ISOLATION AND CHARACTERIZATION OF HYDROCARBON DEGRADING BACTERIA IN CRUDE OIL POLLUTED SOIL IN THE NIGER DELTA

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ABSTRACT

Bioremediation as pollution remediation technique takes advantage of the ability of some microorganisms to degrade organic matter in the environment. Bacteria are important group of microorganism that plays key role in the bioremediation process. Bacteria are ubiquitous organisms that inhabit soil water and air matrices. In this study, some hydrocarbon degrading bacteria have been isolated and identified by morphological and biochemical methods.. Eight different hydrocarbon degrading bacteria were isolated from crude polluted soil collected from oil spill site in Ikarama community in Yenagoa Local Government Area of Bayelsa State in the Niger Delta**.** The total population of heterotrophic bacterium enumerated per gram of each soil sample are $1.42x10^7$, $1.7x10^7$ and $3.1x10^8$ for location 1,2 and 3 respectively While population of total hydrocarbon degrading bacteria enumerated are $4.2x10^6$,: $3.7x10^6$ and $1.43x10^6$ for location 1, 2 and 3 respectively. This population of hydrocarbon degrading bacteria in the soil is enough to initiate natural biodegradation of crude oil pollutant. With the right environmental conditions for the bacteria, bioremediation can be effectively deployed to remediate the spilled area.

Keywords: hydrocarbon, degrading, bacteria, bioremediation, isolation and characterizati

1.1 INTRODUCTION

Bacteria are tiny organisms that are ubiquitous in the environment. Research as shown that certain microorganisms including bacteria are capable of breaking down hydrocarbon pollutant in the environment to water, carbon dioxide (Co_2) and harmless compounds. This has made microorganisms to be applied in environmental restoration technology scientifically known as Bioremediation. Bioremediation is an environmental restoration technology that uses the activities of microorganisms to degrade and neutralize organic pollutants within the environment. It has been given different description and definitions, but all the definition given point to the same thing. Some of these definitions are:"Bioremediation is a process by which chemical substances are degraded by bacteria and other microorganisms." (Atlas, 1995). "Bioremediation is the process of using bacteria and other biological enhancements under controlled conditions to control pollution caused by different components of gasoline and fuel oxygenates in the contaminated land and groundwater"(David, L and Randy, M). Bioremediation offers the possibility of degrading, removing, altering, immobilizing, or otherwise detoxifying various chemicals from the environment through the action of bacteria and fungi {Gadd, 2001 and Singh, 2006). Bioremediation has been globally acknowledged and accepted as a cheaper, environmentally friendly and sound remediation technology. This technology has been shown in literatures that it is only possible by the activities of bacteria and fungi. Many hydrocarbon degrading bacteria species have been discovered both in marine and terrestrial environment. The biodegradation of petroleum in the marine and terrestrial environment is carried out largely by diverse bacterial populations, including various *Pseudomonas* species. Bacllus species, *Arthrobacter* which has been shown to degrade (PAHs), Brevibacterium, Brachybacterium, *Cellulomonas, Corynebacterium, Detzia, Micrococcus, Mycobacterium, Nocardioides , Rhodococcus* and *Flavobacterium* sp e.t.c. These bacteria presumably utilize hydrocarbons that are naturally produced by plants, algae, and other living organisms. They also utilize other organic matters, such as carbohydrates and proteins. When an environment is polluted with petroleum hydrocarbon, the population of hydrocarbon-degrading microorganisms within the polluted environment increases rapidly because these organisms use the hydrocarbon as source of carbon and energy.

In the Niger Delta region of Nigeria, petroleum hydrocarbon pollution has been wide spread and persistent. This has made microorganisms including bacteria in the aquatic and terrestrial

environment to be resilient and adjust to the polluted environment by utilizing the hydrocarbon as source of nutrients and energy, hence making bioremediation feasible, economical, friendly, highly acceptable by the public and attractive option to clean the environment of the Niger Delta. The hydrocarbon-biodegrading bacteria populations are widely distributed in the Niger Delta marine and terrestrial environment indicating that hydrocarbon-degrading microorganisms are ubiquitously distributed in the region. This study is therefore aimed at isolating and characterizing hydrocarbon degrading bacteria in oil spill soil collected from Ikarama community in the Niger Delta region of Nigeria.

2.1 MATERIALS AND METHOD

2.2 Locus of Study.

The study area for this research is Ikarama community where the crude oil polluted soil samples were collected. Ikarama is located in 05°09'16" N, 06°27'11 E within Okordia clan of Yenagoa Local Government Area of Bayelsa State in Southern region of Nigeria. The community is the host to the Okordia-Rumuekpe Trunk line own by Shell Petroleum Development Company (SPDC) in Bayelsa State. The community also hosts the SPDC manifold (Shell Okordia manifold). This manifold receives crude oil from oil wells around Biseni and joinkarama number (JK 4) ie Edagberi community in Ahoada West Local Government Area of Rivers State, passing through the Adibawa Flow station. The community is among the nine fishing community in Okordia – Zarama cluster under a global memorandum of understanding (GMoU) operated between them and the SPDC. Plate 1 below is a photograph showing the oil spilled land with vegetation in Ikarama community, taken during soil sample collection.

