Int.J.Curr.Microbiol.App.Sci (2019) *8*(6): 1291-1302

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 06 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.806.156

Evaluation of Native Bacterial Consortium from Crude Oil-Impacted Tropical Environment for Integration into Bioremediation Process

Olusola A. Ojo-Omoniyi[1, 2*] and Rebecca O. Abegunrin[1]

[1]*Department of Microbiology, Faculty of Science, Lagos State University, Lagos-Nigeria*
P. M. B. 0001 LASU Post office, Lagos-Nigeria
[2]*Department of Biological Sciences, Covenant University, Ota, Nigeria*

**Corresponding author*

Keywords

Antibiotic-resistance, Bacterial consortium, Biodegradation, Bioremediation, Petroleum hydrocarbon, Plasmid DNA

Article Info

Accepted:
12 May 2019
Available Online:
10 June 2019

A B S T R A C T

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. Crude oil-impacted tropical soil and natural water samples (0 - 30cm depth) were obtained from the Niger Delta, Nigeria. A total of twenty seven bacterial species of relevance in bioremediation were isolated and characterized using standard and conventional methods. The predominant species belong to the genera *Pseudomonas* and *Proteus*. The ability of *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Proteus mirabilis*, *Serratia marcescens* and *Bacillus subtilis* to degrade diesel oil was studied. The results showed maximal increase in optical densities and total viable counts, however, with decrease in pH of the culture media after 8 - day incubation period. Typical generation times varied between 0.89 and 4.0d for *Pseudomonas aeruginosa*, 0.85 and 0.32d for *Pseudomonas stutzeri*, 0.8 and 3.9d for *Proteus mirabilis*. All the isolates utilized petroleum hydrocarbon as sole carbon and energy sources for growth on Minimal Salt Medium (MSM) supplemented with (0.2%) diesel oil. The isolates were tested against 7 different antibiotics and were found to be resistant to 5 antibiotics. Consequently, Twenty four isolates were randomly selected for plasmid DNA isolation. The presence of plasmid DNA was confirmed in 22 isolates where the molecular weight size ranged between 7 – 23.1kb. Although, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus subtilis* had low specific growth rate on Diesel, they were the best choice for bioremediation since they had the highest mean generation time respectively. The knowledge of the potentials of these isolates to degrade hydrocarbons will increase the possibilities of developing novel strains and strategies for removing hydrocarbon pollutants from the natural environment.

Introduction

Oil and gas companies operating in Nigeria burn over $3.5 to $5 billion yearly from the over 257 flow stations in the Niger Delta. Specifically the country flared about 17.15 percent of the 95.471 metric tons of gas produced in June, 2015 alone, according to

data from Nigerian National Petroleum Corporation (NNPC) (Anonymous, 2015). The Nigerian environment is characterized by lack of concern, indiscriminate and highly unregulated disposal of petroleum products including diesel and engine oil (Odjegba and Sadiq, 2000). However, increased demand for petroleum as a consequence of industrialization has greatly increased the background level of exposure of the environment to different hydrocarbons (Odell, 2013). However, these compounds are slowly degraded by a large variety of microorganisms. All the activities in the oil industries, including exploration, extraction, transportation, refining and oily waste management, are possible sources of environmental pollution (Hu *et al.*, 2013). The US EPA has classified these compounds as priority contaminants of natural resources (Husain, 2008).

The effluents of petroleum wells, oil refinery operations, industrial wastewaters, fuel consumption, wood-processing activities, detergents, pesticides, paints and other chemicals release huge quantities of hydrocarbons into soil and air (Rosenberg, 2013). Crude oils contain a mixture of different low- and high-molecular-weight aliphatic hydrocarbons and several types of monocyclic and polycyclic aromatic compounds, in which one or more of the carbon atoms in their backbone can also be substituted by Nitrogen, Sulphur or Oxygen. The length and nature of hydrocarbons varies in oil-derived compounds from $C_1 – C_{10}$, such as make up gasoline (Jimeson *et al.*, 2012); $C_9–C_{16}$, such as are found in jet fuel and kerosene (Chou *et al.*, 2002); and $C_{10}–C_{50}$, such as waste oil (Monserud and Schwartz, 2012). On the other hand, metabolism of hydrocarbons by microorganisms is a relatively complex process in which the microorganisms first take up the hydrocarbons and then convert these metabolically inactive molecules to more active forms for further catalysis (Ladygina *et al.*, 2006).

