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Characterization of hydrocarbon utilizing bacteria in tropical marine sediments

C. B. Chikere*, G. C. Okpokwasili and O. Ichiakor

Department of Microbiology University of Port Harcourt, P. M. B. 5323, Port Harcourt, Rivers State, Nigeria.

Hydrocarbon utilizing bacteria present in Nembe waterside sediments, a marine habitat in Port Harcourt, Nigeria, were characterized using standard culture dependent techniques. The sediment samples were collected along the navigational route with an Eckman sediment grab (Wild Life Supply Co., NY). The samples had meant total heterotrophic bacterial count of 6.6×10^7 cfu g^{-1} and hydrocarbon utilizing bacteria of 8.22×10^2 cfu g^{-1}. The hydrocarbon utilizing bacteria isolated and identified belonged to the following genera; *Bacillus*, *Nocardia*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Acinetobacter* and *Enterobacter*. *Bacillus* spp. were the most isolated followed by *Pseudomonas* spp. Gas chromatographic analysis of the sediment sample showed a total petroleum hydrocarbon concentration of 102.02 mg kg^{-1} and presence of higher chain hydrocarbons like C_{14}, C_{16}, C_{18} and C_{28}. Species of the bacteria isolated are known hydrocarbon degraders and it is assumed that the genera identified from the sediment may have the catabolic capability to use petroleum hydrocarbons as source of carbon. Thus the marine sediments of the Niger Delta, Nigeria may habour important genera of bacteria that may have beneficial applications in petroleum microbiology.

Key words: Hydrocarbon utilizing bacteria, total petroleum hydrocarbons, Nembe waterside, Niger Delta.

INTRODUCTION

In Nigeria, the Niger Delta region produces more than 80% of the country's crude oil. There is presently an unprecedented increase in the upstream and downstream activities of the oil and allied companies in this area (Abu and Chikere, 2006). Over the years, these oil companies have generated myriad of pollutants in the form of gaseous emissions, oil spills, effluents and solid waste (Odeyemi and Ogunseitan, 1985; Nweke and Okpokwasili, 2004) that have polluted the marine environment beyond sustainability. Heightened navigational activities in inland and coastal waters of the Niger Delta region is another anthropogenic source of refined petroleum pollution of the aquatic environment. An investigation of the polycyclic aromatic hydrocarbons (PAHs) concentrations in some Niger Delta sediments carried out by Ezemonye and Ezemonye (2005) revealed elevated

elevated values of these priority pollutants in the sediments studied.

The continuous input of petroleum-based pollutants has resulted in an enriched microbial community capable of surviving toxic contamination. Micro-organisms are sensitive to fluctuations/changes in their environment. Whenever their chemical or physical environment is suddenly altered, there is a lag period during which the microbial community adapts to the new conditions (Leahy and Colwell, 1990; Chikere and Okpokwasili, 2004; Nweke and Okpokwasili, 2004). This lag period is also called acclimation period and enables the micro-organisms to acquire the metabolic repertoire necessary for their survival (Rosenberg and Ron, 1996; Abed et al., 2002; Head et al., 2006; Yakimov et al., 2007). This phenomenon has been shown to occur both in terrestrial and aquatic ecosystems (Leahy and Colwell, 1990; Macnaughton et al., 1999; Roling et al., 2004; Margesin et al., 2007).

Bacterial communities in sediments are instrumental in the marine food web, where they are responsible for recycling of nutrients and degradation of pollutants

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*Corresponding author. E-mail: ujuazed@yahoo.com. Tel: +2348057770448.

