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Molecular characterization of autochthonous hydrocarbon utilizing bacteria in oil-polluted sites at Bodo Community, Ogoni land, Niger Delta, Nigeria

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Abstract

Hydrocarbon utilizing bacteria in water, soil and sediment samples collected from crude oil-impacted sites at Bodo community, Ogoniland in the Niger Delta were isolated using Bushnell-Haas agar. Bacterial genera tentatively characterized were *Bacillus, Corynebacterium*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Proteus*, *Pseudomonas* and *Serratia.* A portion of the 16S ribosomal ribonucleic acid (16S rRNA) gene of the genomic DNA extracted from each bacterial isolate was amplified with polymerase chain reaction (PCR) using the universal primer set 27F: GAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACT. The PCR products (*ca*.1500bp) were cleaned up and sequenced with the ABI Big Dye version 3.1 kit on ABI 3730XL genetic analyzer. Electropherograms were inspected with Chromas Lite 2.01 while sequence identification was performed using GenBank's BLAST algorithm. Sequence analysis revealed the presence of distinct known hydrocarbon degrading bacteria like *Acinetobacter radioresistens* strain Philippines-11, *Alcaligenes* sp. PAH-43, *Bacillus* sp. UR2, *Bacillus subtilis* strain B7, *Bacterium* NLAE-zl-H221, *Bacterium* NLAE-zl-H156, *Bacterium* NLAE-zl-H231, *Bacterium* NLAE-zl-H84, *Cedecea davisae* isolate PSB5, *Pantoea agglomerans* strain T224 (formerly *Enterobacter agglomerans*), *Proteus mirabilis* strain SNB5, *Proteus mirabilis* strain NBRC 13300, *Proteus mirabilis* strain ALK418, *Proteus penneri* strain YCY34, *Proteus mirabilis* strain TL14C1, *Proteus mirabilis* strain 73, *Proteus mirabilis* strain SZH18, *Proteus mirabilis* strain CIFR1, *Proteus hauseri* strain ZMd44, *Proteus vulgaris* strain Knp3, *Proteus mirabilis* strain D27, *Pseudomonas* sp. G4 (2013) and *Pseudomonas aeruginosa* strain XM1. It is highly recommended that16S rRNA-gene-based techniques be used when studying the bacterial community diversity and dynamics in oil-polluted ecosystems in order to fully describe the active microorganisms involved in hydrocarbon degradation.

Keywords: Hydrocarbon utilizing bacteria, Bodo Ogoniland, 16S rRNA gene, PCR, GenBank.

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Introduction

The Niger Delta is among the ten most significant wetland and marine ecosystems in the world but unsustainable oil exploration activities has rendered the Niger Delta region one of the five most severely petroleum-damaged ecosystems in the world. (FME, 2006). Oil producing areas of Nigeria especially the Niger Delta area have experienced the devastating consequences of crude oil spills to both terrestrial and aquatic environments in the past 5 decades of crude oil exploration

and production. The pollution of aquatic and terrestrial environments with hydrocarbons is widespread in the environment and is creating major health and environmental problems. The utilization of microorganisms to clean up hydrocarbons from a polluted environment represents a potential solution to such environmental problems (Akinde et al., 2012). Petroleum hydrocarbons can be degraded by microorganisms such as bacteria, fungi, cyanobacteria and microalgae. However, bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in an environment (Brooijmans et al., 2009). Hydrocarbon (petroleum) degrading bacteria are reportedly distributed in marine, soil and sediment habitats and their use in bioremediation of hydrocarbon contaminated environments exploits their ability to degrade or detoxify organic contaminants has been established as an economical, efficient, versatile and environmentally friendly treatment.

The ability to degrade hydrocarbon substrate is demonstrated by a wide range of bacteria such as *Pseudomonas, Alcaligenes, Bacillus, Corynebacterium, Klebsiella, Acinetobacter, Flavobacterium*, *Alcanivorax and Proteus* (Malakootian et al., 2009; Abdusalam et al., 2011) which can be characterized using culture-dependent and molecular based techniques (Brito et al., 2006; Babu et al., 2012; Subathra et al., 2013). Enumeration and monitoring of hydrocarbon degrading bacteria populations in contaminated environments using culture-dependent techniques often provides tentative identities of the cultured isolates. Recent advances in microbial ecology render it possible to combine molecular and culture-dependent approaches in order to describe bacterial diversity and their degradative ability in hydrocarbon polluted environments (Brito et al., 2012). For the past two decades microbiologists have relied on the use of 16S rRNA gene sequencing for identification, classification and estimation of bacterial diversity/dynamics in environmental samples through PCR and DNA sequencing (Chikere et al*.,* 2012). PCR is an extremely sensitive technique that allows the amplification of millions of copies of a portion of a desired gene, entire gene or gene clusters with high fidelity within 3 to 4h with the help of a DNA polymerase enzyme and specific primers (Chikere, 2013).

