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Genomic Identification and Degradation Testing of Hydrocarbon-Utilizing Bacteria Isolated from Soil in Eleme Local Government Area of Rivers State, Nigeria

***Chunwafor, M.I., Ogbonda, K.H., & Okwelle, A.A.**
Department of Biology, Ignatius Ajuru University of Education, Port Harcourt, Nigeria

***Corresponding author email:** michunwafor@gmail.com

Abstract
As a result of industrialization, environmental pollution by hydrocarbon is almost inevitable since they are the highest cause of contamination of soil. Since hydrocarbon pollution is an issue of serious consequence and concern, it is important to devise a means to degrade them without causing more harm to the soil and its inhabitants than it is with chemical methods. This research is therefore focused on the identification and degradability test of hydrocarbon-utilizing bacteria isolated from contaminated soil in the Eleme local government area of Rivers state, Nigeria. Soil samples from different contaminated sites were collected aseptically and isolation of the bacteria was carried out using the spread plate vapour transfer method for hydrocarbon-utilizing bacteria. Zymo Quick DNA Fungal/Bacterial Kit was used for the DNA extraction. A fragment of their 16S ribosomal ribonucleic acid (16S rRNA) gene was extracted for all isolates and amplified with polymerase chain reaction (PCR) with the universal primer set 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA. Bacteria that were identified include: *Sinomonas halotolerant* strain, *Priestia flexa* strain, *Rhizobium freireiI* strain, *Burkholderia stagnalis* strain, *Serratia marcescens* strain, *Burkholderia arboris* strain, *Ectobacillus funiculus* strain and *Priestia megaterium* strain with similarity 98-100% to the closest GenBank match. The bacteria were further examined for hydrocarbon compound degradation ability using turbidity test and they showed different capabilities. Of all the bacteria isolates, *Priestia flexa* strain, *Serratia marcescens* strain, *Burkholderia stagnalis* strain and *Rhizobium freireiI* strain showed very high level of degradation ability of all the hydrocarbons (Used oil, Kerosene DPK, Diesel AGO and Fuel PMS) compounds. PMS was the most difficult to degrade by these bacteria. The bacteria isolated from this research can be used for bioremediation of hydrocarbon-contaminated sites instead of other methods.

Keywords: Genomic, Degradation, Hydrocarbon

Introduction

The contamination of the environment with crude oil has been a serious environmental issue in Nigeria and all over the world. At appropriate concentrations, crude oil can result in measurable toxicity towards living systems since it is a complex mixture of hydrocarbon and non-hydrocarbon compounds that occur naturally. Crude oil, because of its characteristics is one of the most significant pollutants in the environment as it is capable of causing severe pollution and serious damage to humans and the ecosystem in general (Chikere & Ekwuabu, 2014b). The toxicity of crude oil or petroleum products varies widely depending on several factors which include their composition, concentration, environmental factors and the biological condition of the organisms at the time of the contamination (Ogbonna et al., 2020). Hydrocarbon, which is a component of crude oil, is known to cause contamination of air, water and soil. Issues of pollution of soil like that of water and air have been and are increasingly of global interest since it is on the rise daily and human comfort is of the essence.

The rising explorative, productive and transportation exercises combined with inappropriate garbage removal rehearses have prompted inescapable pollution of both the amphibian and earthbound biological frameworks. This

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might be because of the way that hydrocarbon is the most widely recognized and significant wellspring of energy for the two ventures, homegrown (people, schools, chapels and so on) and may stay the main hotspot for the following many years. Oil-creating areas of Nigeria particularly the Niger Delta region have encountered the overwhelming outcomes of unrefined petroleum spills in both earthly and sea-going environments in the previous many years of raw petroleum investigation, creation and transportation (Adati, 2012). Aside from investigation, creation and transportation exercises related to oil, oil spillages happen through big haulier mishaps on the way, well victory, harm and coincidental burst of pipelines because of over matured oil pipes or ill-advised designing administrations, bringing about the arrival of unrefined and refined oil into earthbound and amphibian conditions- a typical example of rupture and sabotage of oil pipelines is the several cases in this study area which is Eleme Local Government Area. Petroleum is indeed Nigeria and the world's energy source as it is a major energy source Worldwide except for a few developed and fast-developing countries that are making sincere and justifiable efforts to diversify their source of industrial and domestic energy.

Eleme local government region is situated in the Niger Delta and oil production and exploration as well as transportation exercises inside and outside of the region since around the 1950s. Eleme is presently described by oil fields and establishments that have stayed torpid for a very long time thus numerous new ones are being found. Taking into account the enormous amount of oil going into the Niger Delta climate, particularly farmlands and streams, the need to clean up raw petroleum-tainted locales has turned into a vital natural issue (Vincent et al., 2011). Ordinary strategies, for example, actual expulsion were the principal reaction choice. It is qualified to take note that they don't accomplish a total cleanup of the oil slicks. Current mechanical strategies regularly recuperate not more than 10-15% of unrefined after a significant spill and quite often leave the getting body in more awful circumstances (Abu & Dike, 2008). The need for an environmentally friendly method of remediation of hydrocarbon-contaminated sites cannot be over-emphasized.

Materials and Methods

The location for the study was in the Alode crude-oil pipeline, Alode Eleme with coordinates (Latitude $4^0$46’33’’N, Longitude $7^0$07’19’’E) and (Latitude $4^0$46’46’’N, Longitude $7^0$07’36’’E) and Ogale pipe line in Okulebo-Ogale with coordinates (Latitude $4^0$49’43’’N, Longitude $7^0$09’29’’E) and (Latitude $4^0$49’45’’N, Longitude $7^0$09’20’’E) both in Eleme local government area of Rivers State where oil spillage was reported to have occurred due to vandalism and rupturing of age long underground pipe belonging to Nigeria Nation Petroleum Company (NNPC) that carries crude oil and other refined products.

A random sampling technique was used to ensure that each soil bacterium within the location of the study area was allowed to represent itself (Ogbonna et al. 2020). Soil samples were collected using a sterile spatula at a tillage depth of 20 cm (Onifade & Abubakar, 2007) randomly from 5 core points at each of the locations on the contaminated area of the pipeline and homogenized to obtain composite soil samples. Soil samples were collected at Alode and Ogale both in Eleme into sterile re-sealable bags and were transported in an ice pack to Ignatius Ajuru University of Education Biology research laboratory within 24 hours of collection for microbiological culturing and biochemical analyses after which the isolates were transported aseptically wrapped with cling film and carried in a sterile container to Regional Centre for Biotechnology and Bioresources Research RCBBR, University of PortHarcourt for gene extraction and then sequencing for bacteria characterization and back to RCBBR PortHarcourt for hydrocarbon degradation test.

The diluents (physiological saline) were prepared by dissolving 8.5g of analytical sodium chloride in 1000m of deionized water. The already prepared physiological saline was used as diluents for the serial dilutions. The diluents were dispensed in 90ml amounts into test tubes and sterilized by autoclaving at 121^0C for 15 minutes at 15p.s.i. Then ten (10) grams of each soil sample was separately diluted in 90ml of sterile physiological saline in a conical flask to get the homogenous stock solution by shaking thoroughly and a six-fold serial dilution was carried out by transferring 10ml of the original solution into 90ml of the diluents (Ogbonna et al., 2020).

