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Molecular Characterization of Hydrocarbon-utilizing Rhizobacteria from Hydrocarbon-impacted Mangrove Soil in Ogoni Land, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AAI designed the study, performed the statistical analysis and wrote the protocol. Authors AAI, CNA and KCO managed the analyses of the study. Authors CAN and KCO managed the literature searches and wrote the first draft of the manuscript. Authors AAI and CNA supervised the whole study which, Author KCO used as part of his M.Sc. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the molecular characterization of hydrocarbon-utilizing rhizobacteria isolates from hydrocarbon-impacted mangrove soil from K-Dere Gokana and Gio Tai, Ogoni land, Nigeria.
Study Design: Cross-sectional study.
Place and Duration of Study: K-Dere Gokana and Gio Tai, Ogoni land, Nigeria, between June 2019 and June 2020.
Methodology: Hydrocarbon-utilizing bacteria were isolated from the rhizosphere of hydrocarbon-impacted mangrove soil. Baseline physicochemical and microbiological characteristics of the rhizosphere soil from the contaminated sites were determined. Hydrocarbon utilization was determined turbidometrically using a spectrophotometer and by growth on Bushnell, Haas agar supplemented with 1% crude oil.
Results: Analysis of the rhizosphere soil revealed that the site was contaminated with crude oil with a TPH value of 547.62±40.15 mg/kg. Based on phylogenetic analysis of these 16S rRNA

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genes the isolates were classified as *Bacillus thuringiensis* SA2-1; *Bacillus velezensis* SA11-2; *Morganella morganii* SA4-3; *Serratia ureilytica* SB1-4; *Serratia marcescens* SB5-5 and *Serratia nematodiphila* SB16-6. The sequences have been deposited under the accession numbers MN631244.1-MN631249.1. These bacteria all demonstrated efficient hydrocarbon utilization in Bushnell medium with 1% crude as the sole carbon source. These bacteria are well documented putative hydrocarbon utilizers.

Conclusion: This study has demonstrated that hydrocarbon utilization from different genera was associated with the rhizosphere of hydrocarbon-impacted mangroves in Gio and K-Dere.

Keywords: Mangroves; hydrocarbon-utilizing bacteria; rhizobacteria; molecular characterization.

1. INTRODUCTION

The Niger Delta region in Nigeria harbours the third largest mangrove ecosystem in the world. Ogoniland, which is situated in this Niger Delta region, precisely in Rivers state is rich in diverse mangrove with great commercial and economic potential [1]. However, crude oil contamination has threatened the existence and economic importance of these mangrove ecosystems. The deleterious effect of crude oil contamination on mangroves are well documented and ranges from mild to severe (usually leading to the death of the mangroves) damages to the ecosystem. Mangroves have been reported to exhibit high susceptibility to oil pollution [2]. During high tide, the oil may enter the swamps and get deposited on aerial roots and surface sediments. When heavy or viscous oil is deposited on the roots of the mangroves, the breathing pores of the trees may be blocked resulting in an anoxic or lack of oxygen condition to the root system, which may eventually kill the mangroves.

Mangrove ecosystems are influenced by tides which makes them more vulnerable to climatic changes. Mangroves ability to enrich the ambient environment with organic matter is believed to keep pace with increasing sea level which destroys associated biota and mangroves due to the sea level rise in the intertidal and supralittoral zone. However, mangroves thrive under conditions of extreme tides, high salinity, high temperatures and muddy anaerobic soils and strong winds.

While most species of mangrove plants are sensitive to oil contamination, a few may show profound resistance, at least, for a longer period. The rhizosphere is a narrow zone, adjacent to and influenced by living plant roots [3]. It could also be said to be a site of high microbial activity in and around roots in soil [4]. However, naturally occurring bacteria known as plant growth-promoting rhizobacteria (PGPR) aggressively colonize plant roots for their optimum growth by several mechanisms [5] and [6]. Rhizobacteria, which are symbiotic bacteria that colonize the root of plant contribute to nitrogen fixation in the soil. Their existence in the rhizosphere provides plants with the capacity to fix free nitrogen in the form accessible to plants. Therefore their presence in the rhizosphere of hydrocarbon-resistant mangroves may signal a symbiotic role that aids the plants' ability to survive the toxic effect of hydrocarbon contamination. This study was aimed at characterizing hydrocarbon-utilizing rhizobacteria isolated from hydrocarbon-resistant mangroves from k-Dere, Gokana using their 16S rRNA genes analysis.

2. MATERIALS AND METHODS

2.1 Sample Collection

Mangrove plant rhizosphere soil samples (root and rhizosphere soil) growing on a hydrocarbon-contaminated soil (Fig. 1) were collected at low tide with the help of a soil auger at different sampling points in the study area. The sampling point co-ordinates were determined using the Global Positioning System (GPS) at Latitude: Latitude: 4°39'21" N and Longitude: 7°14'19" E. The roots were excavated to a depth of between 15 and 30 cm and excess soil removed by vigorous shaking of the root samples. Approximately 2 g of rhizosphere surrounding soil was carefully collected from each root sample by gently brushing off the soil that remained. The rhizosphere soil samples were transferred into a sterile polythene bag and transported to the laboratory in an ice pack for further analysis.