One of the major reasons for prolonged negative impact of oil spill on the environment could probably be due to absence of adequate and qualitative scientific baseline data which are required to provide informed and quick response to emergent environmental challenges. Therefore the objectives of this study were to characterize the *in-situ* culturable active indigenous hydrocarbon degrading bacterial community using culture-dependent and molecular technique and to assess the degradability capabilities of the bacterial isolates. This approach is expected to increase the possibilities of developing models and strategies for the bioremediation of hydrocarbon pollutants in the environment.

Materials and Methods

Sampling: Crude oil polluted water, soil and sediment samples were collected under aseptic conditions from chronically oil-impacted sites in Bodo community in Ogoniland, Rivers state of the Niger Delta. Samples were collected at different parts of each site and bulked for homogeneity and thereafter transported to the laboratory at 4^{o}C.

Enumeration of total culturable heterotrophic and hydrocarbon utilizing bacteria: Total culturable heterotrophic bacteria (THB) were determined using spread plate method on plate count (PC) agar while culturable hydrocarbon utilizing bacteria (HUB) were enumerated by vapour phase transfer method using mineral salt medium (Bushnell Haas agar) according to Hamamura et al. (2006). Individual colonies were phenotypically identified using Bergey's Manual for Determinative Bacteriology (Holt et al., 1994).

Degradation screening: Representative bacteria isolates were further screened for oil degradation capability under aerobic conditions by inoculating a calibrated loop full of 18h old culture of each bacterium into Bushnell Haas Broth containing 1ml of Okono medium crude oil.

Biodegradation was scored by turbidity and emulsification of oil in mineral broth medium after 14days incubation at 30^{o}C (Kostka *et al.,* 2011).

Molecular characterization of bacterial isolates: Genomic DNA was extracted from hydrocarbon utilizing bacterial isolates by boiling method according to Queipo-Ortuno et al. (2008). Universal primers 27F 5'AGAGTTTGATCCTGGCTCAG3' and 1492R 5'GGTTACCTTGTTACGACTT 3' were used to amplify a partial fragment (*ca*.1500bp) of the 16S rRNA gene from the extracted DNA using polymerase chain reaction (PCR). Amplified PCR products were purified and sequenced using an ABI 3130 XL genetic analyzer. Electropherograms of generated sequences were inspected with Chromas Lite 2.0.1 software while sequence identification was performed using GenBank's Basic Local Alignment Search Tool (BLAST) algorithm of National Centre for Biotechnology and Information (NCBI).

Results and Discussion

The presence of microbial activity was determined by the enumeration of total heterotrophic and hydrocarbon utilizing bacterial as presented in Table1. Soil recorded highest THB count with a mean value of 1.0 x 10^{7}cfu/g while sediment recorded highest HUB count of 1.0x10^{6} cfu/g. The result obtained shows that despite the degree of pollution in the various sites, the indigenous bacterial community are able to thrive and withstand the toxic concentration of the crude oil. A similar observation was reported by Eze and Okpokwasili (2011) and Ibiene et al. (2011). Thirty bacterial isolates out of 47 isolated were significant for crude oil degradability assay evidenced by turbidity (increase in cell biomass), emulsification and a concomitant visual gradual decrease in the oil layer after 14 days of incubation when compared with test isolates at zero day of incubation. This assay was also used in the selection of autochthonous hydrocarbon degrading bacteria by Oboh et al. (2006). Molecular-based technique was employed to confirm the tentative identities of bacterial isolates. PCR amplification of the 16S rRNA gene fragment was obtained for all isolates. The products all yielded *ca*. 1500bp when visualized in 1.5% tris acetate EDTA (TAE) agarose gel with ethidium bromide (Fig.1). Lanes 2, 3, 4 (HUBSED) and 3, 4 (HUBW) did not yield visible PCR products. Bands from amplified PCR products as shown in Fig.1 were purified and sequenced.

