https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Distribution, Biochemical Properties and Genetic Relatedness of Endophytic Bacteria of Wet land Plants from Petroleum-Contaminated Sites of the Niger Delta, Nigeria

Juliana Okwena Pondei[1*], Chimezie Jason Ogugbue[2] and Gideon Chijioke Okpokwasili[2]

[1] Department of Microbiology, Federal University Otuoke, Bayelsa State, Nigeria
Email:julipondei@gmail.com
[2] Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria
Email: ceejay55us@yahoo.com; gidsilman@yahoo.com

***Abstract*—** *Microbe-assisted phytoremediation is a recent application of bioremediation with much prospects. The genetic relatedness of culturable endophytic bacteria of wetland plants growing on a six month-old and twelve month-old petroleum-contaminated sites, and an uncontaminated site in Bayelsa State of the Niger Delta Region, Nigeria were compared. Most of the endophyte species isolated from the roots, stems and leaves were common to all the sites and belong to the phyla Proteobacteria, Bacteroidetes Firmicutes, Actinobacteria, Chloroflexi and Actinomicrobia, with the γ-Proteobacteria dominating. Pseudomonas was the most prevalent species in all three sites, but higher in the petroleum contaminated sites. Biochemical properties (API 20E) of the common isolates; Pseudomonas spp.Chryseobacterium indologenes,Bacillus and Proteusvaried with sites while only Providencia rettgeri peculiar to the petroleum-contaminated sites showed the same properties. 16S rRNA PCR-DNA fragments of forty-five species of the isolates (15 from each site) were characterized using RFLP and MspI restriction enzyme and a genetic distance tree of the restriction patterns drawn. The percentage of similarity in the genetic relatedness of isolates ranged from 11.1 – 100%. The genetic tree analysis of the 45 species of identified bacteria revealed 3 major clusters with 17 DNA fingerprinting patterns. Pseudomonas species of the root and leaves of the six month-old petroleum-contaminated site and uncontaminated site were seen to cluster together irrespective of date of isolation. The endophytes may play a role in the in situ degradation of the petroleum hydrocarbon of the sites.*

***Keywords*— Endophytic bacteria, Petroleum, Phytoremediation, Wetlands, Wetland plants.**

I. INTRODUCTION

Endophytic bacteria are non-pathogenic bacteria living inside healthy plants. They are found in all plant species in which one or more endophytes occur [1]. These endophytes generally colonize the intercellular spaces, and have been isolated from all plant compartments including seeds [2]. Endophytic bacterial species isolated from plants, include *Acetobacter, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Herbaspirillum* and *Pseudomonas* among others [3, 4, 5]. Once inside the plant, endophytes either reside in specific plant tissues like the root cortex or the xylem or colonize the plant [6] Roles attributed to endophytes include enhancement of plant growth by producing indole-3-acetic acid, solubilize phosphorous and fix nitrogen [7, 8], increase plant resistance to pathogens, and have biotechnological potential in the improvement and application of phytoremediation due to their ability to resist heavy metals and to degrade organic compounds [9].

The advantages of using endophytes over rhizobacteria in phytoremediation has been highlighted. Endophyte populations are controlled in plants, are specific, reduce problems of competition and are protected from both biotic and abiotic stress in the environment they occur [10]. Some plant species are able to recruit, or selectively augment, the necessary bacteria to remove pollutants, while other

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

resident plants cannot. The efficiency of phytoremediation is attributed to the presence and activity of plant associated microorganisms carrying genes for the degradation process [11]. The use of genes from endophytes in enhancing the efficiency of remediation in genetically engineered plants has continued to be explored [9, 12,13, 14, 15,16]. Inoculating plants with endophytic bacteria have been shown to improve phytoremediation of petroleum [16, 17].

Wetland or aquatic plants are used as food, in water quality assessment and as *in-situ* biomonitors and bioremediators [18]. They are frequently used in wastewater treatment in agricultural landfill and urban storm water runoff, to remove heavy metals and toxic organics from acid mine drainage, and as nutrients [19, 20]. Aquatic plants are used in waste treatmentbecause they accumulate many pollutants and toxic substances efficiently due to their non-complex growth requirements and fast growth rates [20]

Endophytes in wetland plants may be able to remove organic pollutants from wetlands. However, little investigation into the distribution and functions of endophytic bacteria of wetland plants is recorded. The occurrence of Gram-positive and Gram-negative endophytic bacteria ofaquatic plants; *Phragmitescommunis*, *Nymphaeatetragona*, *Najas marina*and*Potamogetoncrispus*were reported in which some of the organisms isolated degraded naphthalene and pesticides and in addition, showed potential to dissolve insoluble phosphate[5].

The study was carried out to investigate and compare the occurrence, distribution and biochemical properties of culturable endophytes in wetland plants of both petroleum-contaminated and uncontaminated soilsin wetlands of Bayelsa State, Nigeria; an oil rich region of the Niger Delta.

II. MATERIALS AND METHODS

2.1 Sampling Location and Collection

A random sampling of wetland plants growing in three freshwater soil locations (Fig. 1) were conducted. The first location is a six month-old petroleum contaminated soil at Oloibiri Oil field, Ogbia LGA, Latitude 4.695^{o}N Longitude 6.35043^{o}E. Crude oil spill occurred in November, 2013 while sampling was done in May, 2014. The second location is a twelve-month old petroleum contaminated soil at Ikarama, Okordia Clan, Yenagoa LGA; Latitude 5.14931^{o}N Longitude 6.45287^{o}E. Oil spill occurred in November, 2013 while sampling was done in November, 2014.The control is a non-petroleum contaminated site located at Federal University Otuoke, Ogbia LGA, Latitude 4.8019^{o}N Longitude 6.3189^{o}E. Sampling was done in June, 2015. Ten different plant species, with a minimum of three plants per specieswere collected per site in clean plastic zip lock bags. Plants were identified by the Plant Taxonomy Unit of the University of Port Harcourt, Nigeria.

2.2 Sample Processing for Culturable Endophytic Bacteria

After the removal of rhizosphere soil, plant samples from each site were rinsed with sterilized water to wash out sediments. The roots, stems and leaves of each plant species were separated to give three subsamples which were cut into 1cm pieces. Each subsample was washed in sterilized water for 5 minutes, then surface-sterilized with a solution containing 5% active chloride (w/v, added as a NaOCl solution) for 3 minutes and 70% ethanol for 1 min. This was then followed by rinsing 4 to 5 times in sterile deionized water. The plant subsets were further cut into 0.3 - 0.5 cm pieces with sterile blades and ground in a mortar using a glass rod plus 0.1ml sterile deionized water. To ensure that there was no cross contamination of plant parts during processing, the glass rod and mortar were surface sterilized using sterile cotton wool dipped in 70% ethanol. Each plant slurry of the roots, stems and leaves was spread onto Luria-Bertani (LB) agar plates in triplicate and incubated at 25^{o}C for 24 – 48 hours after which isolates were identified and stored at -70^{o}C in 20% glycerol. For sterility check, 100µl of the last rinse of each subsample in sterile deionized water was plated out on LB agar and incubated at 20^{o}C for 24 – 48 hours [5, 21]. No growth indicated the absence of contaminants (rhizosphere and phyllosphere bacteria).

2.3 Identification and Characterization of Isolates

Growth characteristics ofpure cultures of the isolates on Luria Bertani (LB) agar were observed after incubation at 25^{o}C for 24 – 48 hours. Characteristics of colonies observed include colour, margin, elevation, consistency, opacity and approximate size. Growth and lactose fermentation on MacConkey agar was noted. The API 20 E identification kit (Biomereux, France) was used for the identification of members of the Enterobacteriaceae and other Gram negative bacteria based on 21 different biochemical tests and a database. The test strip contained tests for beta-galactosidase, arginine dihydrolase, lysine and ornithine decarboxylases, citrate utilization, hydrogen sulphide production, urea hydrolysis, deaminase, indole and acetoin production (Voges-Proskauer), gelatinase and sugar fermentation tests. Gram positive isolates were also identified using some of the biochemical tests mentioned above in addition to sugar fermentation tests. Characterization of strains were done depending on their morphology on gram staining and biochemical tests.