2.2 Determination of Physicochemical Characteristics of the Mangrove Rhizosphere Soil

The temperature was determined *in situ* using a held-held Mercury thermometer. The pH of the soil was determined by making a dilution of the

soil sample with deionized water in the ratio of water soil of 2:1. The titrimetric method as previously described by Stewart et al. [7] was employed in determining the total organic carbon content. Nitrate (NO_3^-) content was determined by the Brucine method while Ammonium-molybdate was employed for the determination of phosphate content. The technique described by Ho et al. [8] and Miroslav and Vladimir (1999) were employed in the determination of heavy metal contents.

Polycyclic aromatic hydrocarbons (PAHs) were analysed using the method described by APHA [9]. A known weight of the soil was poured into an extraction bottle and dried with sodium sulphate. The sample was then extracted with a total volume of 1:1 dichloromethane: hexane mixture in a 25 mL portion. The extract was reduced to about 2 mL in an atmosphere of nitrogen and fractionated in a silica gel column using hexane. This fraction was stored in a refrigerator before the analysis. Each sample was spiked with naphthalene and scalene for quality control purpose. The same sample was further fractionated with chromatographic grade dichloromethane for the analysis of polycyclic aromatic hydrocarbon. Samples for PAH were analysed with programmed gas chromatography (HP, 5890).

The analysis of total petroleum hydrocarbon (TPH) was carried out with dichloromethane (DCM) using the cold extraction method as described in ASTM D-3694. Twenty gram (20 g) of dried soil sample was weighed and added into 100 mL of dichloromethane (DCM) conical flask. Twenty gram of activated anhydrous sodium sulphate as well as 200 mL of DCM was carefully added into the barrier containing the sample and allowed for one hour. This was then filtered into a 5ml flask using a filtration pack with cotton wool. The procedure was repeated on the residual soil till a colourless solution was obtained. The extracts were analysed using HP Agilent 6890 gas chromatography (Agilent Technologies, 610).

Fig. 1. Mangrove plants growing on hydrocarbon contaminated soil

2.3 Determination of Microbiological Characteristics of the Mangrove Rhizosphere Soil

2.3.1 Enumeration of total culturable heterotrophic bacteria (TCHB)

The spread plate method on nutrient agar was applied. Rhizosphere soil suspensions were prepared by 10 fold serial dilution where 1 g of soil sample was dispensed into test-tubes containing normal saline as diluents and shaken thoroughly. 1 mL of soil suspension was pipetted into a test tube containing 9 mL of normal saline till the sample was diluted up to 10^{-6}. Then 0.1 mL aliquots of appropriate dilution were spread on duplicates of sterile nutrient agar. The plates were incubated in the incubator at 28^{o}C for a period of 18-48hrs [10,11].

2.3.2 Enumeration of total culturable heterotrophic fungi (TCHF)

Potato dextrose agar (PDA) was used as the medium of choice with 10% tartaric acid using the spread plate method. The plates were incubated in an incubator for 5 days at room temperature and colonies formed were counted and expressed as cfu/g.

2.3.3 Enumeration of total culturable hydrocarbon utilizing bacteria (TCHUB)

Enumeration of total culturable hydrocarbon utilizing bacteria (TCHUB) was carried out using the vapour phase method described by Abu and Chikere [12]. In brief, dilutions from the soil samples were added onto a solidified sterilized Bushnell Hass agar plates supplemented with 1.0% nystatin to inhibit fungal growth. Filter paper (Whatman No. 1) was soaked with crude oil and the crude oil-impregnated filter papers were aseptically placed on the cover of Petri dishes, then inverted to supply hydrocarbon by vapour-phase transfer to the inoculums [13]. The plates were incubated for 4 days at 37^{o}C and colonies were counted with their mean values recorded in colony-forming units per gram (Cfu/g).

2.3.4 Enumeration of total culturable hydrocarbon utilizing fungi (THUF)

Enumeration of THUF was carried out using mineral salt agar (MSA) containing 10 g of NaCl, 0.45 g of $MgSO_4.7H_2O$, 0.42 g of KCl, 0.29 g of KH_2PO_4, 0.8 g of $Na_2HPO_4.H_2O$, 0.43 g of $NaNO_3$ and 20.0 g of agar powder in 1 L of distilled water. Crude oil was used as both the sole carbon and energy source. Three replicate plates were inoculated by spread plating and inverted over sterile filter paper moistened with sterile crude oil, which was placed on the lid of petri dish covers. The plates were inverted over the dish covers containing the crude oil embedded filter papers [14]. The compound medium for the hydrocarbon utilizing fungal count was amended with 250 mg of chloramphenicol and tetracycline [15]; Numbere [16]. Incubation was done for 7 days at 28^{o}C ± 2^{o}C [17].

