Journal of Petroleum & Environmental Biotechnology

ISSN: 2157-7463

Isiodu et al., J Pet Environ Biotechnol 2016, 7:1
DOI: 10.4172/2157-7463.1000264

Research Article Open Access

Role of Plasmid-Borne Genes in the Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Consortium of Aerobic Heterotrophic Bacteria

Isiodu GG*, Stanley HO, Ezebuiro V and Okerentugba PO

Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, P.M.B 5323, Port Harcourt, Nigeria

Abstract

Background/Aim: Certain plasmids play an important role in adaptation of natural microbial populations to oil and other hydrocarbons. This research investigated the role of plasmid-borne genes in the biodegradation of polycyclic aromatic hydrocarbons (PAHs) by a consortium of aerobic heterotrophic bacteria (AHB).

Methods: Physicochemical and microbiological characteristics of the water sample were analyzed using standard methods. AHB with the capacity for biodegradation were isolated from hydrocarbon polluted water from Bodo Creek using Bushnell Haas Agar. The positive isolates were subjected to plasmid profiling and curing. Three treatments: cured, uncured and control were used in the study. Curing was achieved with acridine orange. The degradation experiment was carried out in a 10 L glass bioreactor and monitored for 56 days using GC-MS analysis.

Results: Seven (7) out of 19 AHB isolates were selected for the degradation study. The 7 isolates, G1, G3, G19, GA5, GB3, GD1 and G12 were classified as *Shewanella haliotis* G1, *Shewanella* sp. G3, *Vibrio alginolyticus* G19, *Pseudomonas putida* GA5, *Bacillus cereus* GB3, *B. pumilus* GD1 and *Shewanella* sp. G12, respectively based on the phylogenetic analysis of their 16S rRNA genes. The sequences were deposited at the GenBank under the accession numbers KT886070-KT886076. The day 0 PAHs for cured, uncured and control treatments was 61.83 mg/L. After 56 days, the PAHs decreased to 2.90, 1.87 and 57.65 mg/L for cured, uncured and control treatments, respectively; representing percentage degradation of 95.31, 96.98 and 6.76%. The respective derived PAH degradation models for cured, uncured and control treatments were $32.614e^{-0.047t}$, $30.09e^{-0.05t}$ and -0.0769t + 61.656. There was no significant difference ($p<0.05$) between PAH degradation in cured and uncured treatments.

Conclusion: The study demonstrated that PAH degradation by the consortium used in the study was chromosomal and not plasmid-borne.

Keywords: Aerobic heterotrophic bacteria; Polycyclic aromatic hydrocarbons; Plasmid; Curing; Gas chromatography-mass spectrometry

Introduction

Petroleum-based products are the major source of energy for industry and daily life. Increase in its exploration and production has brought about high rate of environmental pollution involving both terrestrial and aquatic habitat [1]. The release of hydrocarbons into the environment whether accidentally or due to human activities often pose severe, immediate and long-term ecological and environmental repercussion, since a lot of hydrocarbon constituents are toxic and persistent in terrestrial and aquatic environments. Several physicochemical methods of decontaminating the environment have been established and employed. However, such methods are usually established and labour-intensive and often involve the risk of spreading the pollution because the waste would require disposal elsewhere. A better way would be to use biodegradation [2] (Figure 1).

Ogoniland in the Niger Delta region of Nigeria where the research was carried out has witnessed an intermittent discharge of crude oil into its environment since the inception of crude oil exploration and exploitation. This trend has led to the destruction of its farmlands, aquaculture, rivers and creeks with hydrocarbon compounds [3].

PAHs are formed naturally during thermal geological reactions associated with fossil-fuel and mineral productions and during burning of vegetation in forest and bush fires [4,5]. Anthropogenic sources, particularly fuel combustion, automobiles, spillage of petroleum products, and waste incinerators are significant sources of PAHs into the environment. Aromatic hydrocarbons are among the most prevalent and persistent pollutants in the environment.

Certain plasmids play an important role in adaptation of natural microbial populations to oil and other hydrocarbons. Some of the microbial catabolic pathways responsible for the degradation, including the *alk* (C_5 to C_{12} *n*-alkanes), *nah* (naphthalene) and *xyl* (toluene) pathways have been extensively characterized and are generally located on large catabolic plasmids [6]. Furthermore, many reports have described and characterized microorganisms that can catabolize both aliphatic and aromatic hydrocarbons [7]. Several environmental isolates of *Acinetobacter* sp. and *Alcaligenes* sp. *Arthrobacter* sp. and two *Rhodococcus* strains have been found to degrade both alkanes and naphthalene, although the genes and catabolic pathways responsible were not described [8-10].