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Grouping into various genera was determined using Bergey's Manual of Systematic Bacteria. Filamentous bacteria were identified based on their Gram reaction and morphology [22].

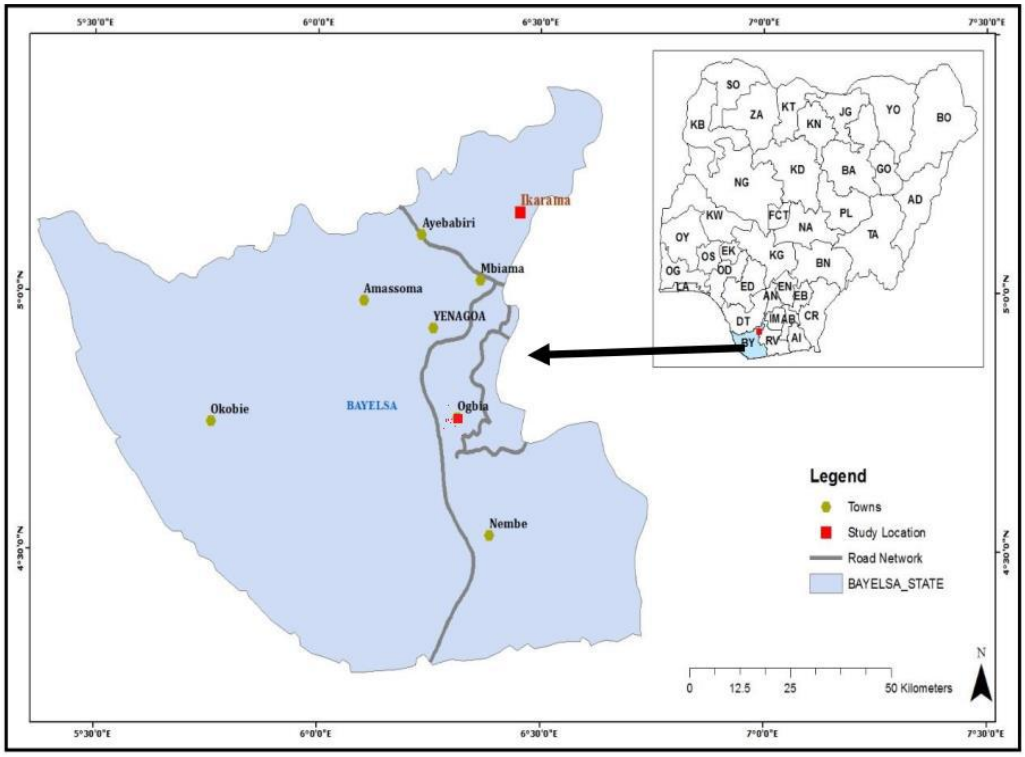


Fig.1: Map of Bayelsa State, Nigeria showing the study locations.

2.4 Molecular Characterization of Endophyte Bacterial Populations.

2.4.1 DNA Extraction

A DNA wash buffer solution containing50mM of Tris, 5mM EDTA, 50mM of NaCl, plus the addition of acetic acid to pH 8.0 was prepared and dispensed into Eppendorf tubes. Fresh colonies of the isolates were added and vortexed for complete mixing. 50mM of Tris, 25 % sucrose and 1mg/ml lysozyme were added and the mixture vortexed. Addition of 5 % SDS and 50mM of Tris was followed by inverting the Eppendorf tubes 3-5 times after which the lysates were incubated at 56^0C for 1hr. The DNA extraction from the lysates involved the addition of 500 µl phenol/chloroform/isoamyl alcohol in ratio 25:24:1. Ultra-centrifugation was carried out at 14,000rpm for 15mins and the supernatant dispensed into new Eppendorf tubes. The phenol/chloroform/isoamyl alcohol procedure was repeated and the supernatant transferred again into another set of Eppendorf tubes. The addition of 200 µl of 3M sodium acetate (pH 6.0 through the addition of glacier acetic acid) and 1ml of absolute ethanol was followed by vortexing, and the mixture allowed to precipitate on ice overnight. This was followed by centrifugation at 30,000rpm for 30mins and the supernatant discarded. The precipitate (chromosomal DNA) was washed twice with 70 % ethanol, centrifuged at 14,000rpm for 5 mins and air-dried after which sterile distilled water was added and kept below freezing temperature before further analysis [23]

2.4.2 Polymerase chain reaction probe for 16s RNA

All primers were purchased from Jena Bioscience GmbH, (Jena, Germany) and were dissolved according to manufacturer's instructions. The primer used was universal 16S rRNA gene primers: 8f: 5'-AGA GTT TGA TCC TGG CTC AG-3' and 1492r: 5'-GGT TAC CTT GTT ACG ACTT-3.The PCR mixture (50 µl) contained 5 µl of 10× PCR buffer with 15 mmol l-1 $MgCl_2$ (Takara), 200 µmol l-1 of each deoxynucleotide triphosphate (Takara), 10 pmol of each primer (Applied Biosystems), 1.5 units of *Taq*DNA polymerase (Takara) and 1 µl of DNA template. The mixture was vortexed for proper mixing. The PCR was performed in a thermocycler, with a thermal profile of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 45 s and extension at 72°C for

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

1.5 min and a final elongation at 72°C for 6 min with 8 µl of PCR fragment of each isolate. The final mixture was loaded on agarose 1.5% gel and electrophoresed for 35 minat 100 V. After electrophoresis the gel bands were observed on U-V trans-illuminator at 312nm. All electrophoretic gel bands were digitally photographed on gel documentation machine.

2.4.3 Fingerprinting of DNA using Restricted Fragment Length Polymorphism

Restriction fragment analysis 16S rRNA fragments amplified by PCR were digested with restriction endonuclease, *Msp*I and separated by electrophoresis in 1.5% agarose gel.

2.4.4 Construction of a genetic distance tree

A similarity matrix of the strains and the related bacterial 16S rRNA was constructed by the neighbor-joining method using computational phylogeny inference package (PHYLIP, Washington DC, USA). Informative DNA fragments, derived from restriction endonuclease digestion longer than 100 bp, were scored for their presence or absence. The similarity and divergence were calculated. The similarity matrix indices and genetic distance trees were constructed based on the RFLP data from the 16S rRNA using DICE and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering methods.

III. RESULTS

Endophytic bacteria were isolated from the roots, stems and leaves of individual plant species picked from all three sites. Table 1 represents the isolates from the six month-old petroleum-contaminated site (Site A). Endophytes had the highest distribution in roots of selected plants with 22 (37.94 %) followed by equal distribution in both stems and leaves with 31.03 % (TABLE 1). In Site B, a twelve month-old petroleum-contaminated soil, endophytes of the plant parts are given in TABLE 2. Endophytes had the highest distribution in roots of selected plants with 21 (42.86 %) followed by stems 18 (36.74 %) and leaves with 20.40 % (TABLE 2). Out of the total of 49 endophytes in the uncontaminated control site, 17 (34.69%) each from the roots and stems, while 15 (30.6%) from the leaves, were isolated (TABLE 3).

Combining all the isolates from the three sites, a total of 156 endophytes were isolated from all the plants with four genera common to all the sites (TABLE 4). *Pseudomonas* spp. had the highest frequency of occurrence of 20.51%, followed by *Chryseobacterium indologenes* (11.54%). *Bacillus* (10.26%) and *Proteus* (7.7%).In addition, *Providencia rettgeri* and *Sphaerotilus natans* were common to only the petroleum contaminated sites with a frequency of 10.26% and 1.92% respectively. There were more isolates of *Providencia rettgeri*, *Bacillus* spp., *Proteus* spp. and *Sphaerotilus natans* in site A than site B while *Pseudomonas* spp. and *Chryseobacterium indologenes* were more in site B than site A. *Corynebacterium*, *Micrococcus*and *Aeromonas* were found in site A only while *Burkholderia*, *Serratia*, *Alcaligenes*, *Vibrio*, *Morganella* and Type 0092 were in site B only. *Staphylococcus*, *Myroides*, *Citrobacter youngae*, *Enterobacter cloaceae*, *Pastuerella pneumotropica* and *Microthrix parvicella* were not isolated in both petroleum contaminated sites.