2.4 Screening of Rhizobacteria for Hydrocarbon Utilization – Turbido - metric Method

The rhizobacterial isolates were screened for their ability to utilize crude oil as their sole source of carbon and energy. The ability of each isolate to utilize hydrocarbon was obtained by using mineral salt broth (Bushnell Haas) in which 1% of the hydrocarbon (crude oil) was added and incubated at 37^{o}C in a shakers incubator for 14 days. The growth of the bacterium was measured using a spectrophotometer with optical density (O.D) readings at 600 nm from day 0 to day15 at regular intervals of 2 days using the mineral salt medium as blank.

2.5 Evaluation of Nitrogen Fixation Capacity of Rhizobacteria Isolates

The rhizobacterial isolates were grown on an autoclaved Jensen's medium (containing per litre of dH_2O: sucrose 20.0 g, $CaCO_3$ 2.0 g, K_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.5 g, NaCl 0.5 g, FeSO4. $7H_2O$ 0.1 g, Na_2MoO_4 0.005 g, and Agar 15.0 g). Isolates that were able to grow after two consecutive subcultures on this medium were selected as nitrogen fixers.

2.6 Identification of the Rhizobacterial Isolates

The rhizobacterial isolates that showed the capacity for either hydrocarbon utilization or nitrogen fixation were identified using their morphological, microscopic, biochemical and molecular characteristics.

2.6.1 Colonial and biochemical identification

The colour of the colonies on agar plates, the size, elevation and edge were observed. Gram's

stain was carried out to determine the Gram's reaction of the isolates and some of the biochemical tests carried out included citrate, catalase, indole, MR-VP, carbohydrates utilization, oxidase, starch and gelatin. The tests were carried out using the methods described by MacFaddin [18], Madigan and Martinko [19] and Mahon et al. [20].

2.6.2 Molecular identification of the isolates

2.6.2.1 DNA extraction

The genomic DNA of the isolate was extracted directly from the freshly grown culture of the isolate using a Qiagen QiaAMP DNA extraction kit. Heavy growth of the isolates was suspended in 200 µL of isotonic buffer into ZR bashing bead lysis tubes; 750µl of lysis solution was added to the tube. The tubes were secured in a bead beaker fitted with a 2 mL-tube holder assembly and processed at maximum speed for 5 min. The ZR bashing bead lysis tubes were centrifuged at 10,000 x g for 1 minute. Four hundred (400) µl of supernatant was transferred to a zymo spin iv spin filter (orange top) in a collection tube and centrifuged at 7000 x g for 1 minute. One thousand two hundred (1200) microlitres of bacterial DNA binding buffer was added to the filtrate in the collection tubes bringing the final volume to 1600µl; 800µl was then transferred to a zymo-spin JIC column in a collection tube and centrifuged at 10,000xg for 1 minute. The flow-through was discarded from the collection tube and the remaining volume was transferred to the same zymo-spin and spun. The ultra-pure DNA was then stored at -20 degrees for further analysis.

2.6.2.2 Agarose gel electrophoresis

One per cent (1%) agarose gel was prepared by adding 1g of agarose to 100ml of Trix borate EDTA (TBE) and soaked to dissolve for 2 minutes. The extraction was heated in the microwave for 5 minutes to enable the agarose to dissolve completely. A volume of 0.5 µl of gel loading dye was added. A comb was fixed in the casting tray in which the liquid agarose gel was poured and allowed to solidify. The agarose gel was then removed and placed in an electrophoresis tank. To each of the extracted samples, the loading dye containing the TBEH was added. The molecular weight ladder was carefully loaded into the first well. The gel was run at 120V for 15 min and visualized using a UV transilluminator.

2.6.2.3 Amplification of 16S rRNA

The 16S rRNA region of the genomic DNA of the isolates was amplified with the primer set 27F 5'-AGA GTT TGA TGC TGG CTC AG -3', and 515R 5'- TTA CCG CGG CKG CTG GCA C 3' according to the method described by Yamada et al. [21] and Katsura et al. [22]. Twenty microliters (20 µL) of the reaction mixture containing 1X PCR buffer (Solis Biodyne, Estonia), 1.5 mM $MgCl_2$ (Solis Biodye Estonia), 0.2 mM of each dNTP (Solis Biodyne, Estonia), 20 pMol of each primer and sterile water was used to make up the reaction mixture. PCR was carried out in an Eppendorf Nexus Thermal Cycler with the following cycling parameters: an initial denaturing step at 95^{o}C for 5 minutes, denaturation at 95^{o}C for 30 seconds, annealing at 55^{o}C for 45 seconds, extension at 72^{o}C for 1 minute and final extension was carried out at 72^{o}C for 10 minutes. The product was resolved on a 1.5% agarose gel. One hundred base pair (100 bp) DNA ladder (Solis Biodyne, Estonia) was used as a DNA molecular weight marker. Electrophoresis was done at 80v for 1 h 30 minutes and the gel was visualized on a UV transilluminator after staining with ethidium bromide (Solis Biodyne, Estonia).