Plasmids are also important in the development (transformation) of new organisms with enhanced degradative capability. Many bacteria of diverse genera have extra chromosomal DNA, suggesting the widespread presence of plasmids in prokaryotic cells. Thus the ability

***Corresponding author:** Isiodu GG, Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, P.M.B 5323, Port Harcourt, Nigeria, Tel: +2348030926873; E-mail: honeygloriano@gmail.com

Receive November 28, 2015; **Accepted** January 10, 2016; **Published** January 10, 2016

Citation: Isiodu GG, Stanley HO, Ezebuiro V, Okerentugba PO (2016) Role of Plasmid-Borne Genes in the Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Consortium of Aerobic Heterotrophic Bacteria. J Pet Environ Biotechnol 7: 264. doi:10.4172/2157-7463.1000264

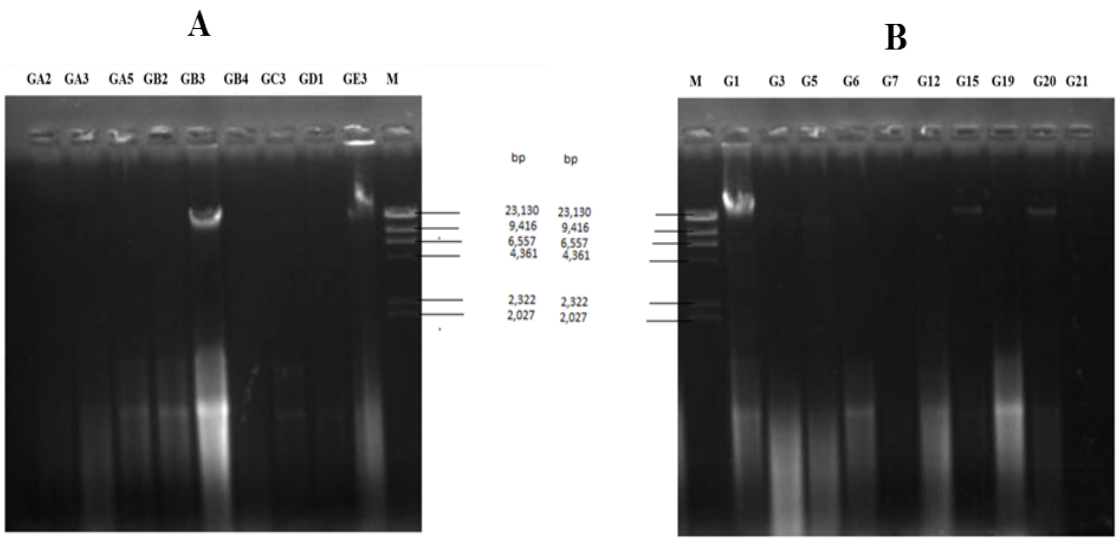


Figure 1: Plasmid profile of bacterial isolates by HIND III digests.

to identify plasmids in bacterial systems is important. Using molecular biology techniques, it is possible to slice pieces of DNA containing genes for specific degradative pathways into plasmids. These plasmids can then be introduced into a host organisms resulting in a recombinant or genetically engineered microorganisms (GEM) with new degradative capabilities. These are used to remediate contaminated sites mainly as organisms for bioaugumentation [11]. Appropriate environmental factors are however, essential for the performance of these organisms [12].

This study was aimed at evaluating the role of plasmid-borne genes in the biodegradation of polycyclic aromatic hydrocarbons (PAHs) by a consortium of aerobic heterotrophic bacteria isolated from Ogoniland in the Niger Delta area of Nigeria.

Materials and Methods

Site description

The aerobic heterotrophic bacteria used in this study were isolated from Bodo Creek brackish water contaminated with crude oil. Bodo Creek is located in Bodo community of Gokana Local Government Area, in the Niger Delta Basin of Nigeria.

Sample collection

Five (5) samples of hydrocarbon polluted brackish surface water from a distance of 50 m apart at 5 points were collected with a sterile container and taken to environmental microbiology laboratory of the University of Port Harcourt for the isolation of hydrocarbon utilizing bacteria.

