.90	± 0.30				
ANT6	0.00	± 0.00	17.00	± ± 0.00	20.0	± 0.00	22.0	± 0.20	0.00	± 0.00	14.00	± 0.00	14.00	± 0.00	9.50	± 0.25				
XYL7	0.00	± 0.00	15.30	± 0.20	13.50	± 0.00	13.30	± 0.00	0.00	± 0.00	14.00	± 0.10	14.00	± 0.20	15.30	± 0.15				
XYL8	0.00	± 0.00	18.00	± 0.10	17.50	± 0.20	16.90	± 0.30	0.00	± 0.00	17.30	± 0.15	8.00	± 0.40	18.00	± 0.30				
PYR9	0.00	± 0.00	11.80	± 0.40	21.10	± 0.70	11.30	± 0.30	0.00	± 0.00	11.00	± 0.00	12.10	± 0.10	14.20	± 0.20				

AMP = Ampicillin; TE = Tetracycline; LEV = Levofloxacin; CF = Cefradoxil; AMO = Amoxicillin; AZM = Azithromycin; C = Chloramphenicol; E = Erythromycin

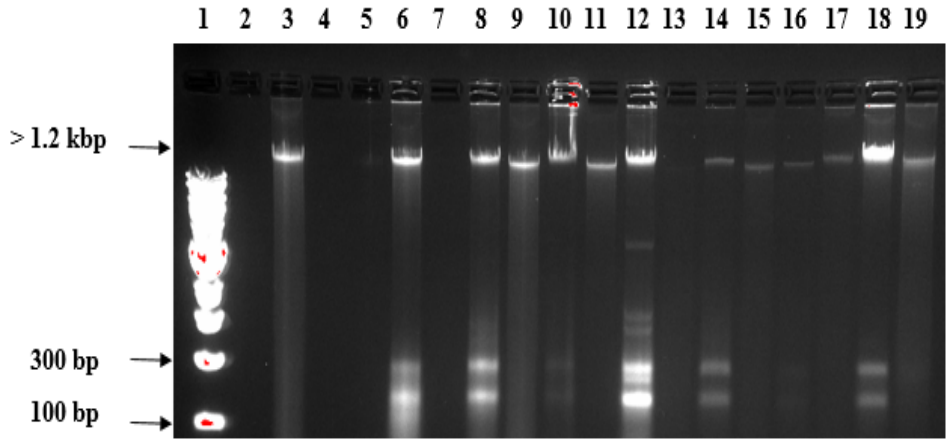


Plate1. Electrophoretic separation profile of plasmid DNAs from cured and non-cured strains of the marine bacteria

1 = 1 kbp DNA ladder; 2 and 3 = Cured and uncured PYR3 isolates; 4 and 5 = Cured and uncured PYR5 isolates; 6 and 7 = Uncured and cured PYR9 isolates; 8 and 9 = Uncured and cured XYL2 isolates; 10 and 11 = Uncured and cured XYL7 isolates; 12 and 13 = Uncured and cured XYL8 isolates; 14 and 15 = Uncured and cured ANT1 isolates; 16 and 17 = Uncured and cured ANT4 isolates; 18 and 19 = Uncured and cured ANT6 isolates

5. CONCLUSION

The whole study revealed that the three sampling sites harbour a lot of efficient aromatic hydrocarbon degrading bacterial strains belonging to the genera: *Providencia, Alcaligenes, Brevundimonas, Myroides,*

Serratia, and Bacillus. It also revealed that the isolated bacteria especially *Serratia marcescens* XYL7 were able to significantly (*P* =.05) degrade simple, low and high molecular weights aromatic hydrocarbons. The plasmid of *Serratia marcescens* XYL7 can also be used in recombinant DNA technology to develop bacteria cells with potentials to degrade aromatic hydrocarbons and hence facilitates bioremediation purposes.

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