2.6.2.4 Sequence analysis

The sequencing was done using the Sanger method of sequencing generated by ABI 3130 automated sequencer (Hitachi, Japan) and visualized using Chromas Lite (version 2.1.1, Technelysium Pty Ltd). BioEdit Sequence Alignment Editor was used for sequence editing, before performing a basic local alignment search tool (BLAST) using NCBI (National Centre for Biotechnology Information) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
Sequences with the highest similarities were downloaded and aligned with ClustalW.

2.6.2.5 Phylogenic tree construction

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.89615307 was constructed. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and were in the units of the number of base substitutions per site. The analysis involved 10 nucleotide sequences. The Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were eliminated for each

sequence pair. There were a total of 655 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [23].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Baseline characteristics of rhizosphere soil

Physicochemical characteristics of the soils from the rhizosphere of crude oil-resistant mangrove plant are presented in Table 1. This showed pH was slightly acidic as it ranged from 5.24±0.06 to 5.8±0.07 for the two sampling locations although they were also lower than the DPR intervention and target limits. Statistically, there is no significant difference $p > 0.05$ between the pH of both soils obtained from the study locations. The temperature was 27±0.001 and 26.93±0.03 for Gio and Kegbara-Dere, respectively. Nitrate was 0.13±0.05 and 0.07±0.05 mg/kg, phosphate was 11.95±0.07 and 5.15±0.05, chromium was 24.58±0.03 and 23.05±0.04 mg/kg with a DPR target and intervention limits of 100 and 380 mg/kg. Iron ranged from 89.18±0.3 to 494.92±0.06 mg/kg. Total petroleum hydrocarbon ranged from 324.6±0.6 to 524.7±0.07 mg/kg. The gas Chromatograms are presented in Figs. 2 and 3 and show the presence of crude fractions between C9-C40.

3.1.2 Microbial population of soil from rhizosphere

Microbial counts of the rhizosphere of the crude oil-resistant mangrove plants are presented in Fig. 4. The results show that the samples obtained from the Gio had a total heterotrophic count of 4.79 Log_{10}Cfu/g, hydrocarbon utilizing bacterial count was 4.3 Log_{10}Cfu/g, and total heterotrophic fungal count 2.84 Log_{10}Cfu/g, K-Dere sample had a lower microbial count 4.04 Log_{10}Cfu/g for the total heterotrophic bacterial count while the hydrocarbon utilizing bacterial was 4.92Log_{10}Cfu/g and the total heterotrophic count was 3.04 Log_{10}Cfu/g.

3.1.3 Hydrocarbon utilization by the rhizobacteria

Hydrocarbon utilization potential of rhizobacterial isolates was presented in Figs. 5 and 6 for Gio and K-Dere locations, respectively. The isolates obtained from the study were observed to possess hydrocarbon utilization potential. *Morganella morganii SA4-3, Bacillus velezensis* SA11-2 and *Micrococcus* sp. (SA 15 and 16) were obtained from Gio. isolates got the K-Dere namely *Serratia nematodiphilila* SB16-6, *Enterobacter Pseudomonas* sp (SB 6), *Bacillus* sp. (SB2), *Azotobacter* sp. (SB12) and *Bacillus* sp. (SB12 and SB15) all demonstrated efficient hydrocarbon utilization.

Table 1. Baseline physicochemical and microbiological properties of hydrocarbon impacted rhizosphere surrounding soil

Parameters	Gio	K-Dere	DPR Target	DPR Intervention
pH	$5.24±0.06^{d}$	$5.8±0.07^{d}$	6.5	8.5
Temperature (^{o}C)	$27±0.001^{g}$	$26.93±0.03^{f}$		
Nitrate (NO_3^-) (mg/kg)	$0.13±0.05^{a}$	$0.07±0.05^{a}$	NA	NA
Phosphate (PO_4^-) (mg/kg)	$11.95±0.07^{e}$	$5.15±0.05^{cd}$	NA	NA
Lead (mg/kg)	$3.45±0.06^{d}$	$3.35±0.05^{bc}$	85.0	530
Cadmium (mg/kg)	$2.5±0.03^{a}$	$0.075±0.05^{a}$	0.8	17
Chromium (mg/kg)	$24.58±0.03^{f}$	$23.05±0.04^{e}$	100.0	380
Zinc (mg/kg)	$3.72±0.06^{c}$	$1.58±0.08^{ab}$	140.0	720
Copper (mg/kg)	$0.035±0.001^{a}$	$4.24±0.007^{d}$	36.0	190
Iron (mg/kg)	$89.18±0.3^{h}$	$494.92±0.06^{i}$	NA	NA
Total organic carbon (%)	$1.22±0.02^{b}$	$1.677±0.009^{ab}$		
Total petroleum hydrocarbon (ppm)	$324.6±0.6^{i}$	$547.62±40.15^{j}$	50	5000
Polycyclic aromatic hydrocarbon (mg/kg)	0	0	1.0	40

