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Bioprospecting of Hydrocarbonoclastic Representative Bacteria

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Abstract

This study was designed and carried out to characterize hydrocarbonoclastic microbial communities in soil polluted with artisanal refined hydrocarbon at Trans Amadi, Phalga Local Government Area of Rivers State, Nigeria. Heterotrophic bacteria count ranged from 8.0×10^5 cfu/gm for sample TSAS1, and 2.1×10^6 cfu/gm for sample TSAS2 while TSAS3 was too numerous to count (TNTC). Hydrocarbon utilizing bacteria count ranged from 1.1×10^5 cfu/gm for TSAS1, and 5.9×10^4 cfu/gm for TSAS2, while TSAS3 was 5.4×10^4 cfu/gm. Physiochemical parameters of the soil were determined. The ranges obtained were pH 6.6, conductivity 125 µs/cm, temperature 27.3˚C, moisture 7.72, total nitrogen 0.056%, phosphate 1.554 ppm, potassium 145.87 ppm, lead 7.02 ppm, cadmium 0.41 ppm, nickel 1.96 ppm, copper 1.14 ppm, total petroleum hydrocarbon 1487.24181 ppm, polycyclic aromatic hydrocarbon 12.85287 ppm. Isolates of hydrocarbon utilizing bacteria characterized belonged to the genera *Escherichia coli*, *Klebsiella sp.*, *Lactobacillus sp.*, *Enterobacter sp.*, *Serratia sp.*, and *Proteus sp.* The findings in this study have revealed the abilities of these groups of bacteria to be employed in bioremediation/biodegradation clean-up practices. Thus the polluted soil may harbour important genera of bacterial species that may have beneficial applications in environmental microbiology for future remediation processes.

Keywords

Bioprospecting, Hydrocarbonoclastic Bacteria, Total Petroleum Hydrocarbons, Polycyclic Aromatic Hydrocarbons, Crude Oil, Soil

1. Introduction

Inadvertent disposal of petroleum products in the terrestrial environment presents a potential public health threat to human and animal populations. Due to the mobility of their toxicity, mutagenicity, and carcinogenic effects, most especially soil pollution which can lead to low output of farm products [1]. These hydrocarbons and their derivatives are capable of disrupting the inherent actions of the reproductive hormones and the ability to affect the neuroendocrine system of humans [2]. Several years of industrialization have led many nations to be dependent on petroleum hydrocarbons as the sole source of energy, which has caused significant damage to the ecological environment globally [3]. Inadvertent disposal of used engine oil, crude oil from multinationals and artisanal refining of hydrocarbons in Port Harcourt, Rivers State has been recognised as the major anthropogenic source of hydrocarbon pollution of soil and water. This has resulted in a high amount of hydrocarbons and their derivatives to run through and contaminate the ocean, farmlands and pipe-borne water. Hydrocarbons released in soil affect the biotic and abiotic components of soil. Therefore, it is essential to have vigorous effective measures for dealing with hydrocarbon contamination problems [4]. Bioremediation has emerged as the most auspicious treatment option for decontaminating polluted soil and water since its fruitful application to clean up the Exxon Valdez in 1989. It is a cost effective act that utilizes ubiquitous organisms carrying out natural attenuation in a polluted environment, most especially bacteria to cause an acceleration of the natural biodegradation process under suitable environmental conditions with nutrient availability [5].

This process proceeds from the ability of the microbes to carry out various energy-dependent processes which involve oxidation-reduction, accumulation, and precipitation, of petroleum hydrocarbon compounds of interest present in the contaminated environment [6]. This study was carried out with the aim of isolating and characterizing hydrocarbonoclastic bacteria species from soil contaminated with artisanal refined hydrocarbon oil and their possible application in bioremediation/biodegradation.

2. Materials and Methods

2.1. Sample Location/Collection

Top soil samples (0 - 15 cm) were collected from different points around the polluted site at Trans Amadi Port Harcourt, Rivers State. Samples were collected in sterile polythene bags and transported to the National Agency for Food and Drug Administration and Control Port Harcourt Area Laboratory for further analysis.

2.2. Isolation of Potential Hydrocarbon-Clastic Bacteria

Detection and enumeration of hydrocarbon degrading bacteria was done by the technique adopted by [1].

2.3. Determination of Physiochemical Parameters of Soil Samples

Soil samples pre-treatment was done according to US-EPA, (Method 3050B) [7]. Physiochemical parameters of soil such as pH, conductivity, temperature, moisture content, total nitrogen, and phosphorus were determined. Heavy metals were analysed with atomic absorption spectrophotometer.

2.4. Chromatographic Analysis

Total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) residual were analysed using gas chromatography flame ionization detector (GC/FID) at Anal Concept Ltd.

