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Bioremediation Potentials of Bacteria Isolated from Rhizosphere of Some Plants of Oil Contaminated Soil of Niger Delta

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Abstract Analysis of the bacterial genera associated with the rhizosphere of *Cyperus*sp., *Parkia*sp., *Panicumpariflorium, Zea mays, Elaeisguineensis* inpetroleum hydrocarbon contaminated soil in Ugada, Imo State, Nigeria was done. The study shows that densities of total culturable heterotrophic and hydrocarbon-utilizing bacteria varied with the type of plant and were higher in the contaminated rhizosphere than in the contaminated bulk soil (non- rhizosphere) of all the plants. Unlike the result of total culturable heterotrophic counts in the bulk soil, the mean counts of the hydrocarbon-utilizing bacteria were higher in the contaminated bulk soil for all plants. *Elaeisguineensis*, gave the lowest mean counts for both total culturable heterotrophic and hydrocarbon-utilizing bacteria of all the plants studied, this may be as a result of some of the plant's exudates not stimulating all the bacterial community. Statistical analysis showed no significant difference ($P>0.05$) between the rhizosphere and bulk of total culturable heterotrophic and hydrocarbon-utilizing bacterial counts in both contaminated and uncontaminated soils. The hydrocarbon-utilizing bacterial genera isolated and identified were *Acinetobacter*, *Arthrobacter*, *Alcaligenes, Bacillus, Flavobacterium, Micrococcus, Pseudomonas* spp. These isolates were able to grow on petroleum hydrocarbon at different growth rates indicating that they can be used as seeds for bioaugmentation during remediation of petroleum contaminated soil.

Keywords: *Niger delta, rhizosphere, crude oil, hydrocarbon utilizing bacteria, potentials*

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1. Introduction

Global industrialization over the past two centuries has resulted in widespread contamination of the environment with persistent organic, inorganic wastes and xenobiotics. Polluted land has generally resulted from past industrial activities where awareness of the environmental health effects connected with the production, use, and disposal of hazardous substances were less recognized than today. The problem is worldwide, and the estimated number of contaminated sites is significant and increasing [1].

The technologies used for contaminated soils such as excavation, incineration and land filling can be quite expensive. Hence, the quest for an effective, non-destructive and inexpensive means of remediating the environment which will be accepted by surrounding communities and regulatory agencies has finally spurred the development of a new remediation technology. This nascent technology which employs the symbiotic relationship between plants and the microorganisms found in their rhizosphere in the breakdown of contaminants to clean up the environment is referred to as rhizoremediation [2].

Many cases of severe crude oil spillage have occurred in the Niger Delta area of Nigeria. These situations not only affect the ecosystem adversely as life may not exist, but the crude may also contain varying levels of polycyclic aromatic hydrocarbons (PAH's); many of these have been reported to have adverse consequences [3].

"Bioremediation of crude oil spill has been accepted as a remediation process [4]". There is still room for improvement and new sustainable methods are needed to guarantee results that are better and more acceptable than conventional methods [5].

Rhizoremediation is presumed to be based on the stimulation of microbial degradation in the rhizosphere by plants [6]. Rhizosphere can be described as a stable micro-environment where there are intense interactions between the plant roots, rhizo-microbes and soil environment. In addition, rhizobacteria improve the tolerance of plants against contaminants such total petroleum hydrocarbon, heavy metals, etc., and promote significantly the accumulation of contaminants into plants [7,8].

This investigation was conducted therefore, to assess the variation, isolation and identification of the rhizosphere bacterial densities of these plants, *Cyperus*sp., *Parkia*sp., *Panicumpariflorium, Zea mays,*

Elaeisguneensis found in oil-contaminated soil in Ugada, Imo State and to explore their applicability in bioremediation.

2. Materials and Methods

2.1. Sample Source, Collection and Processing

Soil samples for the study were collected from rhizosphere of the plants and their bulk soil (30 centimeters from plants' roots) found in crude oil impacted soil, and also from rhizosphere of plants of the same species and their bulk soil from pristine soil. These were transported using marked sterile plastic bags in an ice chest to the laboratory for analysis within 24 hours. The plants were taken to a plant taxonomist for proper identification.