To determine the potentials of the bacterial consortium at utilizing petroleum hydrocarbon as source of carbon and energy for growth

To determine if the metabolic capabilities of the consortium were plasmid-mediated

To evaluate the potentials of native bacterial consortium for integration into bioremediation strategy for a tropical crude oil-impacted environment.

Materials and Methods

Sample collection

Crude oil - polluted soil and water samples were obtained from Lagos and River States in Nigeria in an earlier study, from which twenty - seven petroleum - hydrocarbon utilizers were isolated. These isolates were subsequently cultured on bacteriological media supplemented with Diesel oil at 0.2%.

Bacteriology

The isolates were later sub-cultured on different selective media and Nutrient agar in order to develop pure cultures and identify the bacterial cells.

The pure cultures of bacterial cells were thereafter grown on Mueller Hinton Agar for plasmid extraction and antibiotic sensitivity testing at 26 ± 2º C for 24 hours (Bridson and Brecker, 1970; Olukoya and Oni,1990).

Minimal salt medium

The Minimal Salt Medium (MSM) contained: 12.8g $Na_2HPO_4.7H_2O$, 6g Na_2HPO_4, 3g KH_2PO_4, 0.5g Nacl, 1g NH_4cl, 200ml micronutrients and 0.2% carbon source. The

pH of the medium was adjusted to 7.4 with NaOH before autoclaving (Mills *et al.*, 1978).

Micromorphological and biochemical characterization of isolated bacteria

All isolates were examined and characterized using standard and conventional methods. The biochemical tests used for characterization of isolates includes; Gram staining, carbohydrate fermentation tests, catalase test, Kligler-Iron agar(KIA), Indole test, Oxidase test, Motility test, Methyl Red test, Voges Proskauer test (Gerhardt *et al.*, 1981; Holt *et al.*, 1994; Isenberg, 2004).

Growth of bacteria isolates on hydrocarbon substrate

The Time - course degradation of diesel oil was performed using Minimal salts medium (Mills *et al.*, 1978). The medium was dispensed in 99ml quantity into 250ml Erlenmeyer flasks. Each flask was supplemented with 1ml of diesel as the carbon source.

The flasks were cultivated in a gyratory shaker incubator at 150rpm and 28^{o}C for 8days. The turbidity by optical density measurement (OD600nm) using spectrophotometer (Spectrum lab S23A, Globe Medical, England), total viable count (TVC) and pH of the culture fluids were monitored at pre - determined time intervals as biodegradation indices (Mills *et al.*, 1978).

Antibiotic susceptibility test

Sensitivity and resistance of isolate to antimicrobial agents was determined on Mueller Hinton agar, using the disc diffusion technique (Kasper *et al.*, 1990). Bacteria strains resistant to the antibiotics grew up to the edges of the disc while the growth of those susceptible to the antibiotics was inhibited.