Biochemical tests (API 20E) utilized to differentiate common organisms of the three sites is shown in TABLE 5.*Pseudomonas* spp.*Chryseobacterium indologenes*,*Bacillus* and *Proteus*occurred in all three sites while *Providencia rettgeri* and *Sphaerotilus natans*were of the petroleum-contaminated sites only.Only *Providencia rettgeri* of the contaminated sites and *Ps. putida* of the twelve month-old petroleum-contaminated site and the uncontaminated site exhibited the same biochemical characteristics.

Some strains (total of 45) were selected from the different sites, source and their respective dates of isolation were subjected to DNA fingerprinting analysis using *Msp*I restriction endonucleases(TABLE 6). The percentage of similarity in the genetic relatedness of isolates ranged from 11.1 – 100% (TABLE 7). The genetic tree analysis of the 45 species of identified bacteriarevealed 3 major clusters with 17 DNA fingerprinting patterns (Fig. 2). *Pseudomonas aeruginosa* (Site A, root, May, 2014), *Ps. aeruginosa* (Site C, leaf, June 2015) and *Ps. putida* (Site C, leaf, June 2015 all clustered together showing 100 % genetic homology based on the *Msp*I generated fingerprints of 16S rRNA. The product of the PCR-RFLP of isolated species of *Msp*I restriction endonuclease generated from 16S rRNA fingerprints bands is shown in Fig. 3a-c.

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Table.1: Culturable endophytes obtained from plants growing in the six month-old petroleum contaminated soil site (Site A)

Plant	Endophyte	Root	Stem	Leaf
Commelina benghalensis	*Providencia rettgeri*	-	+	+
	Bacillus sp.	+	-	-
	Corynebacterium sp.	+	-	-
	Chryseobacterium indologenes	-	+	-
	Pseudomonas aeruginosa	+	+	-
Ageratum conyzoides	*Pseudomonas aeruginosa*	+	-	+
	Chryseobacterium indologenes	-	+	+
	Providencia rettgeri	+	-	-
	Bacillus subtilis	+	-	+
	Corynebacterium sp.	-	-	+
	Micrococcus spp.	+	+	+
Chromoleana odoratum	*Providencia rettgeri*	-	+	+
	Pseudomonas spp.	+	+	+
	Bacillus cereus	-	-	+
	Corynebacterium sp.	-	-	+
	Proteus sp.	+	-	-
	Aeromonas salmonicida ssp. *Salmonicida*	-	+	-
Aspilia africana	*Bacillus* spp.	+	+	+
	Corynebacterium sp.	+	-	-
Ipomoea involucrata	*Bacillus* spp.	+	+	-
	Providencia rettgeri	-	-	+
Melastomastrum capitatum	*Sphaerotilus natans*	+	-	+
	Providencia rettgeri	+	-	-
	Chryseobacterium indologenes	-	+	-
Unidentified	*Providencia rettgeri*	+	+	+
	Proteus spp.	+	+	+
Kyllinga erecta	*Pseudomonas* spp.	+	+	-
	Aeromonas salmonicida ssp. *Salmonicida*	+	-	-
	Proteus spp.	+	+	+
Unidentified	*Chryseobacterium indologenes*	-	-	+
	Proteus sp.	-	+	-
	Bacillus sp.	+	-	-
Unidentified	*Aeromonas* spp.	-	+	+
	Chryseobacterium indologenes	+	-	-
	Pseudomonas spp.	+	+	-
Total number of endophytes (58)		22 (37.94)	18 (31.03)	18 (31.03)

*Numbers in parentheses represents percentages

Table.2: Culturable endophytes obtained from plants growing in the twelve month-old petroleum contaminated soil (Site B)

Plant	Endophyte	Root	Stem	Leaf
Fimbristylis littoralis	*Burkholderia cepacia* Type 0092	-	+	-
	Alcaligenes spp.	+	+	-

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

	Providencia rettgeri	+	-	-
	Chryseobacterium indologenes	+	-	-
Kyllinga pumila	*Pseudomonas flourescens*	+	-	-
	Pseudomonas putida	-	-	+
	Pseudomonas aeruginosa	+	-	-
	Burkholderia cepacia	-	+	-
	Chryseobacterium indologenes	+	+	-
	Sphaerotilus natans	+	-	-
Ipomoea involucrata	*Serratia* spp.	+	-	+
	Pseudomonas flourescens	+	-	-
	Pseudomonas putida	+	+	-
	Vibrio spp.	+	+	-
Ageratum conyzoides	*Providencia rettgeri*	-	+	+
	Pseudomonas aeruginosa	+	+	-
	Vibrio sp.	-	+	-
	Burkholderia cepacia	+	-	-
Sacciolepsis africana	*Burkholderia cepacia*	-	+	+
	Providencia rettgeri	+	+	+
	Morganella morganii	+	+	-
Cyperus difformis	*Proteus mirabilis*	-	+	-
	Pseudomonas aeruginosa	-	-	+
	Chryseobacterium indologenes	+	-	-
Chromoleana odorata	*Bacillus* spp.	+	+	-
	Pseudomonas putida	-	-	+
Solenostemon monostachyus	*Alcaligenes* sp.	-	-	+
	Proteus mirabilis	+	-	-
	Pseudomonas aeruginosa	+	-	-
Echinochloa obtusiflora	*Chryseobacterium indologenes*	+	+	-
	Pseudomonasflourescens	-	-	+
Commelina benghalensis	*Chryseobacterium indologenes*	-	+	+
	Pseudomonas flourescens	+	-	-
	Pseudomonas putida	-	+	-
Total number of endophytes (49)		21(42.86)	18 (36.74)	10 (20.40)

*Numbers in parentheses represents percentages

Table.3: Culturable endophytes obtained from plants growing in the non-petroleum-contaminated soil (Site C)

Plant	Endophyte	Root	Stem	Leaf
Diplazium sammatii	*Pastuerella pneumotropica*	-	+	-
	Chryseobacterium indologenes	+	-	-
	Myroides spp.	+	-	+
	Pseudomonas aeruginosa	-	-	+
	Aeromonas salmonicida ssp. *Salmonicida*	-	+	+
Dissotis rotundifolia	*Proteus mirabilis*	+	-	-
	Microthrix parvicella	+	-	+
	Type 0675	-	+	-
	Bacillus spp.	+	+	-

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Anielema sp	*Burkholderia cepacia*	+	+	+
	Corynebacterium spp.	-	+	+
Aspilia africana	Type 0675	-	+	-
	Corynebacterium sp.	+	-	-
	Staphylococcus sp.	+	-	-
	Micrococcus sp.	-	+	-
Panicum laxum	*Proteus mirabilis*	-	+	-
	Pseudomonas putida	-	+	+
	Micrococcus sp.	-	+	-
	Corynebacterium sp.	+	-	-
Scleria verrucosa	*Pseudomonas putida*	-	-	+
	Micrococcus sp.	+	-	-
	Bacillus sp.	+	-	-
	Type 0675	-	-	+
Cyathula prostate	*Bacillus* sp.	+	-	-
	Corynebacterium sp.	-	-	+
	Staphylococcus spp.	-	+	+
Costus sp	*Serratia* spp.	+	+	+
Chromoleana odorata	*Citrobacter youngae*	-	-	+
	Serratia sp.	-	-	+
	Aeromonas hydrophilia	+	-	-
	Burkholderia cepacia	-	+	-
	Chryseobacterium indologenes	-	+	-
Commelina benghalensis	*Enterobacter cloacea*	+	-	-
	Chryseobacterium indologenes	+	+	-
	Pseudomonas aeruginosa	+	+	+
Total number of endophytes (49)		17 (34.69)	17 (34.69)	15 (30.62)