Plate 2.1: Collection of Polluted soil at the study site. (Source: field trip)

2.1 Materials and Method

2.1.1 Materials

Chemicals and media.

The chemicals used in this study include indole stain, catalase, methyl red stain, Voges-Proskaur, coagulase, oxidase, urease and citrate. Media include Bushnell Haas media, nutrient Agar, Blood Agar which were all purchase from commercial shop in Port Harcourt. The Bushnell Haas broth medium contains $MgSO_4$ (0.2g/l), KH_2PO_4 (1g/l), K_2HPO_4 (1g/l), $CaCl_2$ (0.02g/l), NH_4NO_2 (1g/l) and $FeCl_2$ (0.05g/l) Keterazol, Nutrient Agar contains : Peptide digest (5g/l), Beef extract (5g/l) Yeast extract (1.5g/l), NaCl (5g/l) and Agar (1.5g/l) PH (7.4)

2.2 Method

2.2.1 Isolation, enumeration of bacteria.

The bacteria were isolated from the soil sample by culturing them under growth conditions of media. (Bushnell Haas media Blood Agar and Nutrient Agar). 1g of well powdered and sieved oil polluted soil sample was weighed and dissolved in 9ml of sterilized distilled water in nine replicates and properly shaken. Each of the solution that was prepared was made up to $10^{-1.}$ Each of the diluents was further serially diluted up to 10^{-8}. 1ml of each diluent was then inoculated into sterilized Petri dish. The prepared and sterilized growth media containing Bushnell Haas media, Blood Agar and Nutrient Agar media was incorporated with Keterazol to inhibit fungi growth and then poured into each of the Petri dish containing soil water inoculums. The nine Petri dishes were span by rotating on the lab bench several times in clock wise and anti clockwise direction to ensure homogenous spreading of the inoculums. The plates were then kept for some time to set and then incubated in an upside down position at 37^0C for one week, after which the plates were examined for colony growth and enumerated with magnifying hand lens. The plates showing between 30 to 300 colonies were recorded. From the counting, the total viable bacteria cells in each sample were expressed as colony forming unit (cfu/g) per gram of each soil sample. Colonies different in shape, Colour and sizes were selected from different agar plates and sub cultured for further analysis.

2.2.2 Screening of hydrocarbon degrading bacteria.

For the isolation of pure culture of hydrocarbon degrading bacteria in the soil samples, A pure growth of each isolate was inoculated into newly prepared and properly sterilized Bushnell Haas Broth Medium enriched with nutrient agar. The BHBM was prepared according to manufacturers instruction. 1ml of sterilized crude oil was added to it as a source of carbon and energy in a 200ml volumetric flask. 10ml of Keterazol was also added to the newly prepared Bushnell Haas Broth Medium to prevent the growth of fungi. One flask containing the medium and crude oil but without organism inside it was kept as control. The flasks were then incubated at 28-30^{O}C with regular shaking for two weeks. The flasks content were regularly observed for any change in optical density, colour, petroleum hydrocarbon concentration was also measured for the period of two weeks. Changes in TPH concentration were recorded during the period.

2.2.3 **Bacteria identification.**

. The bacteria isolates from the subculture were identified by biochemical test (gram reaction, motility, indole, catalase, methyl red, Voges-Proskaur, coagulase, oxidase, urease and citrate). The resultant characteristics were compared with those of known taxas using Bergey's manual of Determinate Bacteriology by Holt et al, (1994) and the scheme of Cheesbrough (2004)

Table 2.1: Result of Biochemical identification of bacteria.

Organisms	Gram reaction	Motility	Oxidase	Catalase	Citrate	Coagulate	Urease	Indole	Methyl red	VP	Hemolytic	H_2S
Pseudomonas sp	Negative rod	+	+	+	+	--	--	--	--	+	NA	--
Serratia sp	Negative rod	+	+	+	+	--	--	--	NA	NA	NA	NA
Bacillus sp	Positive rod	+	+	+	+	--	--	--	--	+	NA	+
Staphylococcus sp	Positive rod	--	--	+	--	+	--	--	+	+	NA	+
Corrybacterium sp	Positive rod	--	--	+	+	--	--	+	NA	NA	NA	NA
Enterobacter spp	Negative rod	+	--	+	+	--	--	--	--	+	NA	--

Micrococcus p	Positive rod	--	--	+	--	--	--	--	+	--	NA	--
Flavobacterium	Negative rod	--	--	+	NA	--	NA	+	+	--	NA	+
Achromobacter	Negative rod	+	+	+	+	NA	+	--	NA	--	NA	NA
Alcaligenes	Negative rod	+	+	+	+	NA	NA	--	NA	NA		NA
Escherichia	Negative rod		--	+	--	--	--	+	+	--	NA	NA
Enterococcus sp	Positive rod	--	--	+	NA	--	NA	--	--	--	NA	+
Citrobacter sp	Negative rod	+	--	+	+	--	NA	--	+	--	NA	NA

(+ = positive, - negative reaction, NA= Not applicable)

Courtesy: Laboratory analysis.