Physicochemical analysis

The baseline physicochemical characteristics of the water samples were analyzed. The parameters that were analyzed using standard methods as described by APHA included: nitrate, total nitrogen, phosphate, total organic carbon, pH, temperature, electrical conductivity, sulphate and turbidity. Salinity was determined using refractometer (S/mill-E Range 0-100%) [13]. The Gas chromatographic analyses were carried out to estimate the polycyclic aromatic hydrocarbons (PAH) and the total petroleum hydrocarbons (TPH). TPH was analyzed using the USEPA 8015. Sample extraction was carried out with dichloromethane followed by sample clean-up and separation. One microliter (1 µl) of extract after concentration to 1ml was injected into the Agilent 6890 GC-FID for analysis. The concentration of TPH was electronically deduced from the calibration graph.

Polycyclic aromatic hydrocarbons (PAH) were analyzed using the USEPA 8270**.** Sample extraction was carried out with dichloromethane followed by sample clean-up and separation. One microliter (1 µl) of extract after concentration to 1ml was injected into Agilent 7890 GC-MS for analysis. The concentration of the individual component of PAH was automatically deduced from the calibration graph (Figure 2).

Microbiological analysis

Serial dilution of sample: Ten-fold serial dilution was performed on the samples as described by Jalal [14].

Isolation and enumeration of hydrocarbon utilizing bacteria: Exactly 0.1 ml each of 10^{-3}, 10^{-4} and 10^{-5} dilutions of the serially diluted samples were spread onto Bushnell Haas Agar medium (g/L: $MgSO_4.7H_20$- 0.2g; $CaCl_2.2H_2O$- 0.02g; KH_2PO_4- 1g; K_2HPO_4- 1g; NH_4NO_3- 1g; $FeCl_3$- 0.05g; Agar 20g; nystatin- 0.5g; pH 7.0 ± 0.2) in duplicates. The medium was amended with 5% crude oil and sterilized in an autoclave at 121°C (15 psi) for 15 min). The plates were incubated at 37°C for 4 days. The plates with colonies ranging between 30 and 300 were selected and the average taken as the hydrocarbon utilizing bacterial count. Discrete colonies from the agar plates were selected based on their difference in morphology and sub-cultured on nutrient agar to obtain pure culture of the isolates. The pure cultures were maintained in minimal salt agar slant for further studies. The total viable count (TVC), expressed in colony forming units per ml (cfu/ml) was calculated using the formula below:

$$\text{TVC (cfu/ml)} = \frac{\text{No of colonies} \times \text{dilution factor}}{\text{Volume of inoculum}}$$

Preliminary biodegradation test: The preliminary biodegradation test was carried out in test tubes containing Bushnell Haas broth amended with 1% crude oil. The isolates were inoculated into each test