The counts of total heterotrophic bacteria in the soil samples were determined by the spread plate techniques as described by Prescott et al. (2005) using nutrient agar (NA). Nutrient Agar (NA) was prepared according to the manufacturer's standard, boiled to dissolve and autoclaved at 121c, 15mins and 15psi. It was allowed to cool. The media was poured into each petri dish (In duplicates). It was allowed to solidify after which, 0.1 ml of 10^{-1}, 10^{-3} and

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10^{-6} serially diluted samples were pipetted onto the agar and spread with a bent glass rod. The plates were incubated for 24 hours then a total heterotrophic bacteria count was done.
The vapour phase transfer method as described by Ogbonna et al. (2020) was adopted in estimating the population of hydrocarbon utilizing bacteria using spread plate techniques on mineral salt medium (MSM). Aliquots of 0.1ml of 10^{-3} were inoculated onto solidified mineral-enriched media in duplicates and spread with a spreader. The vapour phase method was done by soaking sterile filter paper discs in 2ml of sterile crude oil. The soaked filter paper was aseptically placed onto the inside cover of the Petri dishes and the Petri dishes were inverted before incubating. The plates were incubated at 37^0C for 24 - 48 hours. Total hydrocarbon utilizing bacteria count was done. Other agar (Mannitol salt agar, MacConkey agar and Eosin methylene blue agar) enriched with the mineral medium were also used for the isolation of specific organisms.

The different colonies that developed after incubation were sub-cultured twice and incubated for 24 hours each time to obtain pure cultures (Chikere & Ekwuabu, 2014b). The final isolates were then maintained on properly labelled nutrient agar slants by streaking and incubated for 24 hours before they were preserved by refrigerating for further tests. Then the isolates were characterized and identified according to the methods of Ndubuisi-Nnaji et al. **(**2015). Identification of the bacterial isolates was compared to Bergy's Manual of Determinative Bacteriology (Holt et al., 1994) based on their cellular morphology, colonial morphology, microscopic examination after Gram staining and biochemical tests. The biochemical tests done were catalase test, coagulase test, oxidase test, spore test, motility test, indole test, methyl red test, Vogues Proskauer test, citrate utilization test and sugar fermentation test (sucrose, lactose, mannose and glucose) and they were used for tentative identification of bacteria that were isolated.

Zymo Quick DNA Fungal/Bacterial Kit was used for the DNA extraction. The protocol of the Zymo Quick DNA Fungal/Bacterial kit was used with modifications to elute the DNA.

27F: AGAGTTTGATCMTGGCTCAG
1525R: AAGGAGGTGWTCCARCCGCA

2.5ul of 10x PCR buffer,1ul of 25mM MgCl2, 1ul each of forward preliminary and opposite groundwork, 1ul of DMSO, 2ul of 2.5mMDNTPs, 0.1ul of 5u/ul Taq DNA polymerase, and 3ul of 10ng/ul DNA. The absolute response volume was made up to 25ul utilizing 13.4ul nuclease-free water

Beginning denaturation at 94˚C for 5mins, trailed by 36 patterns of denaturation at 94˚C for 30sec, tempering at 56˚C for 30secs and prolongation at 72˚C for 45sec. Followed by a last lengthening step at 72˚C for 7 minutes and holding temperature at 10 ˚C until the end of time. Enhanced parts were envisioned on Safe view-stained 1.5% agarose electrophoresis gels. The size of the amplicon is around 1500bp and the DNA stepping stool utilized is Hyperladder from Bioline.

DNA quality and immaculateness were taken a look at utilizing a NanoDrop 2000c spectrophotometer (Thermo Fisher Logical Inc. Wilmington, Delaware, USA). Virtue is estimated as a proportion of Bright (UV) light absorbance at 260nm to that of 280nm. The NanoDrop was associated with a PC framework, and the sensor was cleaned utilizing a cotton fleece and 70% ethanol. 1µl of Elution cradle (the arrangement used to re-suspend the DNA) was administered straightforwardly on top of the NanoDrop sensor. The Nanodrop decided the clear and the DNA tests (1µL) were independently stacked onto the sensor. The sensor was typically cleaned when another example was to be stacked to stay away from pollution. Nanodrop estimation was taken in sets of three for each example.

Gel electrophoresis was performed utilizing 1.5% agarose gel. 0.75g of agarose powder was blended in with 50 ml of TrisBoris EDTA (TBE) 1X in an estimating jar and microwaved for 2 minutes to get an unmistakable arrangement. 5µL of EZ seeing colour (Blue Light) was added to the substance in the tapered jar and afterwards filled the projecting plate or gel holder. The brush was set inside the projecting plate and permitted to sit between 20 to 30 minutes at room temperature for the gel to harden. The gel electrophoresis unit was then set up; the gel holder containing the gel was placed into the gel tank and TBE 1X was filled the gel tank until the gel was lowered. The atomic weight marker (1Kb DNA Stepping stool) was stacked into the main path, and the DNA tests were independently stacked into the wells made by the brush on the gel. Every DNA test (3µL) was blended in with 3µL of 2X stacking colour and afterwards stacked in one path on the gel. A control was likewise stacked which contained all parts of the PCR response combination except format DNA. The setup was permitted to run for 40 minutes at 100 volts. Toward the finish of the

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running time, the DNA parts were envisioned under a Bright (UV) transilluminator (Gel Documentation microDOCTM, Cleaver Scientific Ltd, UK).

The neighborhood-joining strategy gathered the developmental history (Saitou & Nei, 1987). The ideal tree is shown. The level of imitate trees in which the related taxa grouped in the bootstrap test (1000 repeats) are displayed close to the branches (Felsenstein, 1985). The tree is attracted to scale, with branch lengths in similar units as those of the transformative distances used to surmise the phylogenetic tree. The transformative distances were registered to utilize the Jukes-Cantor strategy (Jukes & Cantor, 1969) and are in the units of the number of base replacements per site. This examination included 6 nucleotide groupings. Cordon positions included were 1st+2nd+3rd+Noncoding. All questionable positions were taken out for each arrangement pair (match-wise erasure choice). There were a sum of 907 situations in the last dataset. Developmental examinations were directed in Super X (Kumar et al., 2018).

Representative of each HUB isolate was screened for degradation capability using kerosene (DPK), fuel (PMS), diesel (AGO) and used engine oil as a carbon source for the bacteria isolates by inoculating a calibrated loop full of the 24-hour culture of each hydrocarbon utilizing bacᵗerium obtained from the contaminated soil into 9ml of Bushnell Haas Broth medium containing 1 ml of each of the substrate. Biodegradation was scored by turbidity (checking for optical density) and emulsification of oil-in-mineral broth medium on day 7, day 14, day 21 and on the 28th day of incubation at 30°C (Chikere & Ekwuabu, 2014a). Turbidity was screened as each test tube was scored for optical density at 600nm. The reading was taken separately for each of the test tubes every 7 days (days- 7, 14, 21 and 28) and the pH value was also noted.

Results

The result from the total heterotrophic bacteria count (THBC)and hydrocarbon utilizing bacteria count (HUBC) is shown below with very high THBC in Alode and the THBC in Ogale being too numerous to count. While the HUBC were also very high in both communities.

Table 3. 1: Mean bacteria count for total heterotrophic bacteria (THBC) and hydrocarbon-utilizing bacteria (HUBC)

Station	THBC (cfu/ml)	HUBC(cfu/ml)
Alode	$1.95x10^6$	$1.60x10^6$
Ogale	TNTC	$1.88zx10^6$

TNTC= too numerous to count

This table (table 1) shows the mean bacteria count for the total heterotrophic bacteria that were isolated in this study in both Alode and Ogale of Eleme local government area.

The bacteria identified from the DNA sequencing of are shown in the table below. Their accession number and strain identity as well as their closest match in the Genbank is shown. Some of the bacteria have their old name and the corresponding new name shown.