Isolation of plasmid

The bacteria isolate was inoculated into 2ml of trypticase soy broth and grown overnight at 37^{o}C in the shaker. 1.5ml of the overnight culture was transferred to 1.5ml micro - centrifuge tube. It was centrifuged at 10,000rpm for 5 minutes. The supernatant was discarded and the liquid drained by inverting on the blotting paper. The tube was kept on ice. The cell pellet was re-suspended in 100µl of ice cold solution I (50mM Glucose, 25mM Tris; pH 8.0, 10mM EDTA) and mixed by vortexing gently. It was kept at room temperature for 5 minutes. 200µl of solution II (0.2M NaOH, 1% SDS) was added and gently mixed by inverting the tube 5 times. 150µl of solution III (3M potassium acetate, pH 5.5) was added and gently mixed, the tube was placed on ice for 5 minutes. It was centrifuged at 10, 000 rpm for 10 minutes. The supernatant was transferred into a fresh tube and 450µl of isopropanol was added, the content was mixed by inverting the tube. It was centrifuged at 10,000rpm for 10minutes. The supernatant was removed carefully and discarded. The DNA was seen as white precipitate sticking to the wall of the tube. 1ml of 70% ethanol was added to wash the pellet, the contents was mixed by inverting and centrifuged at 10, 000 rpm for 10 minutes. The supernatant was removed completely and the pellet dried by keeping the cap of the tube open. 30µl of autoclaved distilled water was added and gently mixed by tapping the sides of the tube to dissolve the DNA. It was allowed to stand at room temperature with intermittent mixing for 15 – 20 minutes. The DNA was checked by 0.8% agarose gel electrophoresis by loading 5µl of DNA sample (Kado and Liu, 1981; Sambrook *et al.*, 1989; Olukoya and Oni, 1990).

Results and Discussion

Pseudomonas species have been reported by several authors to have capability for

mineralizing petroleum hydrocarbons especially in environment receiving petroleum waste discharges (Scoboszlay *et al.,* 2003; Okoh and Trejo-Hernandez, 2006; Das and Mukherjee, 2007). This corroborated our findings from the degradation – Time course study. Typical doubling times on diesel ranged from 0.89 - 4.0d for *Pseudomonas aeruginosa*, 0.85 - 0.32d for *Pseudomonas stutzeri;* 0.8 - 3.9d for *Proteus mirabilis* (Table 4). *Pseudomonas aeruginosa*, *Proteus mirabilis and Bacillus subtilis* were the best choice for bioremediation because they had low specific growth rate which suggested that the organisms were making use of the diesel oil as their source of carbon and energy (Fig. 1–7 and Table 1–8).

Table.1 Bacterial isolates screened for petroleum- hydrocarbon utilization

Bacteria	Frequency (%)
Pseudomonas aeruginosa	**8 (29.6)**
Pseudomonas stutzeri	**6 (22.3)**
Proteus mirabilis	**8 (29.6)**
Serratia marcescens	**2 (7.4)**
Escherichia coli	**1 (3.7)**
Bacillus subtilis	**2 (7.4)**
Total	**27 (100)**

Table.2 Biochemical characteristics of the bacterial isolates screened for petroleum-hydrocarbon utilization

Biochemical characteristics	N (%)
Gram reaction	
+	**2 (7.4)**
-	**25 (92.6)**
Lactose fermentation	**3 (11.1)**
Glucose fermentation	**2 (7.4)**
Sucrose fermentation	**27 (100)**
Arabinose fermentation	**24 (81.5)**
Urease production	**22 (81.5)**
Oxidase production	**14 (51.9)**
Pyocyanin production	**14 (51.9)**
Growth at 42^{o}C	**16 (59.3)**
Growth in 10% NaCl	**12 (44.4)**
Indole production	**3 (11.1)**
Arginine utilization	**16 (59.3)**
Lysine utilisation	**10 (37)**
Catalase production	**25 (92.6)**
Hydrogensulphide production	**8 (29.6)**
Gas from sucrose fermentation	**25 (92.6)**
Voges-Proskauer	**2 (7.4)**
Methyl red	**24 (88.9)**