*Numbers in parentheses represent percentages

Table.4: Frequency of occurrence of genera of endophytes in all the sites

Endophyte	Site A	Site B	Site C	Total	%
Pseudomonas spp.	11	14	7	32	20.51
Chryseobacterium indologenes	6	8	4	18	11.54
Providencia rettgeri	10	6	0	16	10.26
Bacillus spp.	10	2	4	16	10.26
Proteus spp.	8	2	2	12	7.7
Burkholderia cepacia	0	5	4	9	5.77
Corynebacterium spp.	4	0	5	9	5.77
Aeromonas spp.	4	0	3	7	4.49
Serratia spp.	0	2	4	6	3.85
Micrococcus spp.	3	0	3	6	3.85
Staphylococcus spp.	0	0	3	3	1.92
Alcaligenes spp.	0	3	0	3	1.92
Sphaerotilus natans	2	1	0	3	1.92
Type 0675	0	0	3	3	1.92

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Vibrio spp.	0	3	0	3	1.92
Microthrix parvicella	0	0	2	2	1.28
Morganella morgani	0	2	0	2	1.28
Myroides spp.	0	0	2	2	1.28
Citrobacter youngae	0	0	1	1	0.64
Enterobacter cloaceae	0	0	1	1	0.64
Pastuerella pneumotropica	0	0	1	1	0.64
Type 0092	0	1	0	1	0.64
Total number of genera (156)	58	49	49	156	100

Key: Site A, six month-old petroleum-contaminated; Site B. twelve month-old petroleum-contaminated; Site C, uncontaminated.

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Table.5: Biochemical reactions of common endophytes of the petroleum-contaminated and uncontaminated sites

Microorganism	**Site**	**Biochemical Tests of Bacterial Species (AP1 20 E)**																			
		ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SUC	MEL	AMY	ARA	OX
Chr. indologenes	A	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
	B	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+
	C	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Ps. aeruginosa	A	-	+	-	-	+	-		-	-	-	+	-	-	-	-	-	-	-	+	-	+
	B	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	--	-	-	+
	C	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
***Pseudomonas* spp.**	A	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Ps. flourescens	B	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
	C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ps. putida	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	B	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	C	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
***Proteus vulgaris* gp**	A	-	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	-	-	+	-	-
Proteus mirabilis	B	-	-	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-
	C	-	-	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-
Providencia rettgeri	A	-	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	-	-	+	-	-
	B	-	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	-	-	+	-	-
Sphaerotilus natans	A	-	-	-	-	-	-	-	-	--	-	+	-	-	-	-	-	-	-	-	-	+
	B	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
***Bacillus* spp.**	A	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	-	-	-	-	-	+
	A	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	-	-	+	-	+
	B	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+
	C	-	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Key: Site A, six month-old petroleum-contaminated; Site B, twelve month-old petroleum contaminated; Site C, uncontaminated; ONPG, β-galactosidase; ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; CIT, citrate utilization; H_2S, H_2Sproduction; URE, urease; TDA, tryptophanedeaminase; IND, indole production; VP, Voges–Proskauer; GEL, gelatinase; GLU, glucose; MAN, mannitol;INO: inositol; SOR: sobitol; RHA, rhamnose; SAC; saccharose; MEL, melbiose; AMY: amygdalin; ARA, arabinose; OX, cytochrome oxidase; NA, not available.

Table 6. Selected endophytic bacteria employed in PCR-RFLP analysis

Strains	Microorganism	Site	Source	Date of Isolation
1.	*Sphaerotilus natans*	Oloibiri	LEAF	May, 2014
2.	*Micrococcus* spp.	Oloibiri	STEM	May, 2014
3.	*Chryseobacterium indologenes*	Oloibiri	STEM	May, 2014
4.	*Proteus* spp.	Oloibiri	STEM	May, 2014
5.	*Aeromonas salmonocida*ssp *salmonicida*	Oloibiri	ROOT	May, 2014
6.	*Bacillus* spp.	Oloibiri	ROOT	May, 2014
7.	*Proteus* spp.	Oloibiri	LEAF	May, 2014
8.	*Bacillus* spp.	Oloibiri	ROOT	May, 2014
9.	*Bacillus* spp.	Oloibiri	LEAF	May, 2014
10.	*Pseudomonas*spp.	Oloibiri	LEAF	May, 2014
11.	*Aeromonas hydrophilia*	Oloibiri	STEM	May, 2014
12.	*Providencia rettgeri*	Oloibiri	LEAF	May, 2014
13.	*Providencia rettgeri*	Oloibiri	ROOT	May, 2014
14.	*Pseudomonas oleovorus*	Oloibiri	STEM	May, 2014
15.	*Corynebacterium*	Otuoke	ROOT	June, 2015
16.	*Providencia rettgeri*	Ikarama	LEAF	Dec, 2014
17.	*Morganella morganii*	Ikarama	ROOT	Dec, 2014
18.	*Burkholderia cepacia*	Ikarama	ROOT	Dec, 2014
19.	*Serratia mercescens*	Ikarama	R/S/L	Dec, 2014
20.	*Sphaerotilus natans*	Ikarama	ROOT	Dec, 2014
21.	*Vibrio alginolyticus*	Ikarama	ROOT	Dec, 2014
22.	*Bacillus* spp.	Ikarama	STEM	Dec, 2014
23.	*Chryseobacterium indologenes/Myroides*	Ikarama	ROOT	Dec, 2014
24.	*Pseudomonas putida*	Ikarama	LEAF	Dec, 2014
25.	*Alcaligenes* spp.	Ikarama	LEAF	Dec, 2014
26.	*Pseudomonas aeruginosa*	Ikarama	LEAF	Dec, 2014
27.	Eikelboom Type 0092	Ikarama	STEM	Dec, 2014
28.	*Vibrio* spp.	Ikarama	STEM	Dec, 2014

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

29.	*Pseudomonas aeruginosa*	Oloibiri	ROOT	May, 2014
30.	*Pseudomonas aeruginosa*	Otuoke	LEAF	June, 2015
31.	*Burkholderia cepacia*	Otuoke	ROOT	June, 2015
32.	*Pseudomonas* spp.	Otuoke	STEM	June, 2015
33.	*Proteus mirabilis*	Otuoke	ROOT	June, 2015
34.	*Pastuerella pneumotropica*	Otuoke	STEM	June, 2015
35.	*Enterobacter cloaceae*	Otuoke	ROOT	June, 2015
36.	*Chryseobacterium indologenes*	Otuoke	ROOT	June, 2015
37.	*Citrobacter youngae*	Otuoke	LEAF	June, 2015
38.	*Microthrix parvicella*	Otuoke	R/L	June, 2015
39.	Type 0675	Otuoke	LEAF	June, 2015
40.	*Serratia mercescens*	Otuoke	R/S/L	June, 2015
41.	*Pseudomonas putida*	Otuoke	LEAF	June, 2015
42.	*Corynebacterium* spp.	Otuoke	S/L	June, 2015
43.	*Bacillus* spp.	Otuoke	LEAF	June, 2015
44.	*Staphylococcus* spp.	Otuoke	ROOT	June, 2015
45.	Type 0675	Otuoke	R/S	June, 2015

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Table.7: Similarity matrix index (%) of genetic relatedness of strains from different study sites