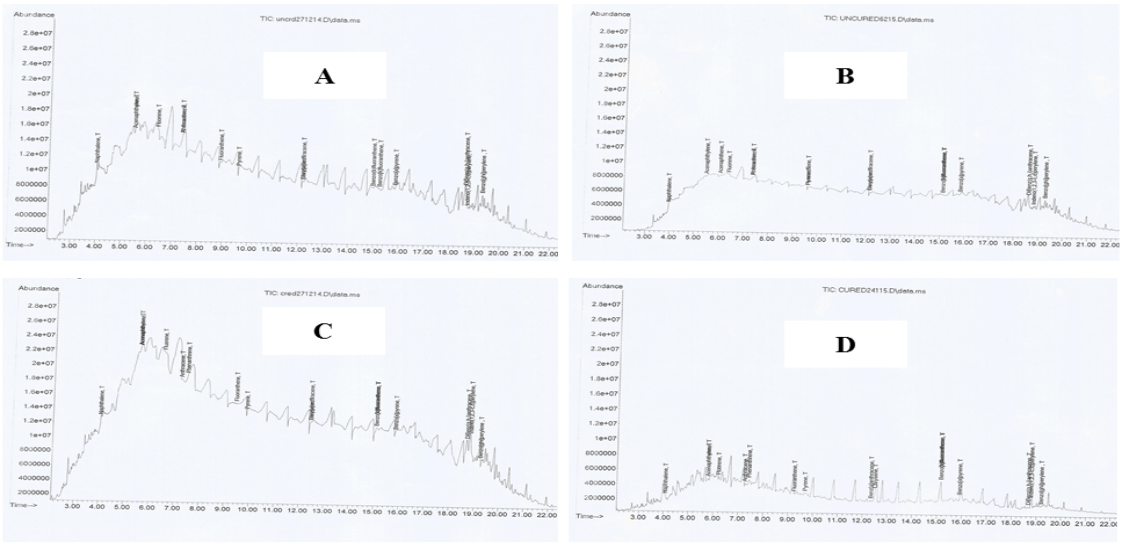


Figure 2: GC-MS chromatography of PAH component degradation over time for different treatments.

tube and incubated at 37°C for 14 days. Biodegradation was monitored spectrophotometrically (Optima SP-300 spec. Japan) by measuring the optical density of the medium at 600 nm wavelength.

Plasmid extraction and curing: plasmid isolation (Gram +ve bacteria): Precisely 1.5 ml of overnight culture was spun for 1 minute in a micro-centrifuge to pellet cells. The cells were suspended in 200 µl of solution A (100 mM glucose, 50 mMTris hydrochloride (pH 8), 10 mM EDTA) containing 10 mg of lysozyme per ml and incubated for 30 min at 37°C. Four hundred microlitre (400 µl) of freshly prepared 1% sodium dodecyl sulfate (SDS) in 0.2 N NaOH was added and the samples mixed by inverting tubes. Exactly 300 µl of a 30% potassium acetate solution (pH 4.8) was added and mixed by vortexing. After incubating on ice for 5 min, debris was removed by a 5-minute centrifugation. The supernatant was transferred and extracted once with a phenol-chloroform mixture (1:1). Plasmid DNA was precipitated with an equal volume of isopropanol. The precipitated plasmid DNA was allowed to dry and dissolved with TE buffer [15].

Plasmid isolation (TENS – Mini prep) for gram -ve bacteria: Exactly 1.5 ml of overnight culture was spun for 1 minute in a micro-centrifuge to pellet cells. The supernatant was gently decanted, leaving 50 – 100 µl together with cell pellet (cells were harvested into 100 µl of nutrient broth since they were grown on agar) and vortexed at high speed to re-suspend cells completely. Thereafter, 300 µl of TENS was added. The tubes were mixed by inverting them 3 – 5 times until the mixture became sticky. When more than 10 min was needed before moving to the next step, samples were set on ice to prevent them from the degradation of chromosomal DNA which may be co-precipitated with plasmid DNA in steps. One hundred and fifty microlitre (150 µl) of 3.0 M sodium acetate pH 5.2 was added and vortexed to mix completely. The mixture was spun for 5 min in micro-centrifuge to pellet cell debris and chromosomal DNA. The supernatant obtained was transferred into a fresh tube; mixed well with 900 µl of ice-cold absolute ethanol. This was spun for 10 minutes to pellet plasmid DNA. (White pellet was observed). The supernatant was discarded; the pellet rinsed twice with 1 ml of 70% ethanol and dried. The pellet was re-suspended in 20 - 40 µl of TE buffer for further use. (TENS composition: Tris 25 mM, EDTA 10 mM, NaOH 0.1 N and SDS 0.5%) [16].

Plasmid curing by treatment with acridine orange: The plasmid curing was performed using the method described by Silhavvy with slight modification. Bacterial cells were grown in broth overnight [17]. Five (5) ml of Nutrient broth supplemented with 0.1 mg/ml acridine orange was prepared. The organisms were sub-cultured into Nutrient broth containing the acridine orange, incubated at 37°C from 48 h to about one week and plated out on nutrient agar. Colonies able to grow on nutrient agar but on solid medium were isolated and considered cured. These colonies were subjected to plasmid profiling to confirm loss of plasmid.

Experimental design

The final experiment comprised three set-ups namely: cured set-up, uncured and control. In the cured set-up the consortium cured of plasmid were used to inoculate the crude oil contaminated water sample with crude oil concentration of 5% v/v. The uncured set-up contained the same crude oil concentration but with isolates still retaining their plasmids while the control set-up contained no inoculum.