Legend: Similar superscripts suggest there was no significant difference at p>0.05 while different superscripts reveal significant difference at p<0.05. Superscripts a,b,c... i are levels/strength of statistical significance using a homogenous subset at approximately ≤0.05 on Duncan and Sheffe

3.1.4 Molecular characteristics of rhizo-bacteria

The amplified DNA all should high quality and purity as shown in Fig. 7 with an amplicons size of 1490. Figs. 8 and 9 show the phylogenetic construct for evolutionary relatedness of the isolates obtained from this study with ones already deposited in GenBank. Table 2 shows the GenBank features of the rhizobacteria based on 16S rRNA genes sequence analysis. The accession numbers are given in the table (in parenthesis) and phylogenetic trees.

3.2 Discussion

This study characterized rhizobacteria isolated from the rhizosphere of hydrocarbon-resistant mangroves in Gio, Tai LGA, and K-Dere, Gokan all in Rivers State, Nigeria. The rhizobacteria were identified using molecular means. The 16S rRNA gene sequences of the bacterial isolates were amplified and sequenced. Based on phylogenetic analysis of these 16S rRNA genes the isolates were classified as *Bacillus thuringiensis* SA2-1; *Bacillus velezensis* SA11-2; *Morganella morganii* SA4-3; *Serratia ureilytica* SB1-4; *Serratia marcescens* SB5-5 and *Serratia nematodiphila* SB16-6. The sequences have been deposited under the accession numbers MN631244.1-MN631249.1. These bacteria all demonstrated efficient hydrocarbon utilization in Bushnell medium with 1% crude as the sole carbon source. These bacteria are well documented putative hydrocarbon utilizers [24,11,25].

Bacterial floras, associated with the rhizosphere of the plant are primarily to enhance geocycling of nutrients and solubilization of nutrients. This study documented the presence of *Morganella morganii* SA4-3, *Bacillus thuringiensis* SA2-1, *Bacillus velezensis* SA11-2, *Paenibacillus* sp. and *Micrococcus* sp that were identified from the Gio soil samples with *Micrococcus* sp. being the most frequent of the bacterial isolates obtained from the investigation; different strains of *Serratia* were also found in Kegbara-Dere samples which are *Serratia ureilytica* SB1-4, *Serratia marcensis* SB5-5 and *Serratia nematodiphilila* SB16-6, *Bacillus* sp. and Azotobacter while the Baranyowa-Dere sample was observed to possess *Lysinibacillus fusiformis* SC2-7, *Bacillus proteolyticus* SC14-8, *Klebsiella* sp., *Actrobacter* sp., *Pseudomonas* sp., *Acinetobacter* sp. and *Micrococcus* sp. This corroborates the report of Omoregbee et al. [26] whose investigation reported *Bacillus substilis, Micrococcus luteus, Clostridium* sp., *Micrococcus varians, Pseudomonas putida, Achromobacter* sp., *Bacillus pumlis* and *Serratia marascens.*

Furthermore, Borowik et al. [27] further support the findings of the previously reported bacterial flora involved in the rhizosphere of polluted soil to include groups such as *Acidobacteria*, *Actinobacteria, Bacteroides* and *Proteobacteria*. Efeovbkhan et al. [28] referred to *Bacillus* sp. and *Pseudomonas* sp. as prolific organisms due to their quick development rate and utilization of waste and toxins. These isolates were obtained during the present study. This demonstrates that *Bacillus* sp. and *Micrococcus* sp. were genuine colonizers of indigenous plants as observed within the current study. The biodegradation capacity of unrefined oil has made a level of categorization of the isolates into functional niches.