Ten-fold serial dilutions of the soil samples were prepared using 0.85% sterile physiological saline. From each dilution of 10^{-3} to 10^{-6}, aliquots (0.1ml) were aseptically plated on sterile Nutrient agar (NA) and Mineral Salt agar in duplicates.

2.2. Enumeration of Total Culturable Heterotrophic Bacteria and Total Hydrocarbon-Utilizing Bacteria

The total heterotrophic bacteria count was performed using Nutrient agar (Oxoid). The medium was prepared according to manufacturer's specifications. Aliquots (0.1ml) of the serially diluted soil samples were plated out in duplicates on Nutrient agar plates using spread plate method. The plates were incubated at 35°C for 24 to 48 hours.

The vapour phase transfer method as described by [9] was adopted. Mineral salt agar with the following composition: NaCl 10.0g, MgSO4 0.42g, KCl 0.29g, KH_2PO_4 0.83g, $NaPO_4$ 1.25g, $NANO_3$ 0.42g, agar 15g and distilled water 1000ml as modified by [10] was used. Sterile Whatman No.1 filter paper saturated with crude oil was aseptically placed on the inside cover of each Petri dish. The Petri dishes were incubated with the agar side up at 35°C for 5 to 7 days. The crude oil saturated filter paper supplied the bacteria with the carbon and energy required through the vapour phase transfer of the hydrocarbon.

2.3. Characterization of Bacterial Isolates

Pure stock cultures of crude oil utilizing bacterial isolates were examined for colonial appearance and used to carry out the following tests: Gram staining, Motility test, Catalase test, Citrate Utilization test, Indole test, Hydrogen Sulphide Production test, Methyl Red – Voges Proskauer test, Oxidase test, Sugar fermentation test. Confirmatory identities of the bacteria were made using the Bergey's Manual of Determinative Bacteriology [11].

2.4. Screen Test for Crude Oil Utilization

Nutrient broth was used to culture the bacterial isolates. They were incubated for 24 hours at 28+2°C. 0.1ml of the young culture in nutrient broth grown was inoculated into each test tube containing 9.9ml of sterile mineral salt broth and 0.1ml of crude oil. A control test tube containing 9.9ml of sterile mineral salt broth with 0.1ml of crude oil remained uninoculated. The cultures were incubated for seven days at room temperature. Crude oil utilizing bacteria would turn the broth into a cloudy suspension as against a clear control.

2.5. Statistical Methods

One-way Analysis of Variance (ANOVA) and t-test were used to analyse the data obtained from the plants, and the contaminated and uncontaminated soil sample of each plant respectively.

3. Results and Discussion

The result (Figure 1) showed that the least mean count of contaminated rhizosphere was obtained from *Elaeisguineensis* as $0.98x10^6$cfu/g while the highest mean count of $1.31x10^6$cfu/g was obtained from *Parkia* sp. The uncontaminated rhizosphere gave its least mean count as $4.11x10^5$cfu/g from *Elaeisguineensis* and the highest mean count was obtained from *Parkia* sp. ($6.95\ x10^5$cfu/g). A least mean count of 2.56×10^5 cfu/g was obtained from *Zea mays* for the contaminated bulk. The highest mean count for the contaminated bulk was $3.28x10^5$cfu/g from *Panicumparifolium*. The uncontaminated bulk gave a least mean count of 3.49×10^5 cfu/g from *Panicumparifolium* while the highest mean count of 3.74×10^5 cfu/g was obtained from *Parkia* sp.