(Bordenave et al., 2004; Edlund and Jansson, 2006; Head et al., 2006). Several studies have revealed that the bacterial community composition in hydrocarbon-polluted sediments tend to comprise mostly bacteria that are specially adapted to use hydrocarbons as carbon sources (Engelhardt et al., 2001; Iwabuchi et al., 2002; Kasai et al., 2002). Other studies on oil-impacted marine environments have also demonstrated that some of these bacteria are rapidly and strongly selected when hydrocarbon degradation is stimulated by addition of nutrients (Iwabuchi et al., 2002; Kasai et al., 2002; Roling et al., 2002; Xu and Obbard, 2003; Xu et al., 2004; Head et al., 2006). Information on the composition of the bacterial populations in a polluted site is of valuable importance in order to estimate the self-purification capability of the ecosystem and the feasibility of biological decontamination if engineered bioremediation should be considered (Allen et al., 2007; Said et al., 2008)

The main objective of the present study was to characterize bacterial community inhabiting the sediment from Nembe waterside Port Harcourt, Nigeria. This water-way is subject to human-induced pressures resulting from urbanization, industrialization and intensive navigation. It links Port Harcourt City with Bonny Island where most of the oil installations in Rivers State are. It also links the Island directly with the Atlantic ocean through which crude oil is exported by massive oil tankers. From the sediment samples, bacterial strains which are able to utilize crude oil as a carbon source were isolated and characterized.

MATERIALS AND METHODS

Sample collection

The sampling site was Nembe waterside which is very close to Creek road market, Port Harcourt, Nigeria. The deepest part of the waterway was chosen as it is part of the navigational route for the boats and other vessels. The sediment samples were collected with an Eckman sediment grab (Wild Life Supply Co., NY) and put into 95% ethanol-sanitized plastic containers, then taken to the laboratory for analysis.

Bacterial enumeration

For total heterotrophic bacterial count (THB), the initial inoculum was transferred in a series of eight 10-fold serial dilutions using physiological saline. Decimal dilutions were conducted and 0.1 ml aliquot of 10^{-6} dilution was inoculated by spread plate method onto nutrient agar plates in duplicates. The plates were incubated at 30°C for 24 h. Hydrocarbon utilizing bacterial counts (HUB) were enumerated by inoculating 0.1 ml aliquot of 10^{-6} sediment suspension onto mineral salts agar plates prepared according to Mills et al. (1978). The hydrocarbon source was supplied through the vapour phase by placing filter papers(Whatman No.1) impregnated with 1 ml of filter-sterilized Bonny light crude oil on the lids of the plates. The plates were incubated at 30°C for 7 d. All plates yielding 30 - 300 colonies were counted.

Isolation and identification of hydrocarbon utilizing bacteria

Discreet colonies were subcultured onto nutrient agar and the purity of each colony was confirmed by Gram staining. Those found to be mixed were further subcultured and gram stained. Pure isolates were identified by routine microbiological tests including gram staining, micro and macro morphological characteristics, catalase and oxidase production, cell motility examined with motility agar. The following biochemical tests were performed according to standard bacteriological methods: gas/acid production from fermentation of glucose, maltose or lactose, indole production, citrate utilization, triple sugar iron fermentation and methyl red-Voges Proskaeur test.

Hydrocarbon analysis of the sediment sample

The hydrocarbons in the sediment sample were quantified using a Hewlett Packard 6890 series gas chromatograph equipped with flame ionization detector (GC-FID). The carrier gas was helium and the column used was DB-5 (DB 5 (125-50320) with the dimensions: 30*320 μm * 0.00 μm. For the detector, its temperature was 350°C, hydrogen gas flow rate was 35 ml/min, air flow rate was 250 ml/min, while nitrogen gas flow rate was 20 ml/min. The inlet which was of electronic pneumatic capture splitless make was operated thus: pressure (psi) 4.18; split flow rate (8 ml/min); total flow rate (11.9 ml/min) and temperature (275°C). For the oven, the initial and final temperatures were 40 and 325°C respectively, the run time was 38.3 min, pressure was 4.18 psi while flow rate was 0.8 ml/min.