Table 3.2: Identity of the bacterial isolates - strain, name of organism, closest GenBank, percentage identity and accession

S/N	Strain	Organism	Old Name	Closest GenBank Match	Similarity (%)	Accession No
1	RCBBR_MIC1	*Sinomonas halotolerans*		*Sinomonas halotolerans* JCM 31751	99	OP143664
2	RCBBR_MIC3	*Burkholderia stagnalis*		*Burkholderia stagnalis* LMG 28156	100	OP143665
3	RCBBR_MIC4	*Priestia flexa*	*Bacillus flexus*	*Priestia flexa* SBMP3	98	OP143666
4	RCBBR_MIC5	*Burkholderia arboris*		*Burkholderia arboris* R-24201	100	OP143667
5	RCBBR_MIC7	*Serratia marcescens*		*Serratia marcescens subsp. marcescens* JCM 1239	100	OP143668
6	RCBBR_MIC8	*Rhizobium freirei*		*Rhizobium freirei* PRF 81	99	OP143669

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7	RCBBR_MIC9	*Ectobacillus funiculus*	*Ectobacillus funiculus* NAF001	100	OP143670
8	RCBBR_MIC10	*Priestia megaterium*	*Priestia megaterium* IAM 13418	99	OP143671

The table below shows the quality and purity of the extracted DNA using a 2000c spectrophotometer. The purity is measured as a ratio of ultraviolet light absorbance at 260nm to 280nm. This was done in connection with a computer system.

Table 3.3: NanoDrop spectrometry characteristics of the isolates

S/N	Strain	Conc. (ng/mL)	A260	A280	Purity (A260/A280)
1	RCBBR_MIC1	245.2	4.927	2.545	1.94
2	RCBBR_MIC3	180.1	3.601	1.871	1.92
3	RCBBR_MIC4	54.8	1.068	0.559	1.91
4	RCBBR_MIC5	213.4	4.288	2.314	1.84
5	RCBBR_MIC7	212.7	4.266	2.295	1.86
6	RCBBR_MIC8	153.8	3.067	1.661	1.85
7	RCBBR_MIC9	170.5	3.299	1.764	1.87
8	RCBBR_MIC10	73.5	1.483	0.745	1.99

The figure below is the phylogenetic tree showing all isolates and their strain identity with their accession number.

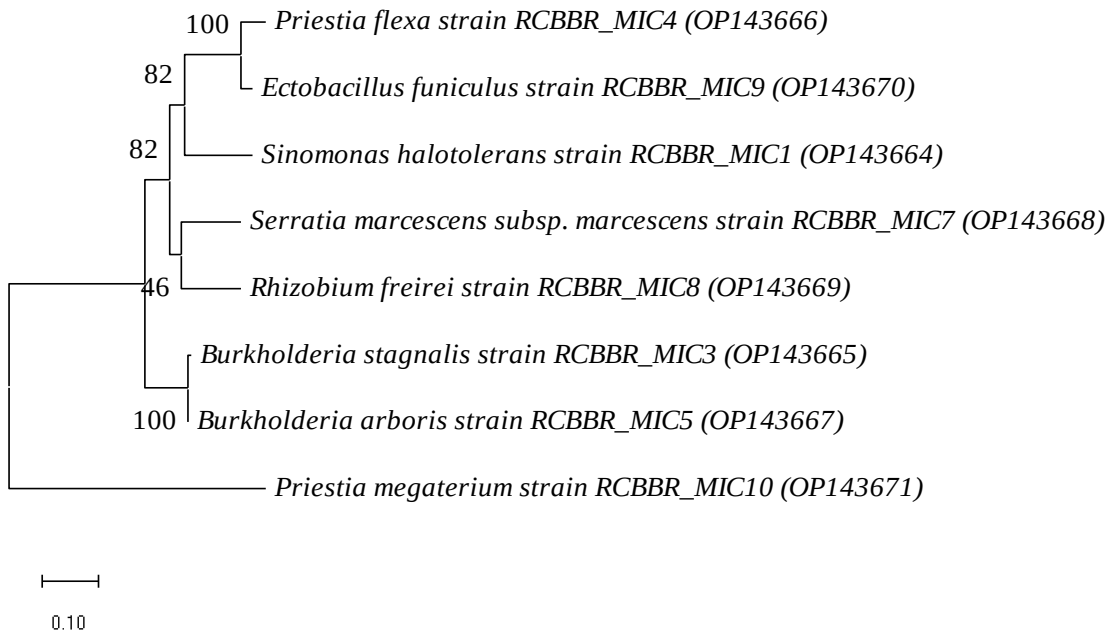

Figure 1: Phylogeny of the isolates

Below is the phylogenetic tree of *Sinomonas halotolerans* in combination with another replicate tree of different strain and accession number.

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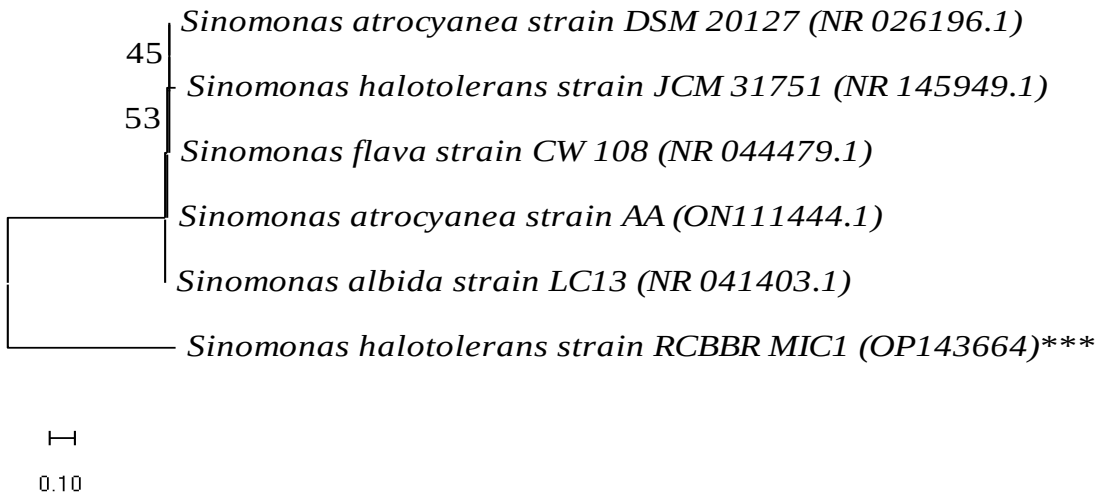

Figure 2: Phylogeny of the isolate RCBBR_MIC1

The figure below is the phylogenetic tree of *Burkholderia stagnalis* in combination with another replicate tree of different strains and accession numbers.

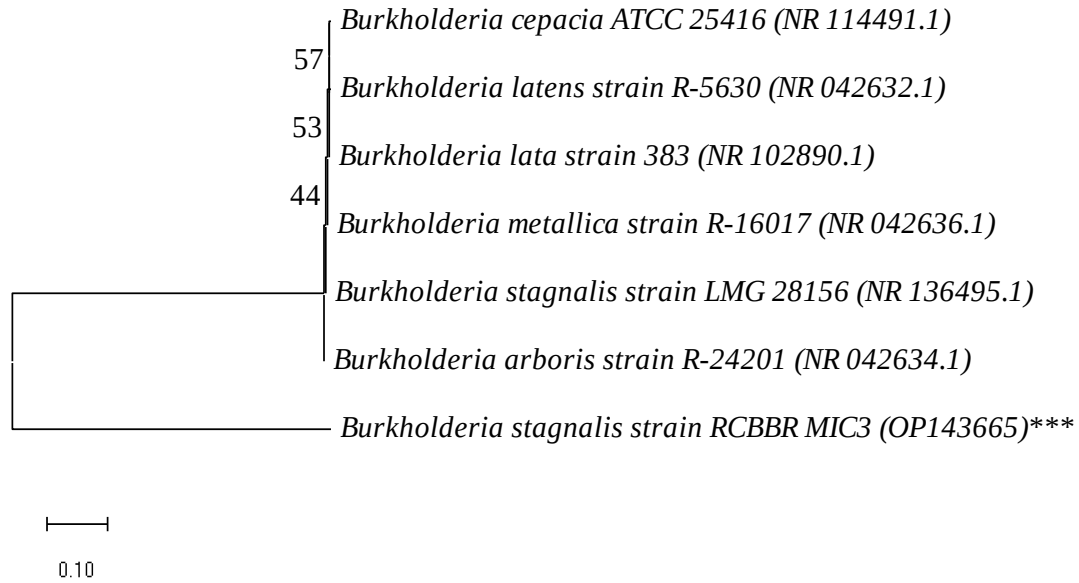

Figure 3: Phylogeny of the isolate RCBBR_MIC3

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The phylogenetic tree below shows *Priestia flexa* in combination with another replicate tree of different strains and accession numbers.