Table.3 The growth potential of hydrocarbon utilizing bacteria screened

Isolates	Hydrocarbon source	
	Diesel	
	m (d^{-1})[a]	T(d)[b]
Pseudomonas aeruginosa01	0.72	2.4
Pseudomonas aeruginosa02	0.75	2.2
Pseudomonas aeruginosa03	1.05	0.89
Pseudomonas aeruginosa04	0.66	3.4
Pseudomonas aeruginosa05	0.62	3.8
Pseudomonas aeruginosa06	0.82	2
Pseudomonas aeruginosa07	0.75	2.2
Pseudomonas aeruginosa08	0.63	4
Pseudomonas stutzeri01	0.91	1.5
Pseudomonas stutzeri02	0.68	3.2
Pseudomonas stutzeri03	0.74	2.3
Pseudomonas stutzeri04	1.06	0.88
Pseudomonas stutzeri05	1.05	0.89
Pseudomonas stutzeri06	1.08	0.85
Proteus mirabilis 01	1.10	0.8
Proteus mirabilis 02	0.69	3
Proteus mirabilis 03	0.73	2.1
Proteus mirabilis 04	0.74	2.1
Proteus mirabilis 05	0.85	1.8
Proteus mirabilis 06	0.69	3.2
Proteus mirabilis 07	0.82	1.9
Proteus mirabilis 08	0.64	3.9
Serratia marcescens01	0.69	3.2
Serratia marcescens02	0.72	2.4
Escherichia coli 01	0.71	2.3
Bacillus subtilis 01	0.59	4.2
Bacillus subtilis 02	0.52	4.4

a - specific growth rate, b - mean generation time

Table.4 Antibiogram profile of bacterial isolates

	AMP	CAZ	OFL	AUG	NIT	GEN	CRX	MAR index
Pseudomonas aeruginosa 01	R	R	S	S	S	S	R	0.42
Pseudomonas aeruginosa 02	R	R	S	S	S	S	S	0.29
Pseudomonas aeruginosa 03	R	S	R	S	R	S	S	0.42
Pseudomonas aeruginosa 04	R	R	S	S	S	S	S	0.29
Pseudomonas aeruginosa 05	R	S	S	S	R	S	S	0.29
Pseudomonas aeruginosa 06	R	S	S	S	S	S	S	0.14
Pseudomonas aeruginosa 07	R	S	S	S	R	S	R	0.42
Pseudomonas aeruginosa 08	R	S	S	S	S	S	S	0.14
Pseudomonas stutzeri 01	R	S	S	S	S	S	S	0.14
Pseudomonas stutzeri 02	S	S	S	S	S	S	S	0
Pseudomonas stutzeri 03	S	S	S	S	S	S	S	0
Pseudomonas stutzeri 04	R	R	S	S	S	S	S	0.29
Pseudomonas stutzeri 05	R	S	S	S	S	S	S	0.14
Pseudomonas stutzeri 06	R	S	S	S	S	S	S	0.14
Proteus mirabilis 01	R	S	S	S	S	S	S	0.14
Proteus mirabilis 02	S	S	S	S	S	S	S	0
Proteus mirabilis 03	R	S	S	S	S	S	S	0.14
Proteus mirabilis 04	R	S	S	S	S	S	S	0.14
Proteus mirabilis 05	R	S	S	S	R	S	S	0.29
Proteus mirabilis 06	R	S	S	S	S	S	S	0.14
Proteus mirabilis 07	R	S	S	S	S	S	S	0.14
Proteus mirabilis 08	S	S	S	S	S	S	S	0
Serratia marcescens01	R	R	S	S	S	S	R	0.42
Serratia marcescens 02	S	S	S	S	S	S	S	0
Escherichia coli	R	S	S	S	S	S	S	0.14
Bacillus subtilis 01	S	S	S	S	S	S	S	0
Bacillus subtilis 02	S	S	S	S	S	S	S	0

Key:
R – Resistance
S – Sensitive
MAR index – ratio of number of antibiotic resisted to number of antibiotics tested.
AMP - Ampicillin
CAZ - Cefrihzidime
OFL - Ofloxacin
AUG - Clavulanate
NIT – Nitrofurantoin
GEN – Gentamicin
CRX - Ceftriroxime

Table.5 Antibiotic resistance of the bacterial isolates

Antibiotics	*P. aeruginosa* (N – 8)	*P. stutzeri* (N – 6)	*Proteus mirabilis* (N – 8)	*Serratia marcescens* (N – 2)	*E. coli* (N – 1)	*Bacillus subtilis* (N – 2)	Total (%)
AMP	8 ((100%)	4 (66.6%)	6 (75%)	1(50%)	1 (50%)	0	20 (74.07%)
CAZ	3 (37.5%)	1 (16.66%)	0	1 (50%)	0	0	5(18.51%)
OFL	1 (12.5%)	0	0	0	0	0	1(3.70%)
AUG	0	0	0	0	0	0	0
NIT	3 (38%)	0	1 (12.5%)	0	0	0	4 (14.81%)
GEN	0	0	0	0	0	0	0
CRX	2 (25%)	0	0	1 (50%)	0	0	3 (11.11%)