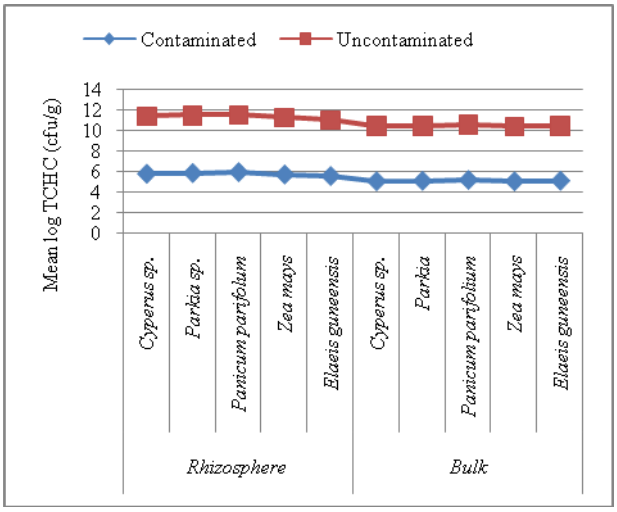

Figure 1. Total culturable heterotrophic bacterial counts of contaminated and uncontaminated rhizosphere and bulk soil samples

The result (Figure 2) indicated that the contaminated rhizosphere of *Elaeisguineensis* gave the least mean count of $7.28x10^5$cfu/g for hydrocarbon-utilizing bacterial count while *Parkia* sp. gave the highest mean count of $1.02x10^6$cfu/g. The uncontaminated rhizosphere yielded a least mean count of $1.95x10^5$ cfu/g from *Elaeisguineensis* while a highest mean count of 3.31×10^5 cfu/g from *Panicumparifolium* was obtained. The contaminated bulk of *Elaeisguineensis* gave the least mean count of $1.10x10^5$cfu/g while the highest mean count was obtained from *Panicumparifolium*as $1.42x10^5$ cfu/g. The uncontaminated bulk had a least mean count of 6.05×10^4 cfu/g (*Elaeisguineesis*) while *Panicumparifolium* gave the highest mean count of $9.75x10^4$ cfu/g.

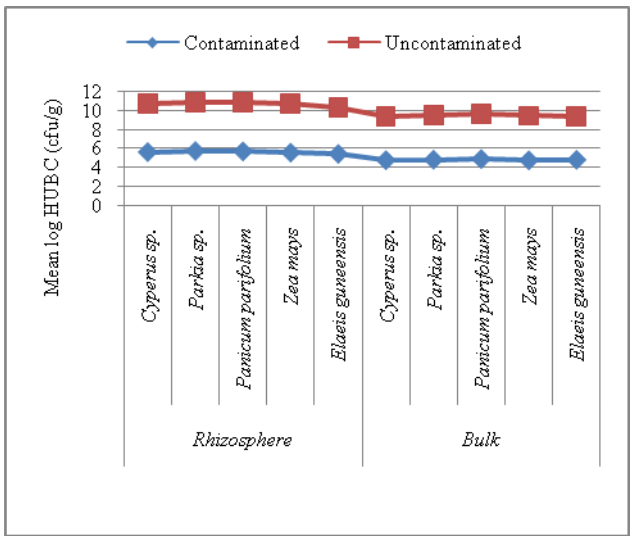

Figure 2. Hydrocarbon-utilizing bacterial counts of contaminated and uncontaminated rhizosphere and bulk soil samples

The result of the screen test for the utilization of hydrocarbon by the bacterial isolates as source of carbon and energy are presented in Table 1. All the bacterial isolates were able to utilize crude oil but at different growth rates.

Table 1. Screen test for the utilization of petroleum hydrocarbon by the bacterial isolates

Isolate Code	Growth in Crude Oil	Bacterial Isolate
PRU 1A	++	*Flavobacterium* sp.
PSU 2B	++	*Micrococcus* sp.
PRU 3A	++	*Alcaligenes* sp.
PSU 3A	++	*Bacillus* sp.
PRU 3B	+++	*Pseudomonas* sp.
PRU 1B	+	*Arthrobacter* sp.
NSU 4B	+	*Arthrobacter*sp.
NRU 5B	++	*Acinetobacter* sp.

Key: +++ = Heavy growth; ++ = Moderate Growth; + = Little Growth.