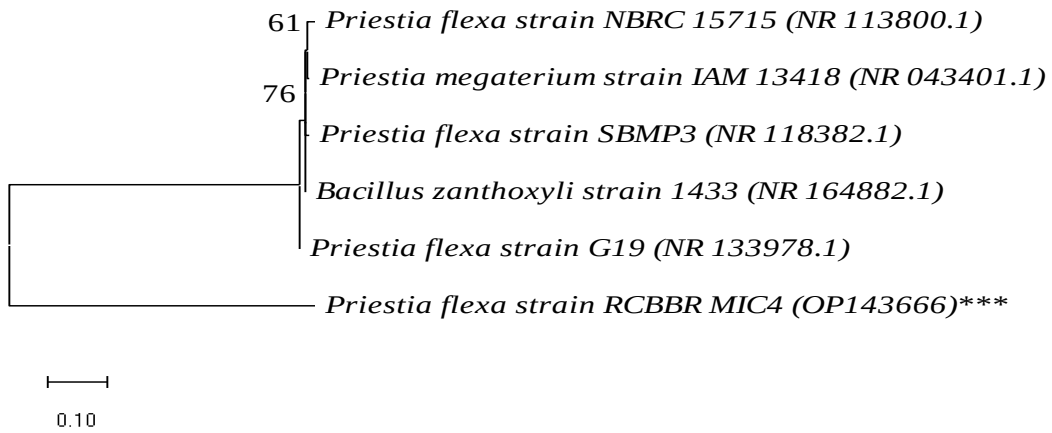

Figure 4: Phylogeny of the isolate RCBBR_MIC4

The figure below is the phylogenetic tree of *Burkholderia arboris* in combination with other replicate trees of different strains and accession numbers.

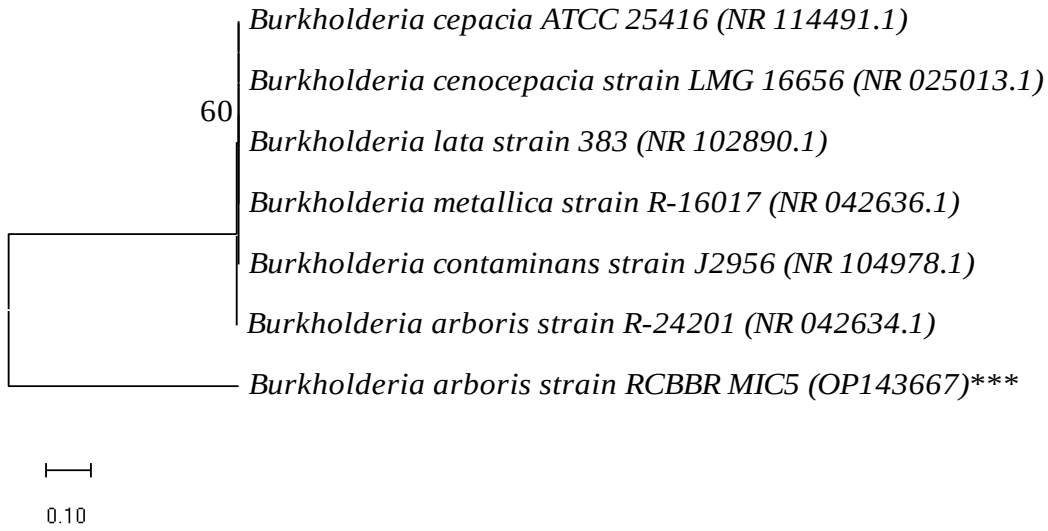

Figure 5: Phylogeny of the isolate RCBBR_MIC5

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The phylogenetic tree of *Serratia marcescens subsp. marcescens* in combination with other replicate trees of different strains and accession numbers is shown below.

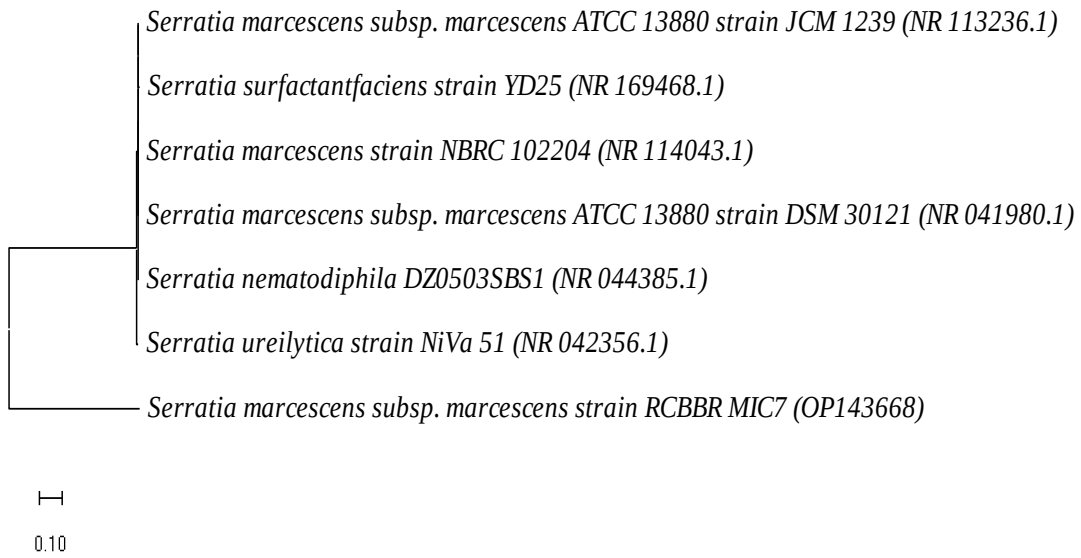

Figure 6: Phylogeny of the isolate RCBBR_MIC7

The figure below shows the phylogenetic tree of *Rhizobium freirei* in combination with other replicate trees of different strains and accession numbers.

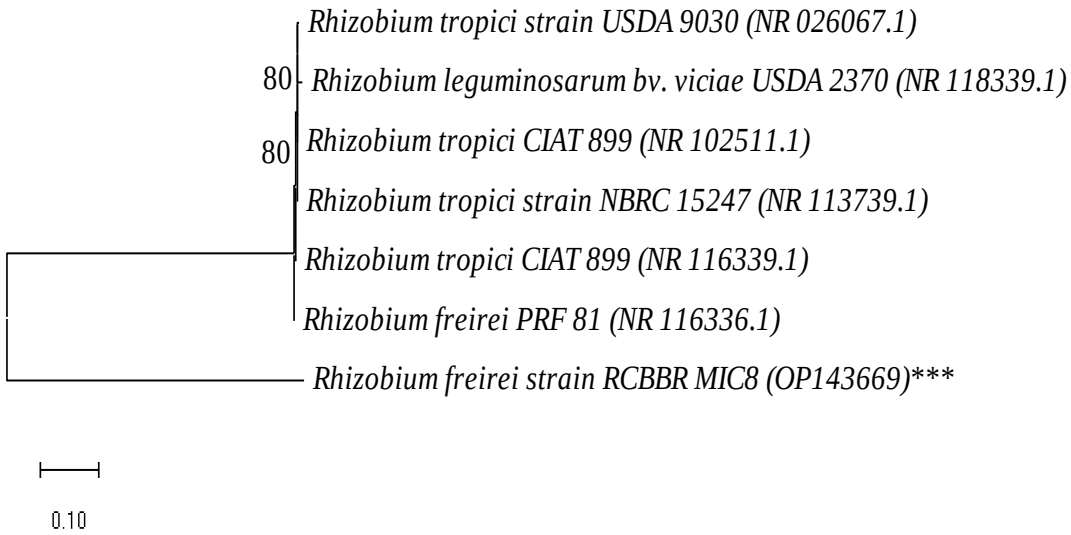

Figure 7: Phylogeny of the isolate RCBBR_MIC8

Ectobacillus funiculus strain is represented below with its phylogenetic tree and accession number.