KEY:
AMP – Ampicillin
CAZ – Cefrihzidime
OFL – Ofloxacin
AUG – Clavulanate
NIT – Nitrofurantoin
GEN – Gentamicin
CRX – ceftriroxime

Table.6 Antibiotic resistance pattern of the bacterial isolates to various antibiotics

Drug resistant pattern	Number of antibiotics	*Pseudomonas aeruginosa*	*Pseudomonas stutzeri*	*Proteus mirabilis*	*Serratia marcescens*	*Escherichia coli*	*Bacillus subtilis*	Total (%)
AMP, CAZ, CRX	3	1	0	0	1	0	0	2 (28.6%)
AMP, CAZ	2	2	1	0	0	0	0	3 (42.9%)
AMP, OFL, NIT	3	1	0	0	0	0	0	1 (14.3%)
AMP, NIT	2	1	0	1	0	0	0	2 (28.9%)
AMP	1	2	3	5	0	1	0	11 (157.1%)
AMP, NIT, CRX	3	1	0	0	0	0	0	1(14.3%)

KEY:
AMP – Ampicillin
CAZ – Cefrihzidime
OFL – Ofloxacin
AUG – Clavulanate
NIT – Nitrofurantoin
GEN – Gentamicin
CRX - ceftriroxime

Table.7 Plasmid profile result of the isolate

Lane	Isolate	No. of plasmids	Size, kb
Lane 1	1 kb DNA marker	10	10 – 1 kb
Lane 2	*Proteus mirabilis 01*	2	23.1, 15
Lane 3	*Proteus mirabilis 02*	1	15
Lane 4	*Proteus mirabilis 03*	3	23.1, 15, 7
Lane 5	*Proteus mirabilis 04*	1	15
Lane 6	*Proteus mirabilis 05*	0	0
Lane 7	*Proteus mirabilis 06*	3	23.1, 17, 7
Lane 8	*Proteus mirabilis 07*	3	23.1, 15, 7
Lane 9	*Proteus mirabilis 08*	3	23.1, 15, 7
Lane 10	*Serratia marcescens 01*	0	0
Lane 11	*Serratia marcescens02*	1	15
Lane 12	*Escherichia coli*	1	15
Lane 13	*Escherichia coli*	1	15

Table.8 Plasmid profile result of the isolates

Isolate	Lane	No. of plasmids	Size, kb
Pseudomonas aeruginosa 01	2	2	10, 9
Pseudomonas aeruginosa 02	3	2	10, 9
Pseudomonas aeruginosa 03	4	1	10
Pseudomonas aeruginosa 04	5	3	10, 9, 7
Pseudomonas aeruginosa 05	6	3	10, 9, 7
Pseudomonas aeruginosa 06	6	3	10, 9, 7
Pseudomonas aeruginosa 07	7	3	10, 9, 7
Pseudomonas aeruginosa 08	8	3	10, 9, 7
Pseudomonas stutzeri 01	9	1	10
Pseudomonas stutzeri 02	10	2	10, 9
Pseudomonas stutzeri 03	11	1	10
Pseudomonas stutzeri 04	12	3	10, 9, 7