	S1	S2	S5	S16	S18	S21	S22	S25	S26	S27	S28	S29	S30	S31	S32	S36	S37	S38	S39	S40	S41	S42	S43	S44
S1	44.4	22.2	33.3	88.9	77.8	22.2	66.7	44.4	55.6	55.6	55.6	55.6	33.3	66.7	77.8	66.7	55.6	55.6	66.7	55.6	44.4	55.6	66.7	55.6
S2	55.6	44.4	33.3	44.4	77.8	77.8	55.6	44.4	44.4	66.7	66.7	44.4	77.8	66.7	77.8	66.7	66.7	55.6	66.7	77.8	66.7	55.6	66.7	
S5	66.7	33.3	44.4	77.8	55.6	55.6	66.7	66.7	66.7	66.7	88.9	55.6	44.4	55.6	66.7	66.7	55.6	66.7	77.8	66.7	55.6	66.7		
S16	44.4	33.3	66.7	44.4	66.7	55.6	55.6	33.3	33.3	77.8	44.4	33.3	22.2	33.3	33.3	44.4	33.3	44.4	33.3	44.4	33.3			
S18	66.7	11.1	55.6	33.3	44.4	44.4	44.4	44.4	44.4	55.6	66.7	55.6	44.4	44.4	55.6	44.4	33.3	44.4	55.6	44.4				
S21	44.4	44.4	66.7	77.8	77.8	77.8	77.8	33.3	66.7	55.6	66.7	77.8	77.8	88.9	77.8	66.7	77.8	88.9	77.8					
S22	55.6	77.8	66.7	66.7	66.7	66.7	66.7	55.6	44.4	55.6	66.7	66.7	55.6	66.7	77.8	66.7	55.6	66.7						
S25	33.3	44.4	44.4	66.7	66.7	66.7	77.8	88.9	77.8	66.7	66.7	55.6	66.7	77.8	66.7	55.6	66.7							
S26	88.9	88.9	66.7	66.7	44.4	55.6	44.4	55.6	66.7	66.7	77.8	66.7	55.6	66.7	77.8	66.7								
S27	100.0	77.8	77.8	55.6	66.7	55.6	66.7	77.8	77.8	88.9	77.8	66.7	77.8	88.9	77.8									
S28	77.8	77.8	55.6	66.7	55.6	66.7	77.8	77.8	88.9	77.8	66.7	77.8	88.9	77.8										
S29	100.0	55.6	66.7	77.8	88.9	100.0	100.0	88.9	100.0	88.9	100.0	88.9	100.0											
S30	55.6	66.7	77.8	88.9	100.0	100.0	88.9	100.0	88.9	100.0	88.9	100.0												
S31	44.4	55.6	44.4	55.6	55.6	44.4	55.6	66.7	55.6	44.4	55.6													
S32	66.7	77.8	66.7	66.7	77.8	66.7	77.8	66.7	77.8	66.7														
S36	88.9	77.8	77.8	66.7	77.8	66.7	77.8	66.7	77.8															
S37	88.9	88.9	77.8	88.9	77.8	88.9	77.8	88.9																
S38	100.	88.9	100.	88.9	100.	88.9	100.																	

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

	0		0		0		0
S39	88.9	100.0	88.9	100.0	88.9	100.0	
S40	88.9	77.8	88.9	100.0	88.9		
S41	88.9	100.0	88.9	100.0			
S42	88.9	77.8	88.9				
S43	88.9	100.0					
S44	88.9						

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

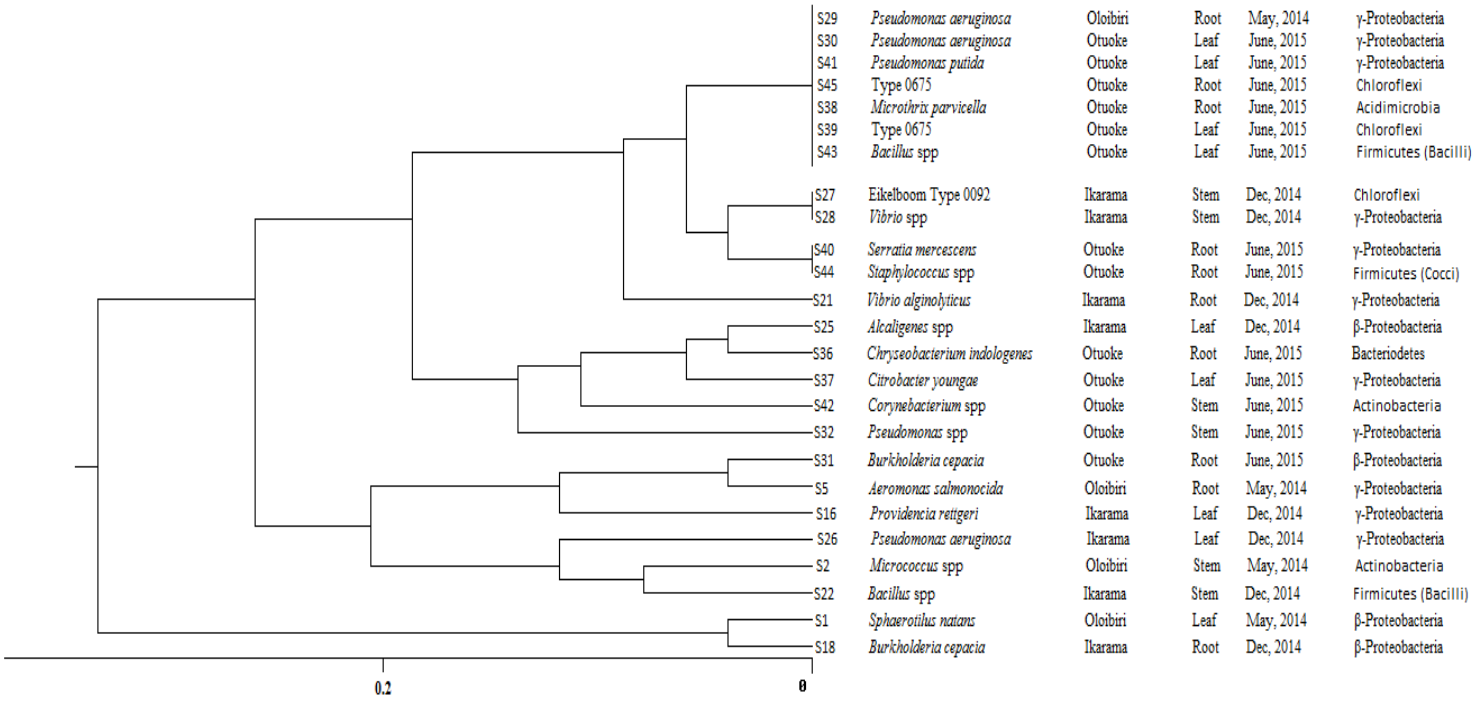
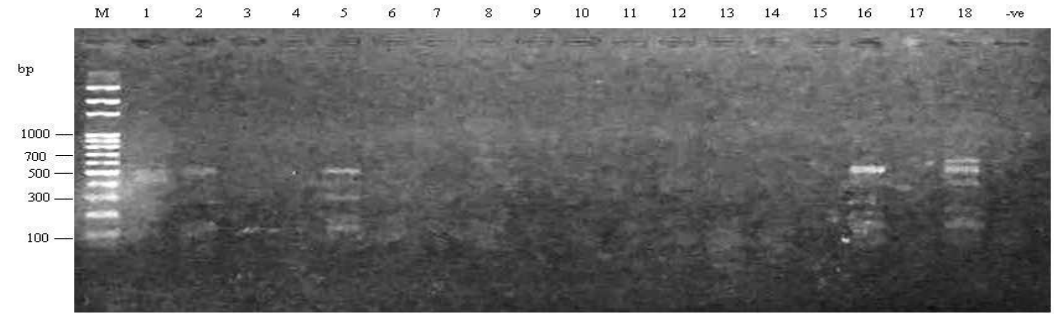
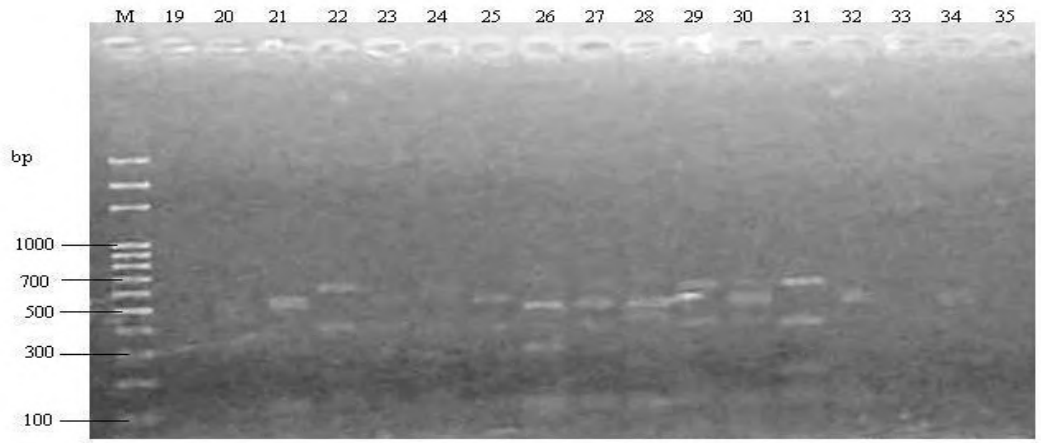


Fig. 2. A genetic distance tree of the MspI restriction patterns of strains based on 16S rRNA PCR-RFLP.