4. Discussion

In this study where the rhizo-bacterial genera were enumerated, the results show that colony forming units per gram of total culturable heterotrophic bacterial counts were higher in the contaminated rhizosphere than in the contaminated bulk of all the plants in the sampled site (Figure1). However, this difference was not significant statistically. [12] stated that when the mean population densities of bacteria in samples from contaminated soil are significantly greater than in background samples, the pollutants are being utilized. They then suggested that microbial enumeration is an evaluated response of microorganisms to hydrocarbons.

The mean counts of total culturable heterotrophic bacteria in the contaminated bulk were less than in the uncontaminated bulk of all the plants. This finding can be employed as an indicator of pollution in soils, even though, statistically, there was no significant difference in the population of the two soil samples. For example, the pollutants can alter the community structure through selection of pollutant degraders. The results obtained in this study, therefore, show that the presence of (crude oil) pollutant and/or the products of its metabolism may be toxic to some microorganisms resulting in population reduction. Microbial characteristics of soils are increasingly being evaluated as sensitive indicators of soil health because of clear relationships between microbial diversity, soil, plant quality and ecosystem sustainability [13].

The hydrocarbon-utilizing bacterial counts were also higher in the contaminated rhizosphere than in the uncontaminated rhizosphere of all the plants (Figure 2). The use of plants in combination with microbes has the advantage of causing an increase in microbial population numbers and metabolic activity in the rhizosphere [2].

Unlike the result of total culturable heterotrophic count, in the bulk soil, the mean counts of the hydrocarbon-utilizing bacteria were higher in the contaminated bulk soil for all the plants (Figure 2). This finding was also reported by [14] who observed that hydrocarbon-utilizing bacteria and fungi were readily isolated from soil and also that the application of oil or oily waste to soil resulted in increased numbers of hydrocarbon-utilizing bacteria and fungi. This phenomenon of selective enrichment causes the numbers of microorganisms that can utilize the compound of interest to increase within the community [14].

[7] stated that it has been generally accepted that bulk soil and rhizosphere microbial community structure can be determined by the local native microbial community, impacted by the soil effects and vegetation. The bacterial isolates obtained from different rhizosphere and bulk of plants found in contaminated and uncontaminated soils in this study were identified to be of the following genera: *Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Micrococcus and Pseudomonas*. This agrees with the findings of [15] who reported that a broad phylogenetic range of bacteria including species/strains of *Achromobacter, Acidovorax, Alcaligenes, Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, Micrococcus, Mycobacterium, Norcadia, Pseudomonas, Rhodococcus, Sphinogomonas* and *Xanthomonas* have been identified in the breakdown of hydrocarbons.

The screen test for petroleum hydrocarbon utilization presented in Table 1 showed that *Pseudomonas* sp. grew luxuriantly (+++), *Flavobacteria* and *Alcaligenes* grew moderately (++) while *Arthrobacter*sp. grew minimally on the crude oil growth medium. These organisms were isolated from contaminated rhizosphere soil. *Micrococcus* sp. and *Bacillus* sp. isolated from contaminated bulk soil grew moderately on the oil growth medium. *Acinetobacter* sp. isolated from uncontaminated rhizosphere grew moderately (++) on the oil growth medium while *Arthrobacter* sp. isolated from uncontaminated bulk grew minimally (+). This means that these bacteria can use hydrocarbon (crude oil) as a source of carbon and energy. The variation in the capacity of the isolates to utilize hydrocarbons could be due to differences in the competence of their crude oil degrading enzyme system. Some isolates may withstand toxic components of the oil and thrive, others may be inhibited. Other investigators [16] have made similar observations. The result also showed that some bacteria isolated from uncontaminated sites had the potential to utilize crude oil but these potentials were not as high as their counterparts isolated from contaminated sites.

5. Conclusion

The results of this study indicate that hydrocarbon-utilizing bacteria can be found in higher magnitude in the rhizosphere of the plants studied than in their bulk soil showing that all the plants' roots exudates induced the increase of bacterial population in the rhizosphere than in the bulk soil. These bacteria were found not to be restricted to petroleum contaminated soils only. The screen test for hydrocarbon utilization by the bacterial isolates showed that these isolates could be used as seeds for bioaugmentation during remediation of petroleum contaminated soil.

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Competing Interests

Authors have declared that no competing interests exist.

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