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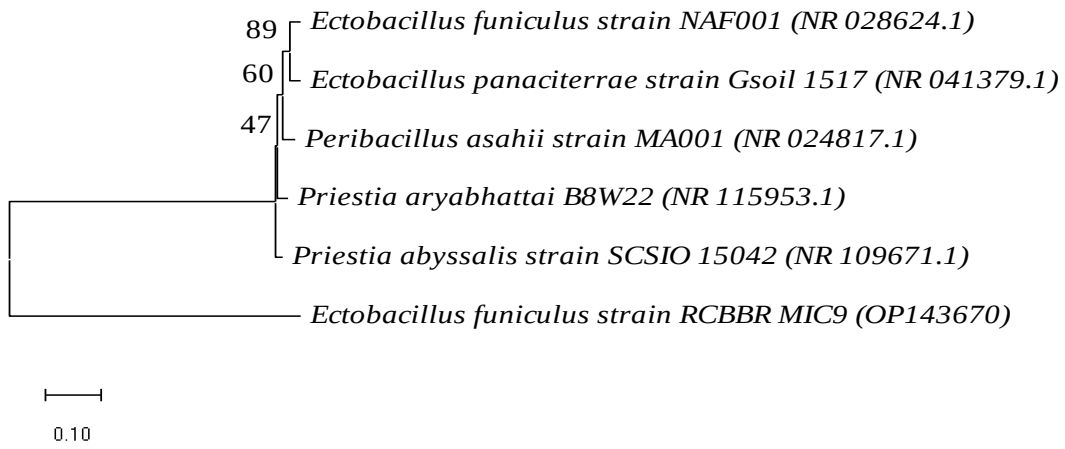

Figure 8: Phylogeny of the isolate RCBBR_MIC9

Below is the phylogenetic tree of *Priestia megaterium* in combination with other replicate trees of different strains and accession numbers.

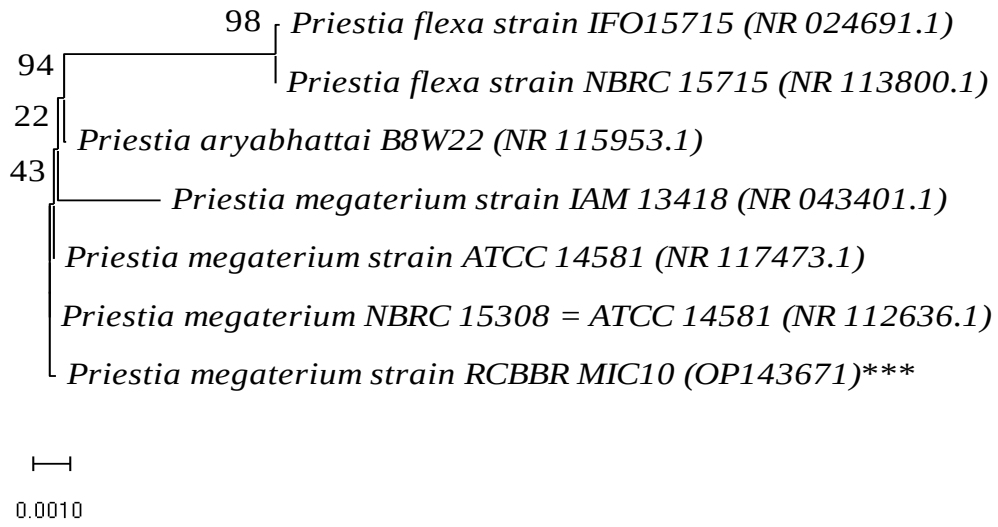

Figure 9: Phylogeny of the isolate RCBBR_MIC10

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The table below shows the statistical analysis using anova for the for the degradability test result of all the bacteria isolates.

Table 4: Degradability screen by different Bacterial Strains

BACTERIAL STRAINS	DAY 7 Mean±SD	DAY14 Mean±SD	DAY 21 Mean±SD	DAY28 Mean±SD
LM2*Sinomonas halotolerans*	.090±.08	.205±.21	.205±.21	.176±.18
LM4*Burkholderia stagnalis*	.105±.11	.215±.24	.215±24	.204±25
LM5 *Priestia flexa*	.078±.06	.224±.25	.224±25	.228±27
LM6*Burkholderia arboris*	.101±.11	.221±.23	.221±23	.293±33
LM8*Serratia marcescens subspmarcescens*	.093±.10	.283±.33	.283±.33	.233±35
LM9 *Rhizobium freirei*	.104±.12	.247±.24	.247±.24	.264±41
LM10 *Ectobacillus funiculus*	.073±.06	.224±.23	.224±.23	.293±37
LM11 *Priestia megaterium*	.08788±.07	.278±.28	.278±.28	.324±38
Control	.02938±.02	.039±.03	.039±.03	.036±03
F	.542	.686	.686	.610
P-value	.821	.702	.702	.766
Decission	NS	NS	NS	NS

LM2-LM11=different bacterial strain, SD=Standard Deviation, NS=Not Significant

The table further showed that there was no significant difference in the mean turbidity of the bacteria strains used for the isolate (f=.542, p=.827) for day 7, day 14, day 21 and day 28.

Table 3.5 below represents the statistical analysis of the degradation ability of all bacteria isolated about one another for all the hydrocarbon products carried out for four weeks.

Table 3.5: hydrocarbon-degradation potentials of the bacterial isolates

BACTERIAL STRAINS	PETROLEUM PRODUCT	DAY 7 Mean±SD	DAY14 Mean±SD	DAY 21 Mean±SD	DAY28 Mean±SD
Sinomonas halotolerans	DPK	.020±.000	.185±.233	.141±.171	.164±.203
	AGO	.145±.103	.265±.273	.242±.240	.249±.250
	USED OIL	.089±.118	.242±.317	.305±.405	.218±.282
	PMS	.105±.120	.013±.009	.132±.178	.076±..098
Burkholderia stagnalis	DPK	.164±130	.217±.205	.269±.352	.391±.524
	AGO	.089±.101	.119±.142	.326±.359	.202±.183
	USED OIL	.143±.104	.143±.194	.218±.282	.157±.196
	PMS	.023±004	.042±.031	.046±.057	.067±.086
Priestia flexa	DPK	.058±.053	.109±.053	.166±.207	.219±.281
	AGO	.086±.020	.199±.256	.359±.299	.195±.174
	USED OIL	.116±.138	.344±.478	.359±.482	.412±.557
	PMS	.116±.069	.088±.096	.086±.113	.088±.115
Burkholderia arboris	DPK	.098±.111	.151±.111	.181±.181	.293±.386
	AGO	.131±.083	.143±.176	.304±.304	.267±.275
	USED OIL	.164±..207	.224±.309	.409±.409	.466±.634
	PMS	.013±.009	.111±.128	.196±.196	.147±.200

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BACTERIAL STRAINS	PETROLEUM PRODUCT	DAY 7	DAY14	DAY 21	DAY 28
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Serratia marcescens subsp marcescens	DPK	.058±143	.173±.142	.195±.247	.229±.295
	AGO	.086±.089	.269±.354	.439±.519	.183±.156
	USED OIL	.116±.012	.238±.328	.388±.523	.512±.698
	PMS	.055±.154	.133±.160	.110±.147	.009±004
Rhizobium freirei	DPK	.058±.124	.170±.138	.329±.436	.345±.459
	AGO	.086±.056	.128±.155	.244±.243	.144±.101
	USED OIL	.116±.244	.249±.343	.238±.311	.570±.781
	PMS	.055±.002	.102±.115	.178±.243	-.001±.009
Ectobacillus funiculus	DPK	.058±.078	.231±.224	.300±..396	.416±.560
	AGO	.086±.137	.291±.386	.286±.302	.190±.166
	USED OIL	.116±.034	.165±.226	.215±.278	.480±.653
	PMS	.055±.039	.159±.197	.097±.129	.089±.117
Priestia megaterium	DPK	.078±.078	.238±.234	.366±.489	.471±.638
	AGO	.137±.137	.224±.291	.345±.386	.319±.350
	USED OIL	.034±.034	.202±.277	.202±.260	.424±.574
	PMS	.039±.039	.078±.082	.202±.260	.084±.110