Biodegradation experiment

The degradation experiment was carried out in a 25 L glass bioreactor and monitored for TPH, PAH, pH, and microbiological parameters (count of hydrocarbon utilizing bacteria and total heterotrophic bacteria) fortnightly within a period of 56 days (Figure 3).

Characterization of the selected bacterial consortium

Phenotypic characterization: The selected bacterial isolates were subjected to several biochemical methods as described by Holts, MacFaddin and Madigan [18-20].

Chromosomal DNA extraction: DNA extraction, PCR amplification of the bacterial 16S rRNA genes and gel electrophoresis of the isolate were carried out at the Molecular Biology Laboratory of National Institute for Medical Research (NIMR), Yaba, Lagos, Nigeria. The PCR product was sent to GATC Biotech AG (European Genome and Diagnostics Centre - Jakob-Stadier-Platz 7, 78467 Constance, Germany) where the Sanger Sequencing was carried out. DNA

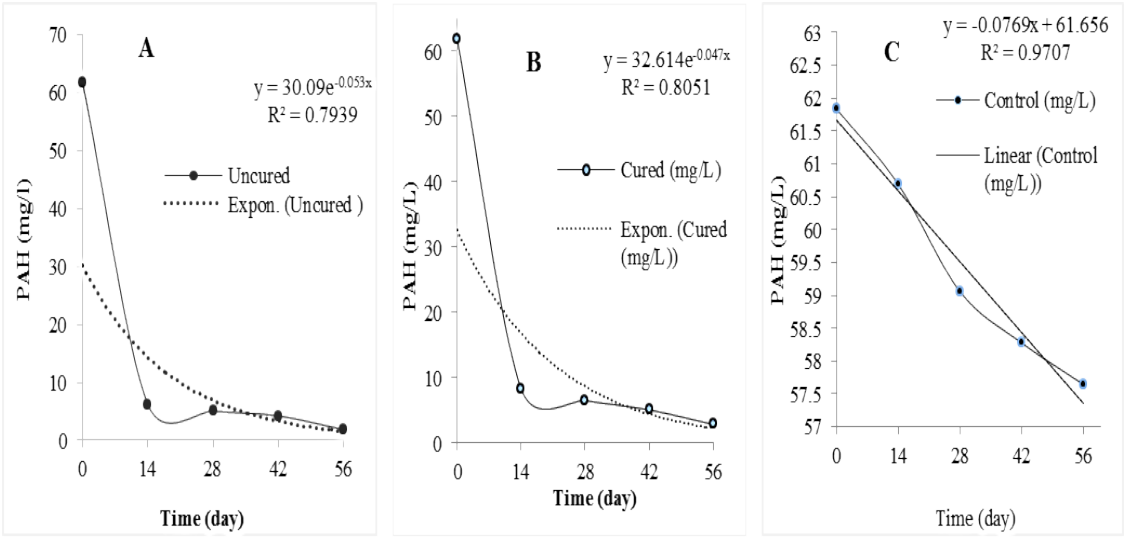


Figure 3: PAH degradation model for the treatments.

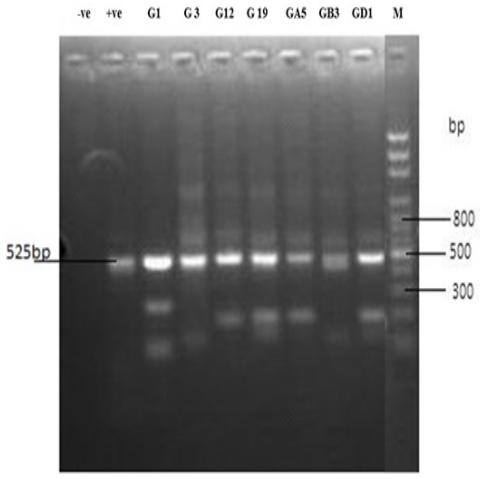
extraction was carried out directly from the sample using a Qiagen QiaAMP DNA extraction kit according to manufacturer's instruction.