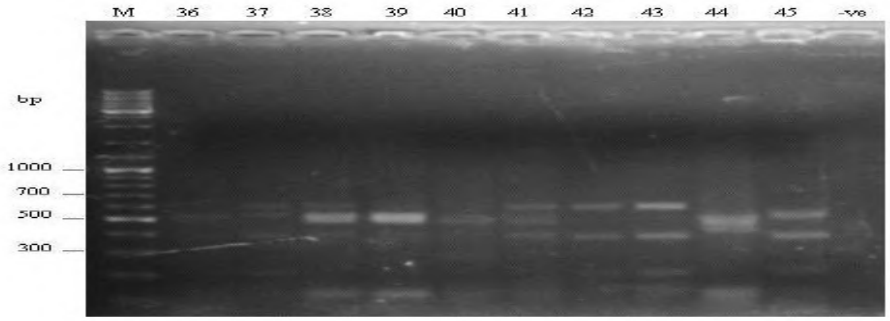


a)



b)

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*



c)

Fig. 3 (a, b, c).Products of PCR-RFLP of isolated strains using MspI restriction endonuclease generated from 16S rRNA

IV. DISCUSSION

Aquatic macrophytes have long been used as bio-monitors of environmental pollution and in the phytoremediation of wastewaters. Aquatic plants common to both petroleum-contaminated and uncontaminated sites were *Commelina benghalensis*, *Chromoleana odorata* and *Asplilia africana*. *Ageratum conyzoides, Ipomoea involucrata* and *Kyllinga* species (*Kyllinga erecta* and *Kyllinga pumila*) were common to the petroleum contaminated sites. These plants have also been found in both petroleum-contaminated and uncontaminated sites, and in the remediation of heavy metals [24].

Other plant species of the twelve-month old petroleum contaminated site were *Fimbristylis littoralis, Sacciolepsis africana, Cyperus difformis, Solenostemon monostachyus* and *Echinochloa obtusiflora*. *Fimbristylis littoralis* was not reported in petroleum polluted sites but as a common weed of the Niger Delta. It has the potential for enhanced phytoremediation of PAHs and heavy metals [25].The presence of these plants in a twelve-month old contaminated site shows its survival and phytoremediation capabilities. *Cyperus difformis* shows the ability to survive in petroleum contaminated soil and to spring up after remediation. *Solenostemon monostachyus, Echinochloa obtusiflora* andother species of *Echinochloa* are potential phytoremediators. These plants could be able to remediate toxic metals since they are present in petroleum. This study is in agreement with past findings [24, 26].Aquatic macrophytes of the non-contaminated sites include *Diplazium sammatii Dissotis rotundifolia, Anielema sp., Panicum laxum, Scleria verrucosa, Cyathula prostrata and Costus* sp.The non-tolerance of *Diplazium esculentum* to chromium [27] and the absence of the genus in petroleum contaminated soils as reported in this study could indicate their inability to survive and grow in the presence of toxic substances, therefore, unsuitable for phytoremediation.

Plants are known to harbour endophytes which are useful for the growth, development and maintaining functional activities of the plant. The association is beneficial for both the plants and the endophytes. Endophtye numbers of the plants varied with the plant species; 2-6 bacterial species were isolated from each plant and at least one species occurred in the root, stem and leaf [1]. The number of endophytes are said to decrease from their point of entry i.e. root region to the shoot and leaf [28]. In this study, more endophytes were found in the roots of the plants from the petroleum-contaminated sites probably because of the increase in number of petroleum degraders in the rhizosphere of the plants. More endophytes are found in the root because these microorganisms from the soil colonize the root zone before entering the plant [29].

Nine genera of endophytes were isolated from the six month-old petroleum-contaminated soil (site A) most of which were Gram negative members of the Proteobacteria, particularly of the Class γ-proteobacteria; *Pseudomonas, Providencia, Proteus* and *Aeromonas*. *Sphaerotilus natans* (β-Proteobacteria), Eikelboom type 0092 (β-Proteobacteria), *Bacillus* (Firmicutes), *Corynebacterium* and *Micrococcus* (Actinobacteria) and *Chryseobacterium* (Bacteroidetes) are other genera isolated. *Providencia* and *Proteus* were the members of the family Enterobacteriaceae present. Many of these species occurred in high numbers. Gram negative bacteria made up 70.69% of the isolates while Gram positive bacteria were 29.31%. More bacterial genera (13) occurred in the twelve month-old petroleum-contaminated soil (site B) with the Proteobacteria dominating as well. *Burkholderia, Alcaligenes* and *Sphaerotilus* are β-Proteobacteria; *Pseudomonas, Vibrio* and members of the family Enterobacteriaceae (*Providencia, Proteus,*

https://dx.doi.org/10.22161/ijhaf.2.5.1 ISSN: 2456-8635

Morganella and *Serratia*) are γ-proteobacteria. Others are *Bacillus*,*Chryseobacterium* and Eikelboom type 0092. Overall, 95.92% were Gram negatives with *Bacillus* (4.08%) as the only Gram positive bacteria. The increase in bacterial genera may be attributed to successive activities of petroleum degradative microorganisms with age of site, and the recovery of site, thus allowing the habitation of those microorganisms unable to utilize specific chain length alkanes.

The uncontaminated soil (site C) had sixteen bacterial genera, again dominated by the Gram negative Proteobacteria and other Gram negatives which made up 59.2% of the isolates and more genera of the Gram positive bacteria; *Bacillus, Corynebacterium, Micrococcus, Staphylococcus* and filamentous bacteria (*Microthrix parvicella* and the Gram variable, Type 0675) making up 40.8%. More bacterial genera were present in this site but their numbers were fewer compared to the petroleum-contaminated sites which suggests the proliferation of specific degraders of the hydrocarbon in these sites.

Past studies on plant endophytic bacteria have reported the dominance of the Proteobacteria, particularly the γ-proteobacteria with *Pseudomonas* as the most frequently isolated, and a large number of the α-proteobacteria and β-proteobacteria, but fewer numbers of the Firmicutes, Actinobacteria and Bacteroidetes [7, 30]. The dominance of the γ-proteobacteria is in agreement with the findings of this work but more Bacteroidetes and Firmicutes were isolated than β-proteobacteria. Endophytes ofsome aquatic plants were found to have γ-proteobacteriaparticularly, *Pseudomonas* spp.and Bacilli predominant [5] and others;*Phragmitesaustralis*and *Ipomoea aquatica*, had predominantly, γ-proteobacteria and mostly *Pseudomonas* spp.*Acinetobacter* spp., *Stenotrophomonas* spp. with less of *Serratia* spp. *Chryseobacterium* spp. and *Erwinia* spp. Of the fewer gram-positive bacteria the predominant strains were *Bacillus* spp., *Paenibacillus* spp., and *Microbacterium*spp.[31]. *Bacillus* spp. were predominant in this study. Gram positive bacterial genera could be few in petroleum-contaminated sites but can actually dominate the bioremediation process considering their metabolic versatility [32].