BACTERIAL STRAINS	PETROLEUM PRODUCT	DAY 7	DAY14	DAY 21	DAY 28
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	DPK	.023±.023	.048±.033	.023±.004	.023±.004
	AGO	.070±.070	.043±.035	.069±.004	.066±.007
	USED OIL	.018±.018	.008±.002	.058±.056	.057±.055
	PMS	.006±.006	.006±.004	.006±.000	-.001±.009
F		.930	.507	..457	.450
P		.519	.847	.878	.882
Decision		NS	NS	NS	NS

SD=Standard Deviation, DPK=Kerosene, AGO=Diesel, PMS=Gasoline, NS=Not Significant

P= Probability, f= ANOVA calculated value. The table further showed that there is no significant difference in the degradability of the different petroleum products and the bacterial strains (p=.882) for day 7, day 14, day 21 and day 28.

The line graph below illustrates the degradation capabilities of the bacteria isolates in the different petroleum hydrocarbons on the seventh day of the composition.

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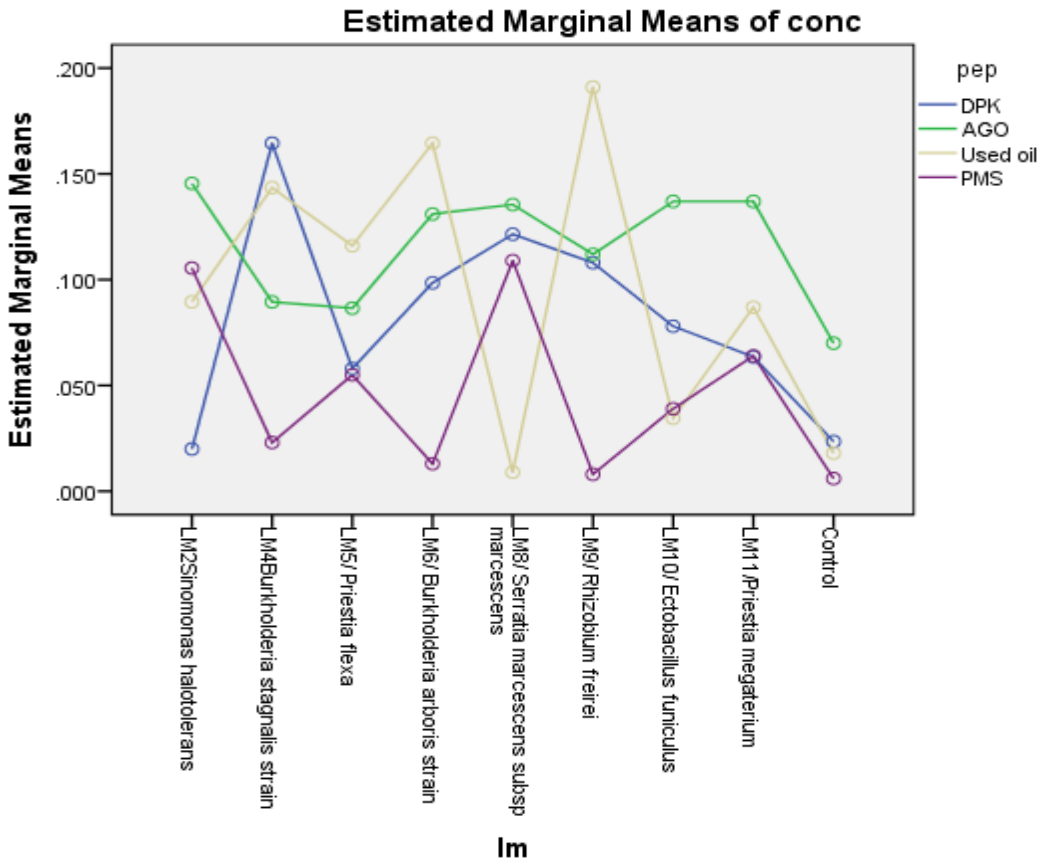

Fig. 10: Hydrocarbon degradability test for day 7

Sinomonas halotolerans had the highest rate of degradability for AGO and PMS, *Burholderia stagnalis* showed the highest degradability rate for DPK while for used oil, it was *Rhizobium freirei* making *Sinomonas halotolerant* dominant on day 7.

Below is a line graph to illustrate the degradation capabilities of the bacteria isolates in the different petroleum hydrocarbons on the fourteenth day of the composition.

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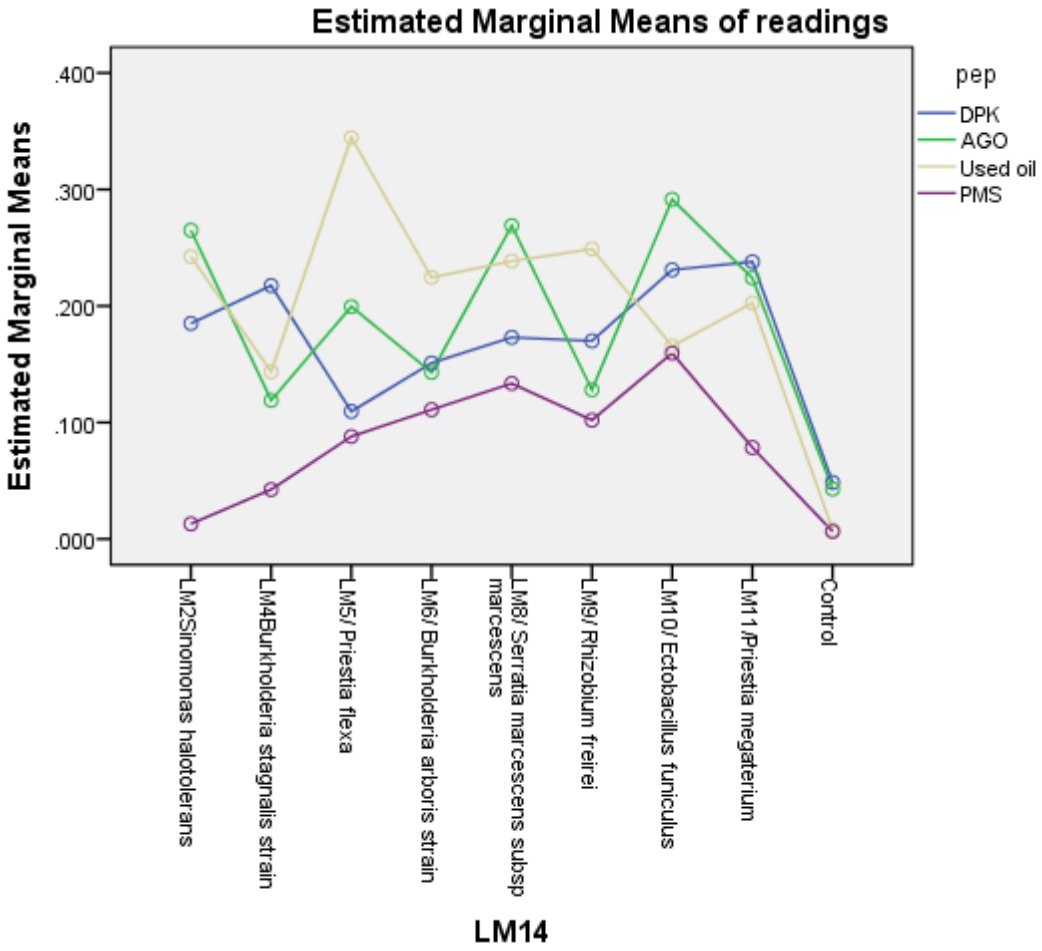

Fig. 11: Hydrocarbon degradability test for day 14

Priestia megatherium and *Priestia flexa* showed the highest degradability rate for DPK and used oil respectively while *Ectobacillus funiculus* proved to be highest for AGO and PMS making *Ectobacillus funiculus* is to be dominant on day 14.