Table 2.2 : Bacteria isolates from the polluted soil samples .

Location1	Location2	Location3
Pseudomonas sp	Pseudomonas sp	Pseudomonas sp
---	Serratia sp	Serratia sp
Bacillus sp	Bacillus sp	Bacillus sp
Staphylococcus sp	Staphylococcus sp	Staphylococcus sp
Corrybacterium sp	Corrybacterium sp	Corrybacterium sp
Enterobacter spp	Enterobacter spp	Enterobacter spp
Micrococcusp	Micrococcusp	Micrococcusp
Flavobacterium	Flavobacterium	Flavobacterium
Achromobacter	--	Achromobacter
Alcaligenes species	Alcaligenes species	Alcaligenes species

Table 2.3: Bacteria density in the various soil samples

Bacterial count	Polluted Soil Samples locations		
	1	2	3
Total heterotrophic bacteria	$1.42x10^7$	$1.71x10^7$	$3.1x10^8$
Hydrocarbon utilizing bacteria	$4.2x10^6$	$3.7x10^6$	$1.43x10^7$

3.1 RESULTS AND DISCUSSION

In this study, isolation and identification of hydrocarbon degrading bacteria in crude oil polluted soil collected from an oil spill site in Bayelsa State in the Niger Delta region of Nigeria has been carried out. The result shows that the Crude oil polluted soil contain a rich consortium of

hydrocarbon degrading bacteria. The Hydrocarbon degrading bacteria isolated and identified by biochemical methods include, *Pseudomonas* sp, *Serratia* sp, *Staphylococcus* sp, *Corrybacterium* sp, *Enterobacter* sp , *Micrococcu* sp, *Flavobacterium*, *Achromobacter*, *Escherichia coli*, *Enterococcus* sp *and Citrobacter* sp. A Total of eleven different bacteria species were solated and identified. eight of the eleven bacteria isolated are petroleum hydrocarbon degraders. This shows that the crude oil spilled soil is capable of undergoing self restoration by natural attenuation given appropriate environmental conditions such sufficient available oxygen within inter particles soil space, sufficient micro and macro nutrients, enough moisture, suitable PH. The self restoration capacity of the soil can be enhanced by aeration of the soil by periodic tilling, sprinkling of water and addition of organic or inorganic fertilizer. Most of the bacteria isolated have been proven to biodegrade different range of petroleum hydrocarbon constituents. For example, Pseudomonas sp has been shown to biodegrade Benzene, toluene, ethyl benzene, xylene, naphthalene, phenanthrene, kerosene and diesel (Watanabe, 2001). *Bacillus* degrade toluene and diesel (Joshi and Pandy, 2011), *Alcaligenes* degrade most PAHs (not specific) (Mao et al., 2012), *Micrococcus* biodegrade low molecular weight PAHs. (Othman, et al., 2011), *Cornebacterium* also biodegrade low molecular weight PAHs. (Othman et al., 2011), *Flavobacterium* also have been discovered to biodegrade PAHs (not specific) (Li et al., 2009). E.t.c The total population of heterotrophic bacterium enumerated per gram of each crude oil polluted soil sampled are $1.42x10^7$, $1.71x10^7$, $3.1x10^8$ for location 1,2 and 3 respectively While total hydrocarbon degrading bacteria $4.2x10^6$,; $3.7x10^6$ and $1.43x10^6$. This bacterial population is a pointer to the fact that natural biodegradation was possible because this population was enough to start biodegradation of crude in the study area. The study by Forsyth, et al, (1995) showed that biodegradation of petroleum hydrocarbon can occur naturally when the population of hydrocarbon utilizing microorganisms is not less than 10^3 cfu/g in the polluted soil. The availability of petroleum hydrocarbon degraders in soil is a pointer to the fact the area has been exposed to crude oil pollution in the past which has therefore encourage the existence of hydrophilic bacteria in the soil. Considering the rich availability of crude oil degrading bacteria in the polluted soil, we are inclined to conclude that bioremediation can be effectively applied to remediate the oil spilled soil given the right conditions to encourage growth and multiplication of the hydrocarbon utilizing bacteria.

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