The γ-Proteobacteria were the most cultured group of bacteria while the other groups differed slightly according to the location where plants were picked. Bacteroidetes was the second dominant phylum occurring more in the twelve month-old contaminated site followed by the Firmicutes occurring more in the six month-old site, Actinobacteria (absent in site B) and β-proteobacteria (more in site B) occurred in equal numbers while other filamentous bacteria (least found) were specific to the uncontaminated site. This suggests that the endophytes may be site specific and their diversity may be dependent on the species of plants present at the sites. *Providencia rettgeri* was present in only the petroleum-contaminated sites. This genus is also a reported soil and rhizosphere isolate of oil-polluted sites but not as an endophyte. Its presence as an endophyte of only the polluted sites is noteworthy.*Providencia rettgeri* is usually an important pathogen of insects and humans [33, 34].*Sphaerotilus natans*, like other filamentous bacteria, are found in sewage and wastewater treatment plants, and occur in marshlands and ponds and water bodies.Thegenus *Sphaerotilus*consist oforganoheterotrophic bacteria, but occurred in plants of the oil polluted sites only. This implies that the organisms also utilized the hydrocarbon as an energy source in the plants.Compartmentalization of the endophytes showed that the β-proteobacteria occurred mostly in the stem, the γ-proteobacteria, Firmicutes and Actinobacteria in the roots and Bacteroidetes in the roots and stems.Increasing petroleum hydrocarbon concentrations was shown to enhance the proliferation of Proteobacteria (γ-proteobacteria) in the root while β-proteobacteria favored the stem [35, 36].

Biochemicalcharacterization of common isolates showed*Chryseobacterium indologenes*of all the sites were citrate utilizers, urea was broken down by the organism in the twelve month-old contaminated site and the uncontaminated site. This ability to produce urease would be related to the presence of urea (a protein of animal origin) in the environment. Gelatinasewas produced by the organisms from the petroleum-contaminated sites. As with other strains of *Ps. aeruginosa*, L-arginine, citrate and gelatin were utilized by the organisms in the three sites, urea was hydrolysed by the strain in site B, again signifying the presence of the protein in the environment, while rhamnose was fermented in the strain occurring in the uncontaminated site and amylose fermented by that of the six month-old contaminated site. These sugar fermentation abilities could be site related. *Pseudomonas* sp. of site A and *Ps. flourescens* of site B (petroleum-contaminated) produced enzymes argininedihydrolase,gelatinase and utilized citrate, andcould be the same species. Both *Ps. putida* of the uncontaminated site and the twelve month-old contaminated site showed the same biochemical properties (L-arginine and citrate utilization).

Proteus vulgaris group was peculiar to the six month-old contaminated site with its biochemical properties while *Proteus mirabilis* of same metabolic properties were at site B and C (TABLE 5).*P. vulgaris* group producing indole and fermenting maltose were reported as petroleum hydrocarbon degraders in tropical soils, and as growth promoters in plants occurring in petroleum-contaminated

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

soils [37]. The same biochemical properties were shown by *Providencia rettgeri* of the petroleum-contaminated sites which suggests their genetic similarities. *Sphaerotilus natans* of site B produced beta galactosidase, giving it an added nutritional advantage over that of site A which did not.

Biochemical properties of *Bacillus* species of the three sites differed. Species of the six month-old contaminated site showed a wide nutritional ability by fermenting sugars and utilizing L-arginine in addition to citrate, urea and production of enzyme deaminase (tryptophan) and gelatinase. The species of the twelve month-old site had limited biochemical properties showing only citrate utilization and gelatinase activity while *Bacillus* of the uncontaminated site produced indole in addition to citrate, urea, tryptophan and gelatin hydrolysis. Differences in biochemical properties of the endophytes could be attributed to conditions in the sites related to soil characteristics; structure and texture of soils, nutrients available, moisture and other environmental factors. These endophytes were isolated from different wetland plants occurring in different habitats and with specific abilities. All these factors could have contributed to the adaptability of the endophytes in them.

Genetic analysis of the clonal relatedness of the bacterial species showed 100 % relatedness of *Pseudomonas aeruginosa* of the six month-old petroleum-contaminated site and the uncontaminated site, as well as *Pseudomonas putida*. The same species of organism was isolated at different times, location and in different plant compartments. This shows strong physiological similarities and probably, same abilities of the two as petroleum degraders. Site A and site C are located in the same region where oil was first discovered in Nigeria (Ogbia Local Government Area). Other members of the γ-proteobacteria also clustered together. The percentage of clonal relatedness ranged from 11.1 – 100% genetic homology, which shows diverse distribution in the genetic components and capabilities of strains. The 16S rRNA generated data of DNA bands showed 100% genetic homology of same species and closely related bacterial group which indicated the possibility of possession of similar traits associated with bioremediation. On the other hand, the clustering of species of different phyla shows some similarities between the species but highlights the possible short-comings of the PCR-RFLP method in phylogenetic analysis [38].

V. CONCLUSION

The present study has highlighted a variety of wetland plants and their accompanying endophytes with potentials for phytoremediation of petroleum polluted wetland soils. A more precise molecular technique for the characterization of the endophytes will reveal the true diversity and functionality of the eendophytes as these were limited in the present work. The presence of these plants indicate the natural recovery process of the vegetation since they were tolerant to the initial impact of the oil. Majority of the above mentioned wetland plants have not been demonstrated in preliminary petroleum hydrocarbon bioremediation laboratory studies as such information would be required for further and more advanced studies on plant-microbe interactions, before a successful implementation of a bioremediation strategy.

ACKNOWLEDGEMENTS

Sincere gratitude to the Departments of Medical Microbiology and Biochemistry, School of Health Sciences, Niger Delta University, Amassoma Bayelsa - Nigeria. Special thanks to the Taxonomic Unit of the University of Port Harcourt, Nigeria for plant identification. Our appreciation goes to all staff in the Department of Microbiology, Federal University Otuoke-Nigeria.

REFERENCES

[1] Z. Stępniewska and A. Kuźniar. “Endophytic microorganisms—promising applications in bioremediation of greenhouse gases,” Appl. Microbiol. Biotechnol., 2013, vol. 97, pp 9589 – 9596.

[2] F. Posada and F. E. Vega. “Establishment of the fungal entomopathogen *Beauveriabassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*),”Mycologia, 2005, vol.97, pp 1195 – 1200.

[3] R. P. Ryan, , K. Germaine, A. Franks, D. L. Ryan and D. N. Dowling. “Bacterial endophytes: recent developments and applications,” FEMS Microbiol. Lett., 2008, vol. 278 no.1, pp 1 – 9.

[4] M. McGuinness and D. Dowling. “Plant-associated bacterial degradation of toxic organic compounds in soil,”Int. J. Environ Res. Public Health, 2009, vol. 6, pp 2226 – 2247.

[5] W-M. Chen, Y-Q. Tang, K. M. Mori and X-L. Wu. “Distribution of culturable endophytic bacteria in aquatic plants and their potential for bioremediation in polluted waters,” Aqua. Biol., 2012, vol. 15 pp 99 – 110.

[6] E. Amora-Lazcano, , L. A., Guerrero-Zúñiga, A. Rodriguez-Tovar, A. Rodriguez-Dorantes and M. S. Vasquez-Murrieta. “Rhizospheric plant-microbe interactions that enhance the remediation of contaminated soils,” in Current Research Technology and Education Topics in Applied

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Microbiology and Microbial Biotechnology. A. Mendez-Vilas ed., 2010, pp 251 – 256.

[7] M. Kukla, T. Płociniczak and Z. Piotrowska-Seget. "Diversity of endophytic bacteria in *Loliumperenne*and their potential to degrade petroleum hydrocarbons and promote plant growth," Chemosphere, 2014, vol. 117 pp 40 – 46.

[8] E. Perez-Rosales, L. Alcaraz-Meléndez, M. E. Puente, ,R. Vázquez-Juárez, E. Quiroz-Guzmán, T. Zenteno-Savín andE. Morales-Bojórquez,. "Isolation and characterization ofendophytic bacteria associated withroots of jojoba (*Simmondsiachinensis*(Link) Schneid)," Curr. Sci., 2017, vol. 112 no. 2, pp 396 – 401.

[9] N. Weyens, D. Van Der Lelie, T. Artois, K. Smeets, S. Taghavi, L. Newman, R. Caarleer and J. Vangronsveld, "Bioaugmentation with engineered endophytic bacteria improves contaminant fate in phytoremediation," Environ. Sci.Technol., 2009, vol. 43, pp 9413 – 9418.