RESULTS AND DISCUSSION

It was observed that the Nembe waterside sediment sample contained a mean THB count of 6.66×10^{7} cfu g^{-1} and a mean HUB count of 8.22×10^{2} cfu g^{-1}. This water-way is continuously exposed to petroleum hydro-carbons owing to navigational activities and this may have enriched the sediment with hydrocarbon utilizing bacteria. However, the paucity of the HUB counts may be attributed to the inadequacy of nutrients at that depth especially nitrogen and phosphorus which deplete with input of hydrocarbons. Another factor that reduces available metabolic nutrients in marine environment according to Xu et al. (2004) is heavy leaching caused by tidal inundation and wave action. The gram positive bacteria belonging to the genus *Bacillus* were the most isolated followed by gram negative *Pseudomonas.* Other genera isolated were *Nocardia*, *Enterobacter*, *Flavo-bacterium*, *Acineto-bacter*, *Staphylococcus* and *Escherichia*. These isolates have also been isolated from hydrocarbon polluted environment by other investigators. Kasai et al. (2002) isolated *Flavobacterium* spp. from oil-polluted marine environment capable of degrading aromatic hydrocarbons in crude oil. In the same vein, Edlund and Jansson (2006) found out that members of the class *Gammaproteobacteria* (*Pseudomonas* spp. inclusive) and *Flavobacterium* spp. were the most dominant bacteria in a highly PAH- and polychlorinated biphenyl- polluted sediment before and after dredging. Said et al. (2008) isolated *Bacillus*, *Staphylococcus*, *Pseudomonas* and

Acinetobacter spp. capable of degrading PAHs from a polluted sediment. It is most likely that the bacterial isolates from Nembe waterside will have the capability of degrading petroleum hydro-carbons as well. *Nocardia* spp. and other *Actinobacteria* are frequently isolated from hydrocarbon polluted sites. The works of Margesin et al. (2003) and Quatrini et al. (2008) demonstrated that *Actinobacteria* play important role during petroleum hydrocarbon degradation. Members of the *Enterobacteriaceae* family have also been isolated from marine environment and most likely they may have been introduced via faecal matter from man and animals.

The GC-FID analysis of the sediment sample revealed that the total petroleum hydrocarbon (TPH) concentration in the sediment was 102.02 mg/kg. The chromatogram showed that C_{14} (49.01 mg/kg), C_{16} (31.26 mg/kg), C_{18} (14.37 mg/kg) and C_{28} (7.38 mg/kg) were the chain lengths present in the sediment. These hydrocarbons are the heavier aliphatics, which are hydrophobic and can easily partition to the sediment following petroleum spill thus the absence of the lighter fraction may be as a result of volatilization immediately after spill (Kasai et al., 2002). The presence of these hydrocarbons may have resulted in the enrichment of these hydrocarbon utilizers in the sediment. Study by Abu and Chikere (2006) revealed the presence of hydrocarbon utilizing bacteria in another marine environment in Port Harcourt, Nigeria. It is most likely that this is a common feature of the marine environment in Niger Delta, Nigeria.

According to Leahy and Colwell, (1990) and Macnaughton et al. (1999) the presence of hydrocarbons in the environment selects for microorganisms capable of surviving toxic contamination which concomitantly may result to the degradation of such hydrocarbons. It may as well be that the bacterial isolates from Nembe waterside sediment could have the catabolic capability to degrade the hydrocarbons in the sediment.

Conclusion

The study revealed the presence of petroleum hydrocarbons in the sediment of Nembe waterside as well as known genera of hydrocarbon utilizing bacteria. The biases associated with culture-dependent microbial enumeration techniques limited the full description of the bacterial diversity in Nembe waterside. It is an established fact that more than 90% of micro-organisms in the environment are unculturable and as such they can only be detected with molecular methods used in the field of metagenomics (Macnaughton et al., 1999; Edlund and Jansson, 2006).

Application of genomic technologies in conjunction with more conventional biochemical and microbial community analysis will help in providing exciting opportunities for increasing our understanding of the vast microbial diversity under the waters in the oil rich Niger Delta, Nigeria (Head et al., 2006).

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