Below is a line graph to illustrate the degradation capabilities of the bacteria isolates in the different petroleum hydrocarbons on the twenty-first day of the composition.

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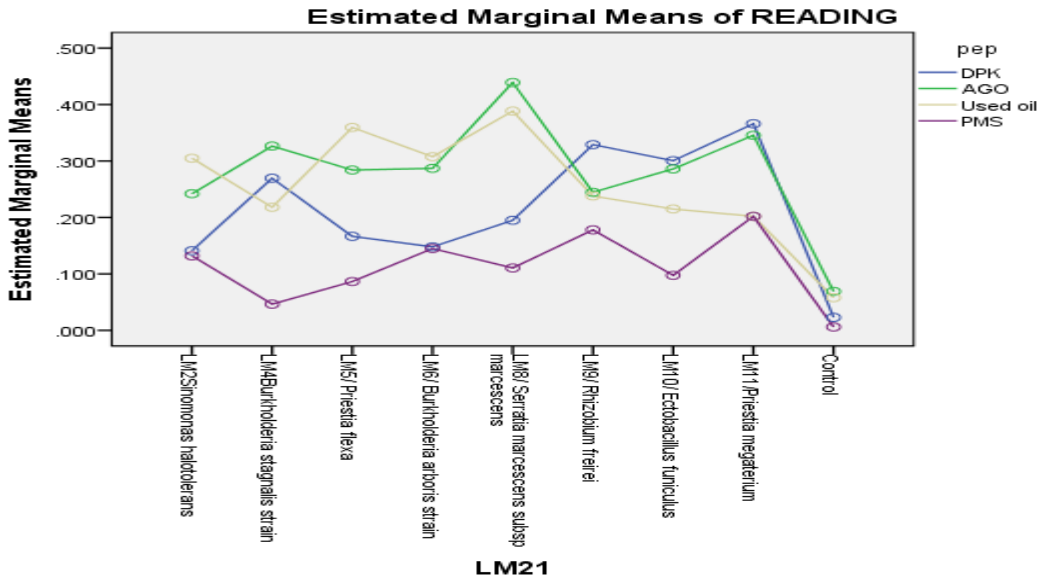

Fig. 12: Hydrocarbon degradability test for day 21

The figure above showed that for DPK and PMS, *Priestia megatarium* showed the highest degradability rate on day 21 Serratia marcescens subsp marcescens proved highest for AGO and for used oil making both bacteria to be dominant on day 21.

The line graph below illustrates the degradation capabilities of the bacteria isolates in the different petroleum hydrocarbon on the twenty eight day of the composition.

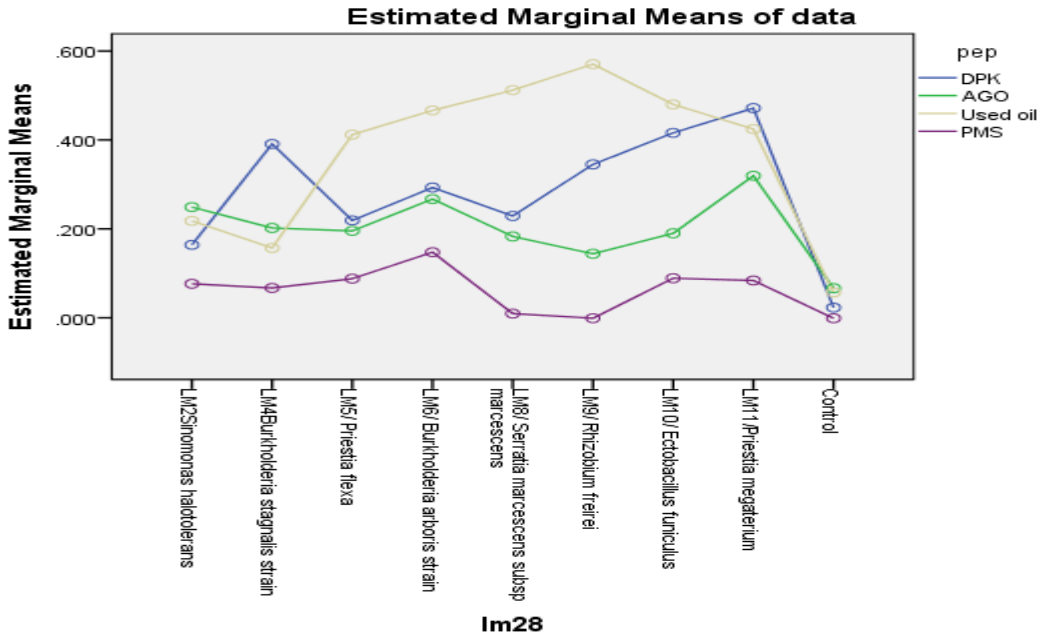

Fig. 13: Hydrocarbon degradability test for day 28

Priestia megatarium showed the highest degradability rate for DPK, AGO and used oil while for PMS, the bacteria with the highest degradability rate was *Burkholderia arboris* for day 28.

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Discussion

Soil has been known to be a conducive habitat for the proliferation of bacteria and other microorganisms. Soil samples that have been long contaminated with crude oil were investigated and the results revealed that they harbored a wide range of bacteria isolates. The presence of microbial activity was determined by the enumeration of total heterotrophic bacteria (THB) count which ranged from $4.5x10^3$cfu/ml in the first site in Alode (A1) as the least count to as high as $2.0x10^9$cfu/ml in Ogale control (Oc) site with a mean bacteria count of $1.0x10^9$(table 1). The results of the total heterotrophic bacteria count in this research were as high as that of Ogbonna et al. (2020) who reported THBC which ranged from $2.1x10^8$ to $2.5x10^8$ from crude oil-contaminated soil. Similarly, there was also an affirmation of the abundance of total heterotrophic bacteria in the soil as evidenced in the result of Onifade and Abubakar (2007) which ranged from 4.10x104 - 2.73x107. The reason for the high count of THB is most likely an indication and affirmation of the abundance of bacteria in the soil as in the environment as a whole.

The enumeration of hydrocarbon utilizing bacteria (HUB) count ranged from $8.8x10^5$cfu/ml for the Ogale control site to $3.32x10^6$cfu/ml for the Alode control site with a mean bacteria count of $2.5x10^6$ (table 2). This high hydrocarbon utilizing bacteria count was similar to the report given by Chikere and Ekwuabu (2014b) with a mean count of $0.5x10^6$ probably due to a similar source of contamination as both regions have suffered similar source of crude or refined oil contamination either by oil pipe rupture or due to sabotage. Ozoude et al (2017) also reported a wide range of HUB counts ranging from $1.8x10^4$ - $1.5x10^8$. This high count is possible as there is an abundant source of carbon resulting from the availability of the hydrocarbon contaminant. It has also been reported by most researchers like Onifade and Abubakar (2007) that residual crude oil in polluted soil enhances the carbon supply in the soil hence encouraging the growth of hydrocarbon-utilizing bacteria as compared to crude oil-free soil. However, there are also cases where HUB counts were lower compared to the findings in this research. $8.0x10^3$ - $5.0x10^4$ as reported by Ogbonna et al. (2020) which may be a function of the soil contamination that was carried out with a certain amount of crude oil to mimic the natural crude oil spill site after allowing to fallow for about 3 weeks.