2.5. Total Heterotrophic Bacteria Count

Heterotrophic bacteria counts were carried out after enrichment procedure on standard plate count agar (SPCA), via serial dilution with ringer's solution. Aliquot (0.1 ml) of the diluents were plated out on Standard plate count agar and incubated at 30˚C for 24 hours. Colony forming units were afterwards counted

2.6. Hydrocarbon Utilization Bacteria Count

Hydrocarbon utilization bacteria counts were enumerated by a method adopted by [8]. The method involved dilution of appropriate homogenate samples and plating out on BHM. Hydrocarbons were incorporated through the vapour phase to potential hydrocarbon utilizers by placing sterile Whatman filter papers impregnated with 5 ml of crude oil on the lids of the inverted plates and incubated for 7 - 14 days at 30˚C.

2.7. Isolates Identification

Discrete colonies that are capable of degrading crude oil were identified with different techniques including morphological characters, biochemical test and microscopically as described in Bergey's manual of determinative bacteriology [9].

2.8. Degradative Screening

Representative hydrocarbon utilizing bacteria isolates were screened for crude oil degradation abilities under aerobic conditions by inoculating a loop full of 24 hours old culture of each hydrocarbon utilizing bacterium into BHM broth containing 1% (v/v) crude oil. Biodegradation was recorded with the discolouration of DCPIP oxidation reduction reagent after 14 days' incubation at 30˚C [5].

3. Results

Bioprospecting of culturable hydrocarbon degrading bacteria has intensified in decays due to the need to remediate and degrade xenobiotic.

Evaluation of soil in this study showed that the soil is chronically polluted with hydrocarbons. Physiochemical parameters of the soil are given in **Table 1**.

Table 2, and **Table 3** presents the chromatographic quantification for total

petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) for TSAS1, TSAS2, and TSAS3 polluted soil samples respectively.

Table 1. Physiochemical characterization of the soil.

Parameters	Concentration in soil	NPRA and NURC intervention limit
TPH	1487.24181 ppm	1000
PAH	12.85287 ppm	40
pH	6.6	_
Conductivity	125 μs/cm	_
Temperature	27.3˚C	_
Moisture	7.72	_
*Total nitrogen	0.056%	_
*Phosphate (PO_4)	1.554 ppm	_
*Potassium (K)	145.87 ppm	_
Lead (Pb)	7.02 ppm	530
Cadmium (Cd)	0.41 ppm	12
Nickel (Ni)	1.96 ppm	210
Copper (Cu)	1.14 ppm	190
THB count (cfu/gm) TSAS1	8.0×10^5	
THB count (cfu/gm) TSAS2	2.1×10^6	
THB count (cfu/gm) TSAS3	TNTC	
HUB count (cfu/gm) TSAS1	1.1×10^5	
HUB count (cfu/gm) TSAS2	5.9×10^4	
HUB count (cfu/gm) TSAS3	5.4×10^4	

* = Variable limits in soil; - = No of Nigerian Midstream and Downstream Petroleum; Regulatory Authority and the Nigeria Upstream Regulatory Commission (NPRA and NURC) Intervention limit.

Table 2. GC/FID Chromatographic quantifications result of TPH in soil.

Real Time (min)	Type	Area counts*s	Amt/Area	Amount (ppm)	Name
4.435	vv	2.57883e4	2.70035e-5	6.96374e-1	n-C12
5.909	vv	1.45722e5	2.72055e-5	3.96443	n-C14
6.575	vv	3.86092e5	2.70642e-5	10.44930	n-C15
7.515	vv	4.51266e5	2.72156e-5	12.28144	n-C16
8.482	vv	2.10111e5	1.73722e-5	3.65009	n-C17
8.623	vv	3.83646e5	7.01398e-5	26.90882	Pristine
9.450	vv	5.00713e5	1.93834e-5	9.70552	n-C18
9.604	vv	5.79529e4	4.75187e-5	2.75385	Phytane
10.547	vv	9.37087e4	2.83355e-5	2.65529	n-C19
11.460	vv	9.85314e5	3.42932e-5	33.78958	n-C20
12.211	vv	2.82517e5e	3.33571e-5	94.23967	n-C21
13.327	vv	1.94252e6	3.25046e-5	63.14066	n-C22
14.129	vv	6.78156e5	3.23021e-5	21.90584	n-C23