Table1. Total culturable heterotrophic and hydrocarbon utilizing bacteria counts

Sample	Mean values of THB	Mean values of HUB
Soil(cfu/g)	1.0x10^{7}	0.5x10^{6}
Water(cfu/ml)	3.1x10^{6}	3.9x10^{5}
Sediment(cfu/g)	2.7x10^{6}	1.0x10^{6}

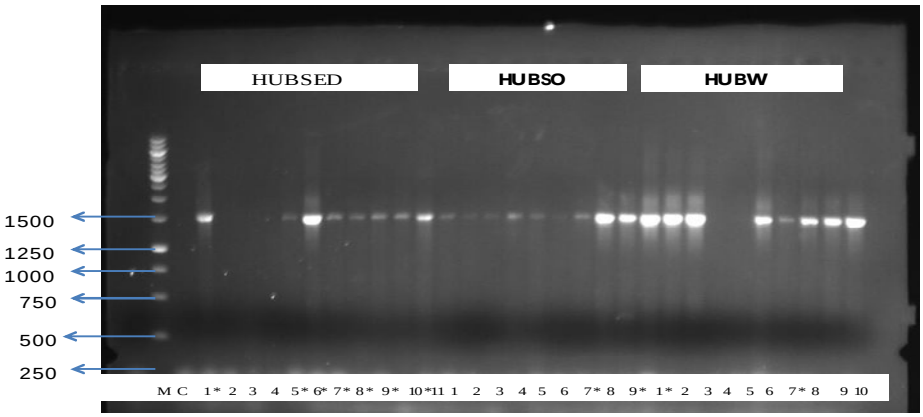

Fig. 1: Gel electrophoresis of PCR products amplified from genomic DNA extracted from sediment, soil and water samples in 1.5% agarose. (M) 250 bp ladder, (C) negative control, 1 -11(HUBSED), 1 - 9(HUBSO), 1-10(HUBW).

Bacterial 16S rRNA sequences were aligned with BLAST algorithm of National Centre for Biotechnology Information (NCBI) database. Sequences aligned showed 90-100% similarity with those deposited in GenBank and they were *Acinetobacter radioresistens* strain Philippines-11, *Alcaligenes* sp. PAH-43, *Bacillus* sp. UR2, *Bacillus subtilis* strain B7, *Bacterium* NLAE-zl-H221, *Bacterium* NLAE-zl-H156, *Bacterium* NLAE-zl-H231, *Bacterium* NLAE-zl-H84, *Cedecea davisae* isolate PSB5, *Pantoea agglomerans* strain T224 (formerly *Enterobacter agglomerans*), *Proteus mirabilis* strain SNB5, *Proteus mirabilis* strain NBRC 13300, *Proteus mirabilis* strain ALK418, *Proteus penneri* strain YCY34, *Proteus mirabilis* strain TL14C1, *Proteus mirabilis* strain 73, *Proteus mirabilis* strain SZH18, *Proteus mirabilis* strain CIFR1, *Proteus hauseri* strain ZMd44, *Proteus vulgaris* strain Knp3, *Proteus mirabilis* strain D27 and *Pseudomonas aeruginosa* strain XM1.

All of these bacteria are well known for their capacity to degrade hydrocarbons (Hamamura et al., 2006; Kostka et al., 2011) except *Cedecea davisae* which has not been reported in association with hydrocarbon degradation. The use of 16S rRNA in the characterization of hydrocarbon utilizing microorganisms is more reliable and sensitive than culture-dependent techniques alone (Brito et al., 2006; Brooijmans et al., 2009; Babu et al., 2012; Al-Awadhi et al., 2013) and the results obtained in this investigation are consistent with past field studies (Kostka et al., 2011; Kadali et al., 2012). The dominant genus obtained was *Proteus* and species of which have been isolated from various ecological and geographical environments as hydrocarbon degraders (Eze and Okpokwasili, 2010; Ibiene et al., 2011; Chikere and Azubuike, 2013).

Soil and marine hydrocarbon utilizing bacteria have been demonstrated to produce bioemulsifiers and biosurfactants which greatly enhance bioavailability by transporting hydrocarbons into cell through efficient uptake mechanisms (Cho et al., 2011) and as such species of *Pseudomonas* and *Bacillus* obtained in this study may have such potential (Nikolopoulou et al., 2009; Satpute et al., 2010). The isolation of high number of hydrocarbon utilizing microorganisms from petroleum-polluted environment is commonly taken as evidence that those organisms are the active hydrocarbon degraders in that environment. The microorganisms capable of surviving in such environment are those that have developed enzymatic and physiological responses that allow them use the hydrocarbons as substrates (Thenmozhi et al., 2012). These findings have revealed that there is an appreciable population of active indigenous hydrocarbon utilizing bacteria in oil-polluted sites in Bodo community which can be monitored and enhanced to bring about bioremediation in this area. The 16S rRNA gene marker employed in this study to identify and characterize bacteria isolates provided an efficient molecular approach to elucidate the bacterial diversity of the study area.

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