PCR amplification of bacterial 16S rRNA gene: The PCR amplification of the 16S rRNA genes was carried out using the primer set 27F- 5'- AGA GTT TGA TYM TGG CTC AG -3', and 515R 5'- TTA CCG CGG CKG CTG GCA C-3'. The reaction was carried out according to the method described by Yamada and Katsura [21,22]. Twenty microlitres (20 µl) reaction mixture containing 1X PCR buffer (Solis Biodyne, Estonia), 1.5 mM Magnesium chloride (Solis Biodyne, Estonia), 0.2 mM of each dNTP (Solis Biodyne, Estonia), 2 U Taq DNA Polymerase (Solis Biodyne, Estonia), 20 pMol of each primer and sterile water was used to make up the reaction mixture. PCR was carried out in an Eppendorf Nexus thermal cycler with the following cycling parameters: an initial denaturation step at 95°C for 5 min, followed by 30 consecutive cycles of denaturation at 95°C for 30 sec., annealing at 55°C for 45 sec., and extension at 72°C for 1 min. After this, a final extension at 72°C for 10 min was carried out.

Agarose gel electrophoresis: After the PCR reaction, PCR product was separated on a 1.5% agarose gel (Solis Biodyne, Estonia). One hundred base pair (100 bp) DNA ladder (Solis Biodyne, Estonia) was used as DNA molecular weight marker. Electrophoresis was done at 80 V for 1 h 30 min, and the gel was viewed under UV light after staining with ethidium bromide (Solis Biodyne, Estonia) (Figure 4).

Sequence analysis: The sequences generated by the sequencer were visualized using Chromaslite for base calling. BioEdit was used for sequence editing and Basic Local Alignment Search Tool (BLAST) performed using NCBI (National Centre for Biotechnology Information) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Similar sequences were downloaded and aligned with ClusterW and phylogenetic tree was drawn with MEGA 6 software [23].

Statistical analysis: The results were compared by one-way analysis of variance (one-way ANOVA) and multiple range tests to find the differences between the measurement means at 5% (0.05) significance level using IBM® SPSS® Statistics Version 20.0 (Gailly and Adler, US).



(Level 1 & 2: control 3-9; 16S rRNA of the isolates; lane 10(M): Ladder)

Figure 4: Agarose gel electrophoresis showing 16S rRNA gene bands.

Results

Baseline physicochemical and microbiological analysis of the water sample

The values of the baseline characteristics of the water sample where the basic bacterial isolations were made are presented in Table 1. The total heterotrophic bacteria (THB) and hydrocarbon utilizing bacterial counts were 1.70 ± 0.13×10^6 and 5.70 ± 0.91×10^6, respectively. The respective TPH and PAH values were 7691 ± 13 and 7.49 ± 0.25 mg/L while the phosphate and nitrogen contents were 40.8 ± 1.2 and 673.3 ± 5.6 mg/L, respectively.

Isolation of aerobic hydrocarbon utilizing bacteria

A total of nineteen (19) aerobic hydrocarbon utilizing bacteria (HUB) were isolated from the polluted water samples using Bushnell Haas agar (Figure 5).

Parameter	Value
Physicochemical	
pH	7.54 ± 0.10
Electrical conductivity (µS/cm)	3160 ± 12
Turbidity(NTU)	252 ± 8
Salinity (ppt)	19.67 ± 1.03
TOC (%)	3.06 ± 0.20
Total phosphate (mg/L)	40.8 ± 1.2
Total nitrogen (mg/L)	673.3 ± 5.6
Nitrate (mg/L)	40.61 ± 2.3
TPH (mg/L)	769 ± 13
PAH (mg/L)	7.49 ± 0.25
Sulphate (mg/L)	305 ± 11
Microbiological	
Total heterotrophic bacteria (THB) (cfu/ml)	$1.70 \pm 0.13 \times 10^6$
Hydrocarbon utilizing bacteria (HUB) (cfu/ml)	$5.7 \pm 0.91 \times 10^5$

Table 1: Mean baseline physicochemical and microbiological parameters of Bodo Creek surface water.

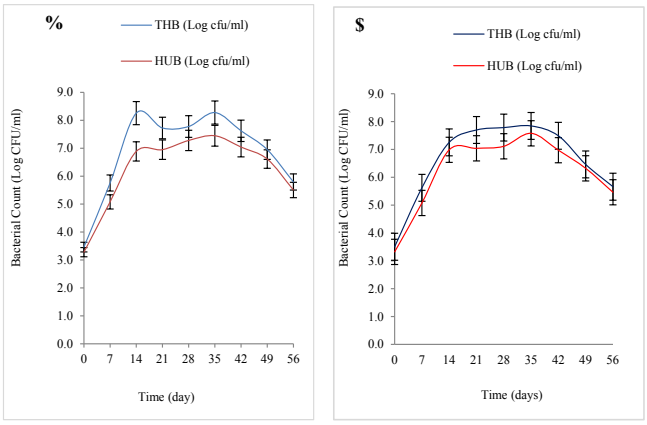


Figure 5: Bacterial growth over time in the treatments. A (uncured treatment); B (cured). (T H B = total cultural heterotrophic bacteria; HUB = hydrocarbon utilizing bacteria; cfu/ml = colony forming unit per millilitre of sample).