Continued

15.077	vv	4.78370e5	3.27415e-5	15.66257	n-C24
15.823	vv	6.14654e5	3.5254e-5	19.99189	n-C25
16.601	vv	1.40229e6	3.19577e-5	44.81392	n-C26
17.477	vv	3.68851e5	3.11710e-5	11.49747	n-C27
18.352	vv	9.59616e6	3.15125e-5	302.39905	n-C28
18.881	vv	2.13279e6	3.33975e-5	71.22990	n-C29
19.781	vv	1.36560e7	3.55050e-5	484.85578	n-C30
20.281	vv	2.53412e5	3.84915e-5	9.75420	n-C31
20.814	vv	1.58633e6	4.32260e-5	68.57063	n-C32
21.728	vv	1.60820e5	4.69189e-5	7.54551	n-C33
22.170	vv	1.96030e5	5.16250e-5	10.12005	n-C34
22.898	vv	3.46856e5	5.87826e-5	20.38908	n-C35
23.595	vv	1.06153e6	6.69752e-5	71.09642	n-C36
24.506	vv	2.64804e5	7.74242e-5	20.38908	n-C37
25.606	vv	1.36196e5	8.67544e-5	11.81564	n-C38
26.809	vv	2.64665e5	1.00185e-4	26.51546	n-C39
28.334	vv	3.83355e4	1.13240e-4	4.34113	n-C40

Totals: 1487.24181.

Table 3. GC/FID Chromatographic quantification result of PAHs in the soil.

Real Time (min)	Type	Area counts*s	Amt/Area	Amount (ppm)	Name
5.134	vv	1.77280e5	3.83831e-7	6.80455e-2	Naphthalene
6.098	vv	3.25172e5	5.60811e-7	1.82360e-1	2-methylnaphthalene
6.180	vv	5.67243e5	3.13213e-6	1.77668	Acenaphthylene
7.315	vp	3.06163e4	5.08046e-7	1.55545e-2	Fluorene
7.500	vv	2.14267e4	3.72572e-7	7.98298e-3	Acenapthene
8.514	vv	5.51225e5	4.61774e-7	2.54541e-1	Phenanthrene
10.250	vv	1.28226e6	7.05793e-7	9.05009e-1	Anthracene
10.549	vv	8.23008e4	6.06617e-7	4.99250e-2	Fluoranthene
13.120	vv	1.07493e5	1.10602e-6	1.18889e-1	Pyrene
13.659	vv	1.151680e5	6.67458e-7	1.01240e-1	Benzo [a]anthracene
16.638	vv	1.92495e6	2.92436e-6	5.62926	Chrysene
16.694	vv	7.76423e5	4.48817e-7	3.48472e-1	Benzo [b] fluoranthene
19.128	vv	7.00175e5	2.54496e-6	1.78192	Benzo [k] fluoranthene
19.285	vv	5.26407e4	4.99167e-7	2.62765e-2	Benzo [a] pyrene
20.084	vv	1.94323e5	3.03305e-6	5.89392e-1	Dibenz [a,h] anthracene
22.565	vv	1.52135e5	4.70690e-6	7.16086e-1	Indeno [1,2,3-cd] pyrene
22.871	vv	1.07519e5	2.61576e-6	2.81243e-1	Benzo [g,h,i] perylene

Totals: 12.85287.

4. Discussion

The results of the physiochemical parameters of the soil in **Table 1** indicate that the soil had been exposed to hydrocarbon pollution with organic and inorganic contaminants for years [10]. The pH of the soil is 6.6, which shows that the soil is slightly acidic. Soil pH is an important determinative factor that controls various physiochemical reactions that involves microbial growth and ability. It exhibits profound degradative efficiency on hydrocarbons through biotic and abiotic paths [11]. According to [12] [13], pH is an important catalogue parameter which exert a controlling influence on the biodegradation of petroleum hydrocarbon contaminants. The soil total nitrogen which comprises the concentrations of ammonia, ammonium, nitrite, nitrate, and dissolved particulate organic nitrogen ranged at 0.056%, phosphate 1.554 ppm, potassium 145.87 ppm, lead 7.02 ppm, cadmium 0.41 ppm, nickel 1.96 ppm, copper 1.14 ppm and the temperature of the soil ranged at 27.3˚C. The soil conductivity in this study measures the soluble salt content in the soil and is used as an overall indicator of the level of micro and macro nutrient availability in the soil [14]. Conductivity for the soil sample in this present study ranged at 125 μs/cm. This is lower than the figure gotten by [5], who reported 6000 μs/cm for soil sample around a crude oil polluted soil in Bie-Ama community. The identified soil pollutants in this study causes damages to humans, animals and the ecosystem. In humans the hydrocarbon residues such as PAHs are well known carcinogens, mutagens that cause alteration in genetic material (DNA), teratogens causing prenatal toxicity characterized by defect in developing embryo and congenital abnormalities [15]. The human health impact of TPH was reported by [16], while toxigenic and carcinogenic effect of several PAHs like benzo [a] anthracene, benzo [a] pyrene, benzo [b] fluoranthene, benzo [k] fluoranthene, indenol [1,2,3-cd] pyrene, anthracene, benzo [g, h, i] perylene, chrysene, fluoranthene, fluorine, phenanthrene and pyrene was reported by [15].