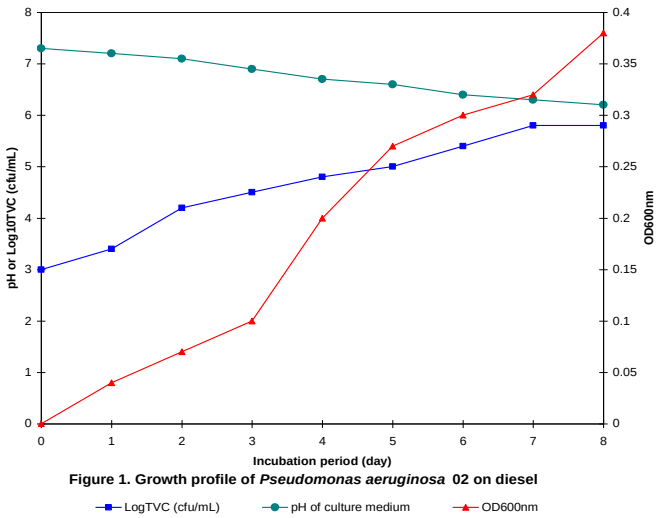


Figure 1. Growth profile of *Pseudomonas aeruginosa* 02 on diesel

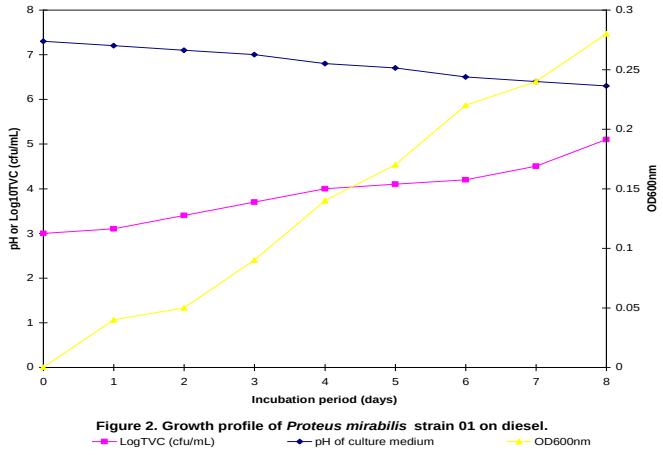


Figure 2. Growth profile of *Proteus mirabilis* strain 01 on diesel.

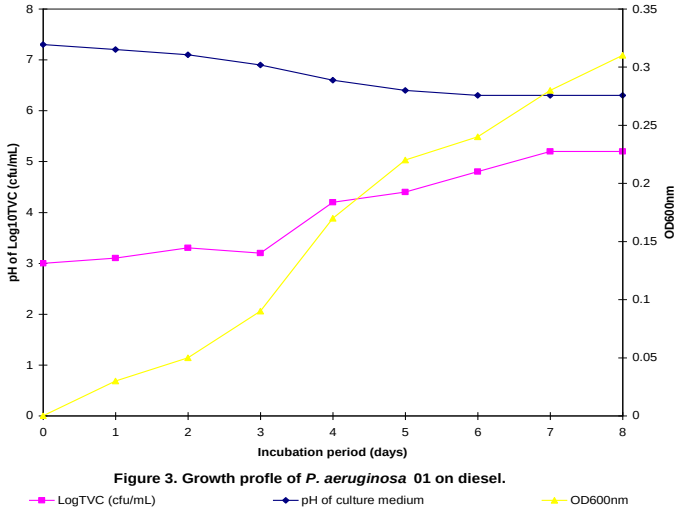


Figure 3. Growth profile of *P. aeruginosa* 01 on diesel.

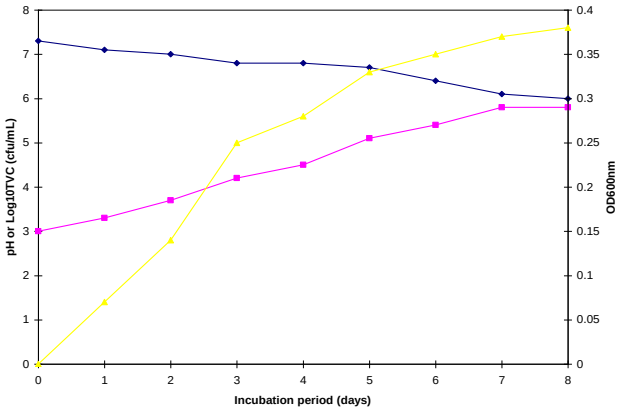


Figure 4. Growth profile of *Pseudomonas stutzeri* on diesel.