Plasmid profiling and curing

Twelve (12) out of the nineteen (19) bacterial isolates showed the presence of degradative plasmid with size ranging between 2027 and 23130 bp.

Selection of consortium for biodegradation

The result of the preliminary biodegradation experiment showed that the twelve isolates were capable of degrading PAH hydrocarbon components however 7 isolates (G1, G3, G12, G19, A5, B3 and D1) which showed higher optical density values were selected for the proper biodegradation test.

PAH analysis of bioreactor treatments

The PAH components present in the Bonny light crude oil as detected by GC-MS analysis were naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benz(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benz(a)pyrene, dibenz(g,h,i)anthracene, indeno(1,2,3-c,d)perylene and benzo(g,h,i)perylene for both cured and uncured treatment on day 0. The total amount of PAH was 61.83 mg/L. The total amount of PAH after contamination of water with crude oil and inoculums showed that cured, uncured and control had PAH of 61.83 mg/L respectively (Table 2). PAH values during the experiment showed reduction for the cured treatment from 61.83 mg/L to 2.90 mg/L, uncured from 61.83 mg/L to 1.87 mg/L and control from 61.83 mg/L to 57.65 mg/L for day 0 and day 56 respectively. The percentage degradation of PAH was 96.98, 95.31, and 6.76% for the uncured, cured and control treatments, respectively in 56 days.

PAH degradation modeling in cured, uncured and control treatments

PAH degradation was monitored for a period of eight (8) weeks (56 days) for the uncured, cured and control treatments. Mathematical modeling of PAH degradation through non-linear regression method revealed that PAH content degradation followed an exponential pattern for the uncured and cured samples while that of the control followed a linear curve The respective derived PAH degradation models for uncured, cured and control treatments are $30.09e^{-0.05t}$, $32.614e^{-0.047t}$ and -0.0769t + 61.656. The R^2 values for the uncured, cured and control models are 0.7939 (79%), 0.8051 (81%) and 0.9707 (97%) respectively.

PCR amplification of 16S rRNA gene

All the 7 isolates showed amplification with an amplicon size of 500 bp.

Phylogenetic tree analysis of the isolates

Phylogenetic tree analysis of the sequences generated from the isolates revealed that the isolates belonged to four genera namely: *Bacillus*, *Pseudomonas*, *Shewanella* and *Vibrio*. The phylogenetic tree constructions are given in Figure 6. The 7 isolates, G1, G3, G19, GA5, GB3, GD1 and G12 were classified as *Shewanella haliotis* G1, *Shewanella* sp. G3, *Vibrio alginolyticus* G19, *Pseudomonas putida* GA5, *Bacillus cereus* GB3, *B. pumilus* GD1 and *Shewanella* sp. G12.

Discussion

In this study, 19 hydrocarbon utilizing bacteria were isolated from Bodo Creek brackish water and screened for hydrocarbon degradation as well as the presence of plasmids. Out of these 19 isolates, 7 isolates (G1, G3, G19, GA5, GB3, GD1 and G12, classified as *Shewanella haliotis* G1, *Shewanella* sp. G3, *Vibrio alginolyticus* G19, *Pseudomonas putida* GA5, *Bacillus cereus* GB3, *B. pumilus* GD1 and *Shewanella* sp. G12, respectively based on the phylogenetic analysis of their 16S rRNA genes) were chosen for the biodegradation study based on their first, hydrocarbon utilizing capabilities and then the presence of degradative plasmids.