The result of this research is in line with the report of Chikere and Ekwuabu (2014a) who also tentatively reported a number of the bacteria isolated here using their phenotypic appearance. There was a great level of contrast between this study and that of Ozoude et al. (2017) as only *Bacillus* sp was in common with this study in their morphological characterization. Environmental factors as well as the nature of contamination may be the reason for the great difference that was observed in the bacteria isolates that were reported. However, most research reports *Bacillus* sp was predominantly reported by researchers as a hydrocarbon utilize most probably due to its ability to produce spores and be able to survive in very harsh environments like food or nutrient scarcity and environmental conditions for a long period till conditions are favourable again.

Following the atomic portrayal strategy that was utilized to affirm the speculative personalities of bacterial detaches utilizing morphological and biochemical responses, the sub-atomic portrayal was considered since the utilization of 16S rRNA in the portrayal of hydrocarbon using microorganisms is more solid and delicate than culture-subordinate techniques alone (Babu et al., 2012; Al-Awadhi et al., 2013, Chikere & Ekwuabu, 2014a). After PCR enhancement of the 16S rRNA, quality part was acquired for all confines, the sub-atomic Distinguishing proof uncovers the singular microscopic organisms recognized to their separate strain levels by the Polymerase chain response (PCR) technique. Bacterial 16S rRNA arrangements were lined up with the Shoot calculation of the Public Community for Biotechnology Data (NCBI) data set. Arrangements adjusted showed 98-100 percent likeness to those saved in GenBank and they were: *Sinomonas halotolerans* strain RCBBR_MIC 1, *Burkholderia stagnalis* strain RCBBR_MIC 3, *Priestia flexa* strain RCBBR_MIC 4, *Burkholderia arboris* strain RCBBR_MIC 5, *Serratiamarcescens* strain RCBBR_MIC 7, *Rhizobium freirei* strain RCBBR_MIC 8, *Ectobacillusfuniculus* strain RCBBR_MIC 9 and *Priestiamegaterium* strain RCBBR_MIC10 .

These organisms are organisms that have specialized metabolic capabilities hence they were able to thrive in hydrocarbon-contaminated environments. This is an affirmation of the report previously given by Ozoude et al. (2017). The phylogenetic tree of the above-mentioned bacteria strains. *Sinomonas halotolerans* which was also isolated in this study is a novel strain of *Actinobacteria* which has similarly been isolated from soil (Guo et al., 2016), which may be a result of similar composition of the soil materials which enabled its isolation of the novel bacterium. *Burkholderia stagnalis and Burkholderia arboris* are most commonly misidentified as *Pseudomonas*, particularly by researchers who only stop at identifying bacteria morphologically and biochemically (Onifade & Abubakar, 2007, Varjani et al.,

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2013, Ozoude et al., 2017, Ebakota et al., 2017, Ogbonna et al., 2020). Only a few researchers who go beyond the culture-dependent level to the genomic level can identify these bacteria as they are. *Priestia megaterium* and *Priestia flexa* are also commonly identified morphologically as *Bacillus* species. The *Priestia* is the recent genus name for the *Bacillus* genus. *Ectobacillusfuniculus* also originally belonged to the genus *Bacillus* which normally shows a diverse range of biochemical characteristics which makes their identification more complex but with the advent of genomic studies, their identification is a lot easier. In conclusion, the increase in turbidity in the composition indicates that the inoculated bacteria isolates were able to utilize the hydrocarbon for their cellular activities which further implies that the bacteria isolates could be used for bioremediation purposes.

Conclusion

The study found that soil contaminated with crude oil harbors a diverse range of bacteria capable of metabolizing hydrocarbons. Molecular analysis identified specific bacterial strains with potential for bioremediation. The findings support using these bacteria for mitigating crude oil contamination in soil.

Recommendation

The study therefore recommends that these bacteria be used for bioremediation of hydrocarbon-contaminated soils in Eleme and other regions and that further studies be carried out on these bacteria to the benefit of man bioremediation.

References

Abu G. O., & Dike P. O. (2008). A study of natural attenuation process involved in a microcosm model of a crude oil-impacted wetland sediment in the Niger Delta. *Biological Technology* 4761 - 4767.

Adati, A. K. (2012). Oil exploration and spillage in Niger Delta of Nigeria.*Civil Environmental Restoration.2*,2222 - 2863.

Al-Awadhi, H., Dashti, N., Khanafer, M., & Radwan, S. (2013). Bias problems in culture independent analysis of environmental bacterial communities: a representative study on hydrocarbonoclastic bacteria. *SpringerPlus.2*, 369

Babu, K.S., Jyothi, K., Nancy, C.K.. & Kashyap, A. (2012). Identification and isolation of hydrocarbon-degrading bacteria by molecular characterization. *Helix. 2*, 105-111.

Chikere, C. B., & Ekwuabu, C. B. (2014a). Molecular characterization of autochthonous hydrocarbon utilizing bacteria in oil-polluted site at Bodo community, Ogoni land, Niger Delta, Nigeria. *Nigerian Journal of Biotechnology, 27*, 28-33.

Chikere, C. B., & Ekwuabu, C. B. (2014b). Culture dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. *Africa journal of Environmental Science and Technology 8*(6), 401-406.

Ebakota, O. D., Osarueme, J. O., Gift, O. N., Odoligie, I., & Joseph, O. O. (2017). Isolation and characterization of hydrocarbon-degrading bacteria in top and subsoil of selected mechanic workshop in Benin City metropolis, Nigeria. *Journal of applied science and environmental management 21*(4), 641-645.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39, 783-791.

Guo. Q. Q., Ming, H., Meng, X. L., Huang, Jr., Duan, Y. Y., Li, S. H., Li, S., Zhang, J. X., Li,W. J.,& Nie, G. X. (2016). *Sinomonas halotolerans* specie, a novel *Actinobacterium* isolated from soil sample. *Antonie Van Leeuwenhoek 108*, 887-895.

Holt, J. G., Kreig, N. R., Sneath, P. H., Stanley, J. T. & Stanley, S. T. (1994). Bergey's manual of determinative bacteriology 9th Edition. William and Wilkins, Baltimore, USA. 45-98.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In Munro HN, editor, *Mammalian Protein Metabolism*, 21-132, Academic Press, New York.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547-1549.

Ndubuisi-Nnaji U. U., John, O.U.M. &Ofon, U. A. (2015). Population dynamics and distribution of hydrocarbon-utilizing bacteria in Automobile workshop within Uyo metropolis Akwa Ibom State. *Journal of Applied Science and Environmental Management. 19*(4), 585-589.

Ogbonna, D. N., Douglas, S. I. &Awari, V. G. (2020). Characterization of Hydrocarbon Utilizing Bacteria and Fungi Associated with Crude oil contaminated soil. *Microbiology Research Journal* 30(5), 54-69.

Onifade, A. K. & Abubakar, F. A. (2007). Charaterization of Hydrocarbon-degrading microorganisms isolated from crude oil contaminated soil and remediation by enhanced natural attenuation. *Research Journal of Microbiology. 2*(2), 149-155.

***Cite this article as*:**
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Ozoude, T. O., Eleanya, E. U., Uzoaru, N. S., & Okey-Ndeche, N. F. (2017). Isolation and characterization of some hydrocarbon utilizing bacteria isolated from contaminated soil in Zuma, Bwari Area Council, FCT Abuja, Nigeria. *Microbiology Research Journal International, 22*(6), 1-8.

Prescott, L. M., Harley, J. P. & Klein, D. A. (2005). Microbiology. 6th Edition, McGraw Hill, London. 23-67.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406-425.

Varjani, S. J., Rana, D. P., Bateja, S.,&Upasani, V. N. (2013). Isolation and screening for Hydrocarbon Utilizing Bacteria (HUB) from Petroleum samples. *International Journal of Current Microbiology and Applied Science* 2(4), 48-60.

Vincent, A. O., Felix, E., Weltimore, M.O., Ise-Iyamu, O. K.,& Daniel, E.E. (2011). Microbial degradation and its kinetics on crude oil polluted soil. Res. J. Chem. Sci. *1*(6), 8-14.