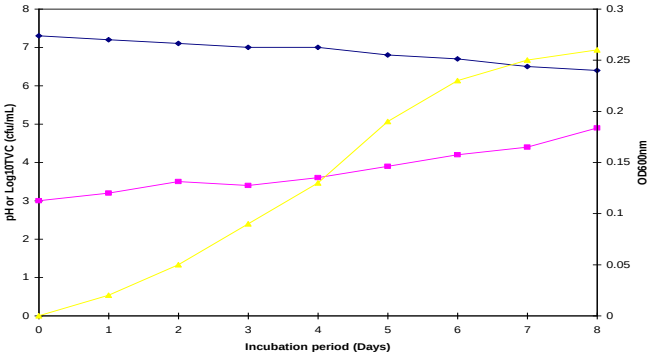
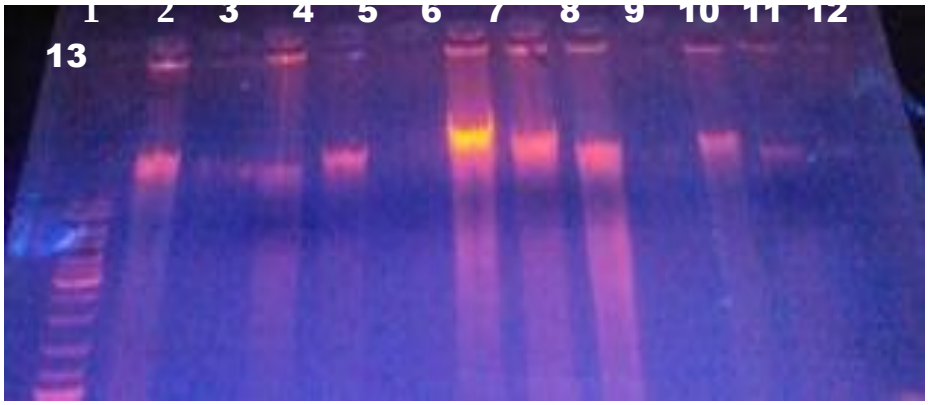


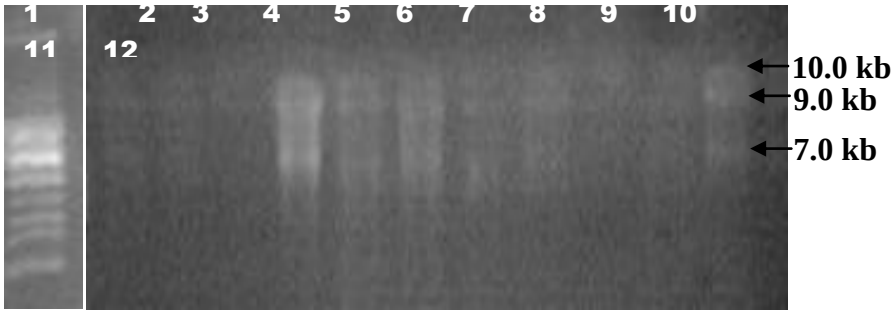
Figure 5. Growth profile of *Bacillus subtilis* strain 01 on diesel

Fig.6 Plasmid profile of hydrocarbon degrading bacterial isolates



Lane 1 – DNA marker, Lane 2to 9 – *Proteus mirabilis*, Lane 10 to 11 – *Serratia marcescens*, Lane 12 to 13 – *Escherichia coli*

Fig.7 Plasmid profile of bacterial isolates



Lane 1 – DNA marker, Lane2 to 8 – *Pseudomonas aeruginosa*, Lane 9 to 12 – *Pseudomonas stutzeri*

It was observed that organisms with the highest specific growth rate had the lowest mean generation time (*Pseudomonas aeruginosa 04, Pseudomonas stutzeri 06, Proteus mirabilis 02, Serratia marcescens 02 and Bacillus subtilis 01*) while the organisms with the lowest specific growth rate had the highest mean generation time (*Pseudomonas aeruginosa 07, Pseudomonas stutzeri 02, Proteus mirabils03, Serratia marcescens 01 and Bacillus subtilis 02*).