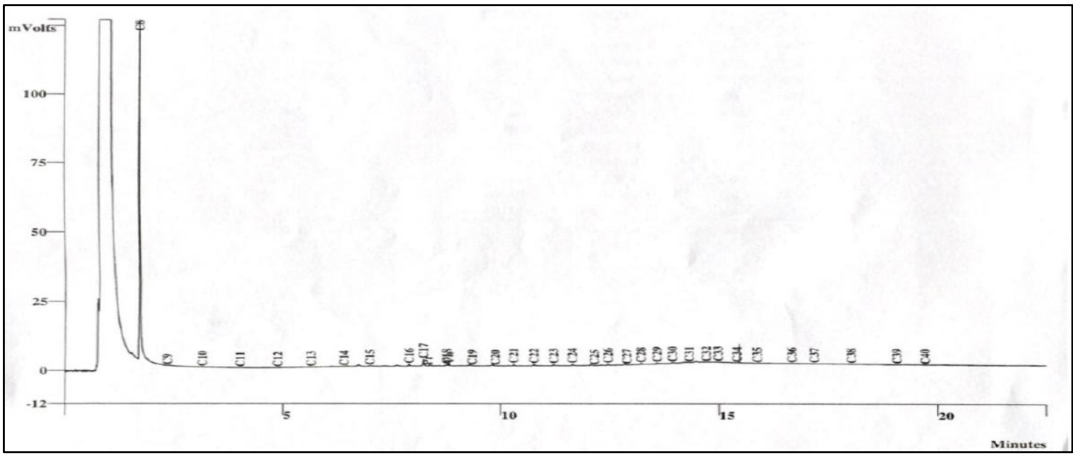

Fig. 2. Chromatogram for TPH of rhizosphere soil obtained from Gio, Tai

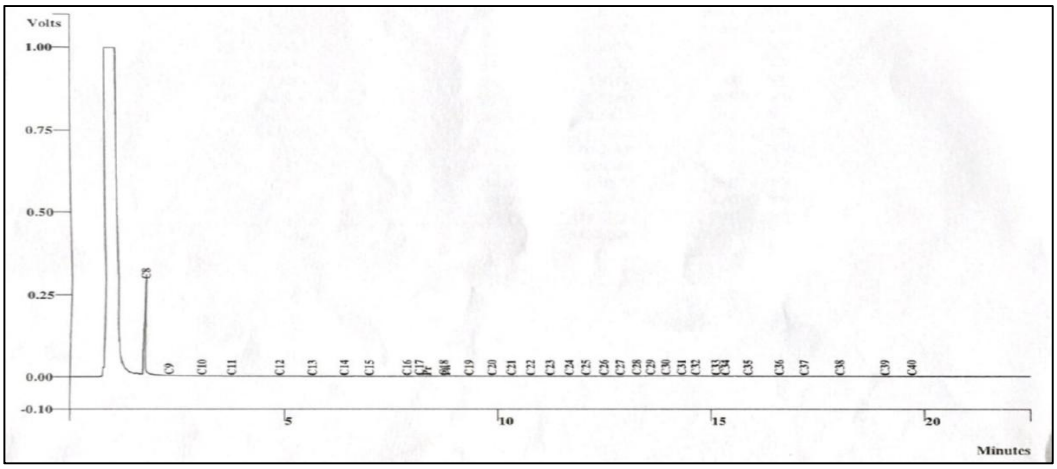

Fig. 3. Chromatogram for TPH of rhizosphere soil obtained from K-Dere, Gokana

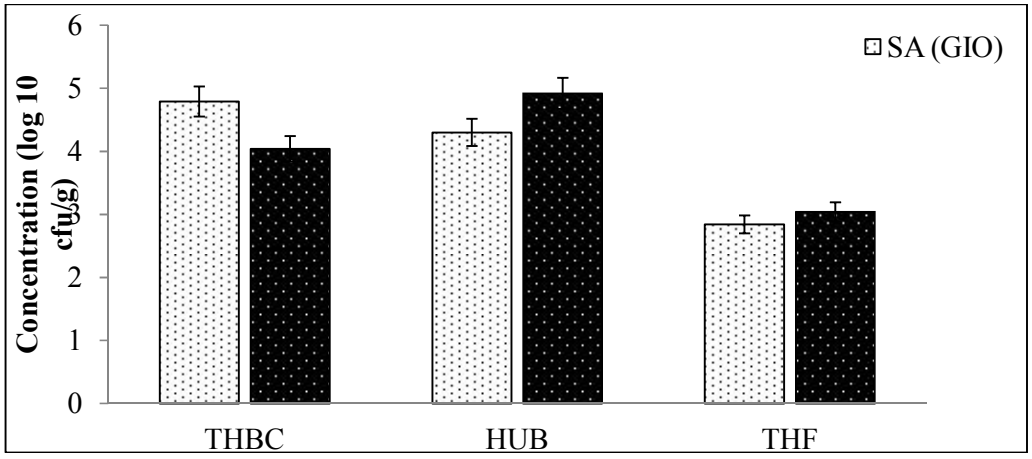

Fig. 4. Microbial count of rhizobacteria from soil obtained from Gio and K-Dere

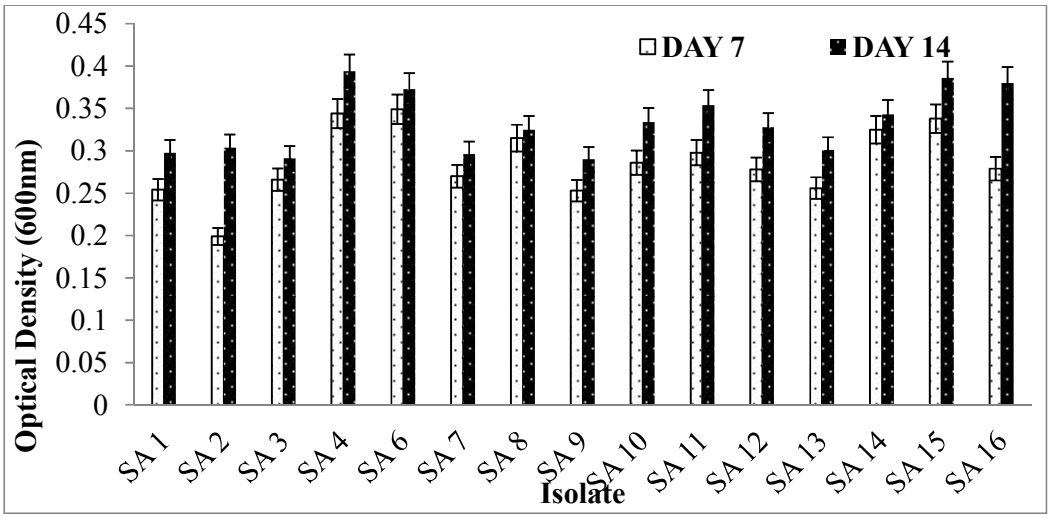

Fig. 5. Hydrocarbon utilization by rhizobacteria isolated from Gio

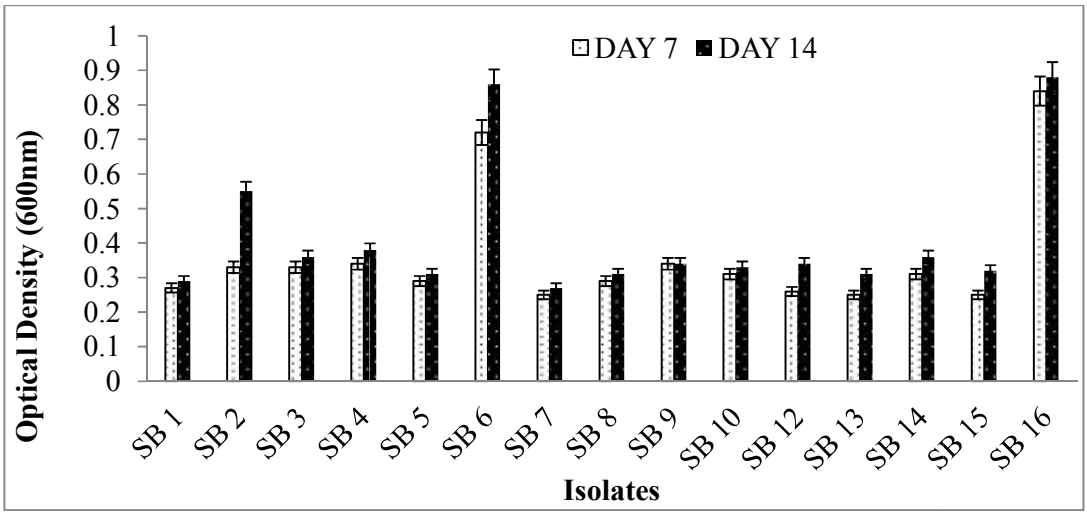

Fig. 6. Hydrocarbon utilization by rhizobacteria isolated from K-Dere

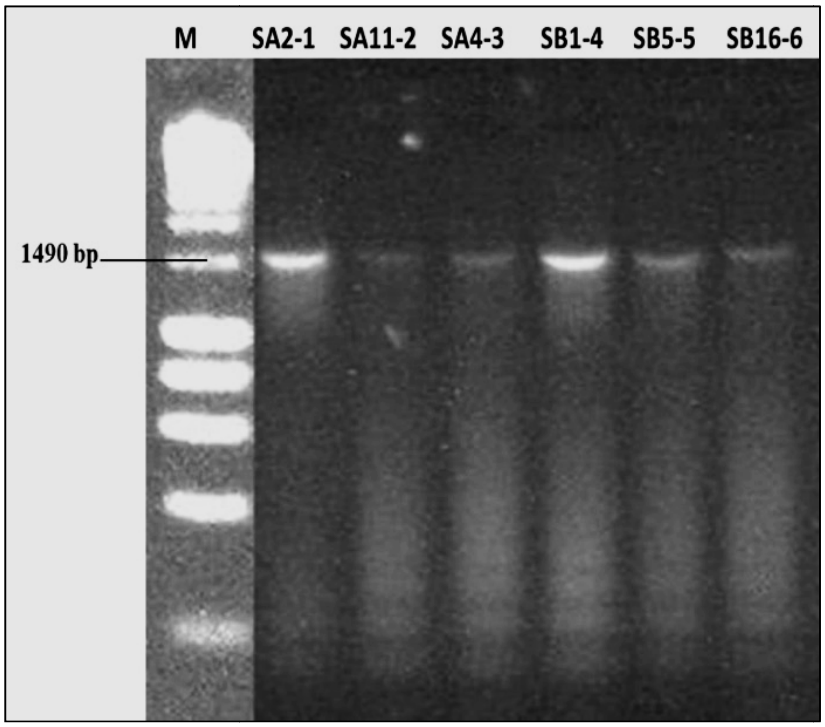

Fig. 7. Gel electrophoresis of amplified regions of the bacterial isolates (M=ladder)

Rhizobacteria that can utilize hydrocarbon were distributed more in K-dere than in Gio. There is relatively presence of hydrocarbon utilizers in the two sampling locations is a testament to the richness of these site in hydrocarbon-utilizing rhizobacteria. However, poor hydrocarbon utilization was observed in Gio compared to K-Dere. This was in agreement with the report of Romero et al. [29] that indigenous species in polluted media lack functional capacity, citing certain features that are induced by the chemical pollution. Geophysical concerns have been identified in recent times as a pivotal factor for the accumulation of bioactive materials. This recently has backed the application of exogenous organisms with the capacity to degrade or utilize waste for the bioremediation of hydrocarbon-contaminated media.