The presence of microbial activities in this study was determined by the enumeration of total heterotrophic bacteria and total hydrocarbon utilizing bacteria as presented in **Table 1**. Soil sample TSAS3 recorded highest cfu/gm for THB counts with isolates too numerous to count. This may be attributed to the pH of 6.6 that favours the growth of autochthonous soil microorganisms which agrees with the findings of [12] [13], which stated that the pH range optimal for biodegradation of hydrocarbons is 6 - 7.

Sample TSAS1 recorded the highest in HUB count with 1.1×10^5, followed by TSAS2 with 5.9×10^4, and TSAS3 with 5.4×10^4. In primary screening for microbial hydrocarbon degradation potential with 2, 6 dichlorophenol indolphenol, these microbes were tested in Bushnell Haas broth medium containing 1% crude oil and 0.1% DCPIP for 3 weeks and all the isolates showed better potential for crude oil degradation. DCPIP test was a rapid primary screening procedure that was performed to assess the indicator dye 2, 6 dichlorophenol indolphenol decolorization efficiency of the isolates for confirmation of crude oil

biodegradation [17].

The residual TPH and PAHs found in the soil exceeded Nigerian Midstream and Downstream Petroleum Regulatory Authority, NPRA, and the Nigerian Upstream Regulatory Commission, NURC intervention limit of 1000 mg/kg and 40 mg/kg for TPH and PAHs [18].

The population of hydrocarbonoclastic bio-prospects investigated in this study showed that the bacteria were coliform Gram negative rods belonging to the phylum Gamma proteobacteria group, this corroborates with the study of [19], who stated that the growth of coliform bacteria on a wide range of aliphatic and aromatic hydrocarbons reflects a high potential of hydrocarbon utilization. Although *Lactobacillus sp.* isolates belonging to the phylum Bacillota was obtained in this study.

Several isolates such as *Escherichia coli, Klebsiella sp., Lactobacillus sp., Enterobacter sp., Serratia sp.,* and *Proteus sp.,* were screened in this study that had the ability to degrade crude oil presented in **Table 4**.

The isolation of *Lactobacillus sp.,* and *Klebsiella sp.,* in this study corroborates with the study of [20], and [21], who reported similar microbes for their effectiveness in the mineralization of hydrocarbons.

In the study conducted by [22] *Proteus sp.,* was dominantly used for hydrocarbon biodegradation.

The detection and enumeration of *Enterobacter sp., Escherichia coli, Serratia sp., Klebsiella sp.,* and *Proteus sp.,* corroborates with the findings of [8]. The findings of [23], also reported these isolates as known crude oil degraders in a study conducted in Bodo, Ogoniland and Nembe waterside Port Harcourt.

[24] characterized *Klebsiella sp.* for their biosurfectant production in a hydrocarbon polluted soil in ogoni land.

In the study conductecd by [25], *Escherichia coli, Enterobacter sp., Klebsiella sp., Proteus sp.,* and *Serratia sp.,* was reported for their crude oil utilization ability which is in line with the findings of this current study.

Table 4. Characterization of bacterial isolates.

Isolate code	Gram reaction	Morphology	Isolate identity	Degradative screening
HUBTSAS1A	_	R	*Escherichia coli.*	Yes
HUBTSAS1B	_	R	*Klebsiella sp.*	Yes
HUBTSAS1C	+	R	*Lactobacillus sp.*	Yes
HUBTSAS2A	_	R	*Enterobacter sp.*	Yes
HUBTSAS2B	_	R	*Serratia sp.*	Yes
HUBTSAS2C	+	R	*Lactobacillus sp.*	Yes
HUBTSAS3A	_	R	*Proteus sp.*	Yes
HUBTSAS3B	_	R	*Proteus sp.*	Yes
HUBTSAS3C	+	R	*Lactobacillus sp.*	Yes

R = rod, - = negative, + = positive.

5. Conclusion

The present study revealed the presence of indigenous microorganisms from petroleum hydrocarbon contaminated soil in Trans Amadi, Port Harcourt as well as known genera of hydrocarbon utilizing bacteria. There is a propensity that the increasing viability of hydrocarbon utilizing microbes in this polluted soil could influence the mechanism of biodegradation. The experimental result determined shows that indigenous petroleum hydrocarbon utilizing bacteria could be isolated and used effectively in the polluted site for hydrocarbon bioremediation/biodegradation. Furthermore, the application of metagenomics analysis, and other OMIC techniques friendly to the ecosystem should be employed to help increase our understanding of the vast microbial diversity in polluted soil.

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Authors' Contribution

Asime Oba conceived and carried out the work; Asime Oba, Barka John and Okeke Uchechukwu were involved in data analysis and interpretation; Asime Oba, Barka John and Okeke Uchechukwu analysed the data and took note. Asime Oba and Barka John drafted the manuscript

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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