[10] S. L. Doty, "Review*:* Enhancing phytoremediation through the use of transgenics and endophytes," New Phytol., 2008, 179 pp 318 – 333.

[11] S. D. Siciliano, N. Fortin, A. Mihoc, G. Ouellette, R. Roy, G. Lyle, M. Whyte, K. Banks, P. Wisse, S. Labelle, D. Schwab, K. Lee and C. W. Greer, "Selection of specific endophytic bacterial genotypes by plants in response to soil contamination," Appl. Environ. Microbiol.,2001, vol. 67 no.6 pp 2469 – 2475.

[12] T. Barac, S. Taghavi, B. Borremans, A. Provoost, L. Oeyen, J. V. Colpaert, J. Vangronsveld and D. van der Lelie, "Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants," Nat. Biotechnol., 2004, vol. 22 no. 5 pp 583 – 588.

[13] S. Taghavi, T. Barac, B. Greenberg, B. Borremans, J. Vangronsveld and D. van der Lelie, "Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene," Appl. Environ. Microbiol., 2005, vol. 71, pp 8500 - 8505.

[14] K. Germaine, X. Liu, G. Cabellos, J. Hogan, D. Ryan and D. N. Dowling 2006, "Bacterial endophyte- enhanced phyto-remediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid," FEMS Microbiol. Ecol., vol. 57 pp 302 – 310.

[15] M. Afzal, S. Yousaf, T. G. Reichenauer and A. Sessitsch, "The inoculation method affect colonization and performance of bacterial inoculant strains in the phytoremediation of soil contaminated with diesel oil," Int. J. Phytorem., 2012, vol. 14, no. 1 pp 35 – 47.

[16] M. Afzal, S. Khan, S. Iqbal, M. S. Mirza and Q. M. Khan, "Inoculation method affects colonization and activity of *Burkholderia phytofirmans*PsJN during phytoremediation of diesel-contaminated soil," Int. Biodeterior. Biodegradation, 2013, vol. 85 pp 331 – 336.

[17] K. Fatima, A Imran,, I. Amin, Q. M. Khan and M. Afzal, "Plant species affect colonization patterns and metabolic activity of associated endophytes during phytoremediation of crude oil-contaminated soil," ESPR, 2016, vol.23 no. 7, pp 6188 – 6196.

[18] R. M. Wersal and J. D. Madsen, "Aquatic plants: their uses and risks," in International Plant Protection Convention, Food and Drug Administration of the United Nations, Rome, Italy, 2012, pp 1 – 13.

[19] M.N.V. Prasad, **"**Aquatic Plants for Phytotechnology," in Environmental Bioremediation Technologies, S. N. Singh and R. D. Tripathi Eds. Springer, Berlin, 2007, pp 259 – 274.

[20] N. Shafi, A. K. Pandit, A. N. Kamili and B. Mashtaq, "Phytoremediation potential of free floating macrophytes – a review," MRJEST, 2015, vol. 3 no. 1, pp 012 – 016.

[21] S. J.Bhore, R. Nithya and C. Y. Loh,"Screening of endophytic bacteria isolated from leaves of SambungNyawa [*Gynuraprocumbens* (Lour.) Merr.] forcytokinin-like compounds,"Bioinformatics, 2010, vol. 5 no.5 pp 191 – 197.

[22] P. H. Nielsen, C. Kragelund, R. J. Seviour and J. L. Nielsen, "Identity and ecophysiology of filamentous bacteria in activated sludge," FEMS Microbiol. Rev., 2009, vol. 33 pp 969 – 998.

[23] Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. Eds., "Preparation and analysis of DNA," in Current Protocols in Molecular Biology. John Wiley & Sons, Inc., New York, 2003.

[24] U. D. Chima and G. Vure," Implications of crude oil pollution on natural regeneration of plant species in an oil-producing community in the Niger Delta Region of Nigeria," J. For. Res., 2014, vol.25 no.4 pp 915 – 921.

[25] E. O. Nwaichi, M. Frac, P. A. Nwoha and P. Eragbor, "Enhanced phytoremediation of crude oil-polluted soil by four plant species; effect of inorganic and organic bioaugmentation," Int. J. Phytoremediation, 2015, vol. 17 no. 12 pp 1253 – 1261.

[26] A. A. El Falaky, S. A. Aboulroos, A. A. Saoud and M. A. Ali, "Aquatic plants for bioremediation of waste water," Eighth International Water

https://dx.doi.org/10.22161/ijhaf.2.5.1 *ISSN: 2456-8635*

Technology Conference, IWTC8, Alexandria, Egypt, 2004, pp 361 – 367.

[27] A. Kumari, Y. B. Pakade, P. Chand, M. N. V. Prasad and B. Lal, "Comparative accounts of chromium accummulation in three ferns under hydroponic system,"J. Sci.Ind. Res., 2014, vol. 73 pp 553 – 558.

[28] J. E. Dombrowski, V. G. Hollenbeck and R. C. Martin, "Isolation and identification of bacterial endophytes from grasses along the Oregon Coast,"Am. J. Plant Sci., 2017, vol. 8 pp 574 – 601.

[29] M. Rosenblueth and E. Martinez-Romero, "Bacterial endophytes and their interactions with Hosts," Mol. Plant Microbe Interact., 2006, vol. 19 pp 827 – 837.

[30] V. Oliveira, N. C. M. Gomes, A. Almeida, A. M. S. Silva, M. M. Q. Simões, K. Smalla andA. Cunha, "Hydrocarbon contamination and plant species determine the phylogenetic and functional diversity of endophytic degrading bacteria," Molecular Ecology, 2013, pp 1 – 10.

[31] Y-N. Ho, D. C. Mathewa, S-C. Hsiaoa, C-H. Shih, M-F. Chien, H-M. Chiang and C-C. Huang, "Selection and application of endophytic bacterium *Achromobacterxylosoxidans* strain F3B for improving phytoremediation of phenolic pollutants," J. of Hazard. Mater., 2012, vol. 219-220 pp 43 – 49.

[32] J. B. van Beilen, E. G. Funhoff, A. van Loon, A. Just, L. Kaysser, M. Bouza, R. Holtackers, M. Röthlisberger, Z. Li and B. Witholt, "Cytochrome P450 alkane hydroxylases of the CYP153 family are common in alkane-degrading eubacteria lacking integral membrane alkane hydroxylases. Appl. Environ. Microbiol., 2006, vol. 72 pp 59 – 65.

[33] J. Manos and R. Belas, "The Genera *Proteus*, *Providencia*, and *Morganella,"* Prokaryotes, 2006, vol. 6 pp 245 – 269.

[34] M. MsaadGuerfali, W. Djobbi, K. Charaabi, H.Hamden, S. Fadhl, W. Marzouki, F. Dhaouedi and C. Chevrier, "Evaluation of *Providencia rettgeri*pathogenicity against laboratory Mediterranean fruit fly strain(Ceratitiscapitata)," PLoS ONE, 2018, vol. 13 no.5, e0196343.

[35] P. Gkorezis, M. Daghio, A. Franzetti, D. Van Hamme, W. Sillen and J. Vangronsveld, "The Interaction between plants and bacteria in the remediation of petroleum hydrocarbons: an environmental perspective," Front. Microbiol.,2016, vol. 7 pp 1836.

[36] S. Tardif, É. Yergeau, J. Tremblay, P. Legendre, L. G. Whyte and C. W. Greer, "The willow microbiome is influenced by soil petroleum-hydrocarbon concentration with plant compartment-specific effects, "Front. Microbiol., 2016, vol. 7 pp 1363.

[37] D. Drzewiecka,"Significance and Roles of Proteus spp. Bacteria in Natural Environments,"Microb. Ecol., 2016, vol. 72 pp 741–758.

[38] H. B. Rasmussen,"Restriction Fragment Length Polymorphism analysis of PCR-amplified fragments (PCR-RFLP) and gel electrophoresis - valuable tool for genotyping and Genetic fingerprinting," in Gel Electrophoresis - Pinciples and Basics, S. Magdeldin Ed., In Tech Croatia, China, 2012, pp 315 – 334 .