The growth profiles showed that none of the bacterial isolates exhibited lag phases which suggested acclimation time being zero as well as inherent presence of the metabolic system for utilization of petroleum hydrocarbon. This agreed with previous findings (Amund, 1984; Okerentugba and Ezeronye, 2003; Nwaogu *et al.,* 2008).

The randomly selected 24 isolates were screened for the presence of plasmid DNA. The marker used was EcoATCC25922 with 10kb pair as standard molecular weight marker. Some of the isolates screened carried multiple plasmids of molecular size varying from 7kb – 23.1kb. Among the isolates, plasmids were not detected in 2 isolates (*Proteusmirabilis* 05 and *Serratia sp*. 01).This study revealed that organisms with more number of plasmids were resistant to less number of antibiotics. Contrary to literature, there was no positive correlation between the number of plasmid harbored by an isolate and resistance to various antibiotics tests.

Indigenous bacterial consortium made up of *Pseudomonas* species, *Proteus mirabilis* and *Bacillus subtilis* could be a cheap natural resource for the removal of petroleum hydrocarbons from natural environment.

The integration of this bacterial consortium into the bioremediation process in tropical countries and the world at large would rescue our oil-polluted environment since these organisms are environment - friendly.

Further understanding of the metabolic process through which microorganisms mineralize petroleum hydrocarbons and the application of biotechnological process could generate novel bacterial that would singly metabolize petroleum hydrocarbon from oil impacted environment into carbon (IV) oxide, water and energy.

References

Abbasian, F., Lockington, R., Mallavarapu, M. and Naidu, R. (2015). A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. *Applied Biochemistry &*

*Biotechnology*176 (3): 670-699.

Adebusoye, S.A., Ilori, M.O., Amund, O.O., Teniola, O. D., and Olatope, S.O. (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical steam. *World J. Microbiology & Biotechnology*. 23:1149-1159.

Das, K., and Mukherjee, A. K. (2007). Crude petroleum – oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum – oil contaminated soil from North – East India. *Bioresources Technology*. 98: 1339 – 1345.

Gerhardt, P., Murray, R.G E., Costilow, R.N., Nester, E. W., Wood, W. A., Krieg, N. R. and

Phillips, G. B. (1981). Manual of methods for general bacteriology. American Society for Microbiology Washington, DC 20006. Pp. 409 – 425.

Hu, G., Li, J., & Zeng, G. (2013). Recent development in the treatment of oily sludge from

petroleum industry: a review. *Journal of Hazardous Materials*261: 470–490.

Kado, C. I., and Liu, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriology*145: 1365-1373.

Kasper, C. W., Burgess, J. J., Knight, I. T. and Colwell, R. R. (1990). Antibiotic resistance

indexing of *Escherichia coli* to identify sources of fecal contamination in water. *Canadian J. Microbiology* 36: 891 – 894.

Nwaogu, L. A. Onyeze, G. O. C., and Nwabueze, R. N. (2008). Degradation of diesel oil in a polluted soil using *Bacillus subtilis*. *African Journal of Biotechnology*. 7(12): 1939 – 1943.

Okoh, A. I., and Trejo – Hernandez, M. R. (2006). Remediation of petroleum hydrocarbon polluted systems: exploiting the bioremediation strategies. *African J. Biotechnology*5: 2520 – 2525.

Olukoya, D. K. and Oni, O. (1990). Plasmid profile analysis and antimicrobial susceptibility patterns of Shigella isolates from Nigeria. *Epidemiology &Infections*105:59-64.

Rosenberg, E. (2013). Hydrocarbon-oxidizing bacteria. In: *The prokaryotes—Prokaryotic physiology and biochemistry* New York: Springer. Pp. 201–214.

How to cite this article:

Olusola A. Ojo-Omoniyi and Rebecca O. Abegunrin. 2019. Evaluation of Native Bacterial Consortium from Crude Oil-Impacted Tropical Environment for Integration into Bioremediation Process. *Int.J.Curr.Microbiol.App.Sci.* 8(06): 1291-1302.
doi: https://doi.org/10.20546/ijcmas.2019.806.156