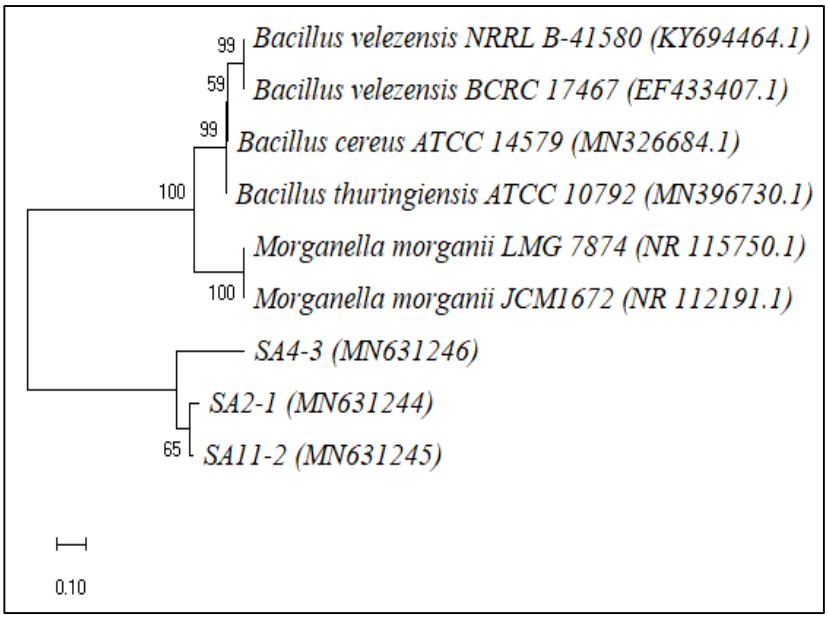

Fig. 8. Phylogenetic tree constructed for bacterial isolates obtained from the Gio

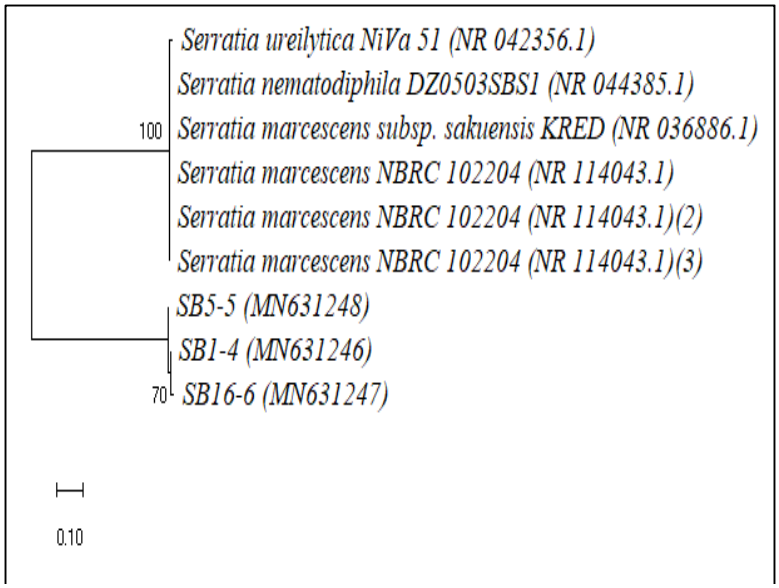

Fig. 9. Phylogenetic tree constructed for bacterial isolates from K-Dere

Table 2. Gen bank features of the rhizobacteria based on 16S rRNA genes sequence analysis

S/N	Isolate code	Organism	Strain	Accession number
Seq1	SA2-1	*Bacillus thuringiensis*	SA2-1	MN631244
Seq2	SA11-2	*Bacillus velezensis*	SA11-2	MN631245
Seq3	SA4-3	*Morganella morganii*	SA4-3	MN631246
Seq4	SB1-4	*Serratia ureilytica*	SB1-4	MN631247
Seq5	SB5-5	*Serratia marcescens*	SB5-5	MN631248
Seq6	SB16-6	*Serratia nematodiphila*	SB16-6	MN631249

4. CONCLUSION

This study has demonstrated that hydrocarbon utilization from different genera was associated with the rhizosphere of hydrocarbon-utilizing resistant mangroves in Gio, Tai and K-Dere, Gokana, Rivers State Nigeria. The finding of this present study highlights the untapped bioresource potentials of the Niger Delta mangroves and the possibility of recovering bioactive Materials from crude oil-contaminated sites.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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