Many researchers (Onifade and Abubakar, Chikere and Ekwuabu, Ichor) have reported the isolation of hydrocarbon utilizing bacteria from contaminated brackish water [24-26]. The bacterial strains used in this study showed polycyclic aromatic hydrocarbon degradation ability. Said reported the degradation of PAH by *Shewanella* species

Time (day)	Treatments		
	Uncured (mg/L)	Cured (mg/L)	Control (mg/L)
0	61.83	61.83	61.83
14	6.21	8.32	60.69
28	5.06	6.54	59.06
42	4.24	5 .13	58.28
56	1.87	2.90	57.65
Percentage degradation (%)	96.98	95.31	6.76

Table 2: Summary of PAH content of the treatments during the biodegradation experiment.

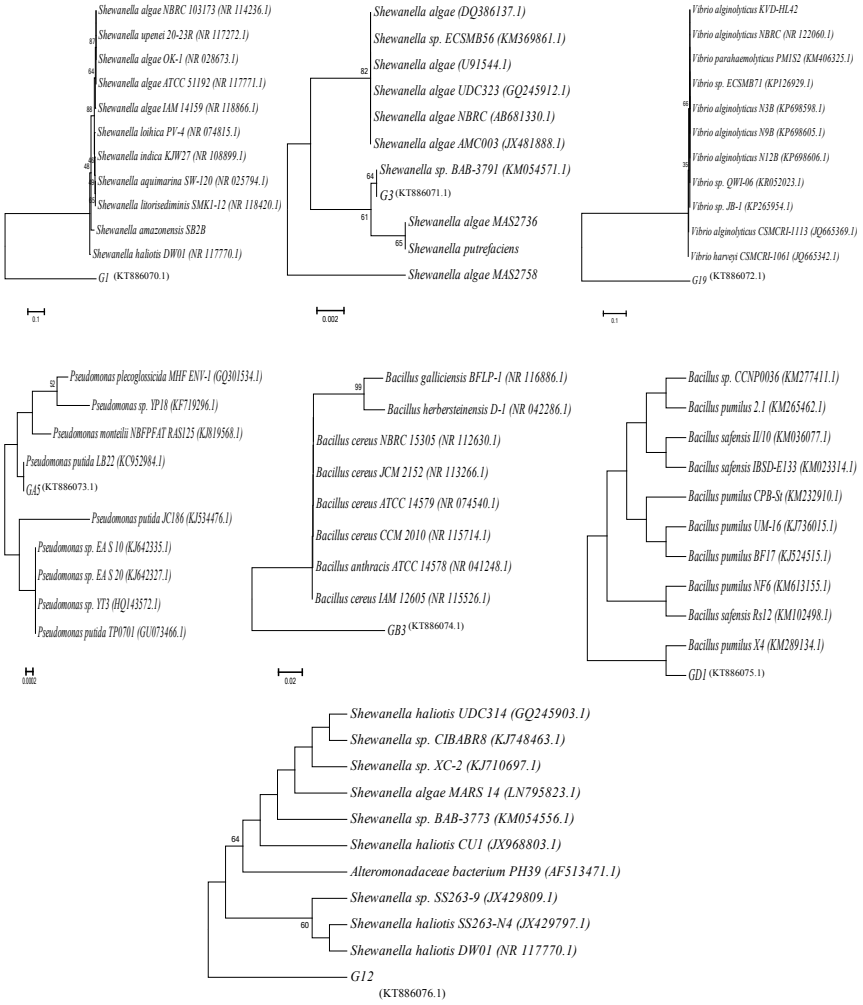


Figure 6: Neighbor-joining phylogenetic tree of isolates G1, G3, G19, GA5, GB3, GD1 and G12 made by MEGA 6.0. Bootstrap values of >50% (based on 1000 replicates) are given in the nodes of the tree. NCBI accession numbers are given in parentheses.

[27]. Other researchers Head, Pucci have reported the involvement of *Shewanella* species in the degradation of crude oil [28,29].

Hydrocarbon degradation by *Bacillus* species is well documented (Okpokwasili and Nnorom, Odokuma and Dickson, Okerentugba and Ezeronye, Ibiene, Chikere and Ekwuabu, Ichor) [30-33]. Borah and Yadav [34] reported hydrocarbon degradation by *Bacillus cereus* strains. The result of this investigation is consistent with the above report as the *Bacillus cereus* IAM 12605 used in the study was able to degrade both the PAH components of the crude oil. Patowary reported biodegradation of polyaromatic hydrocarbons employing biosurfactant-producing *Bacillus pumilus* KS2 [35].

There are supports on the degradation of petroleum hydrocarbon by *Pseudomonas putida* strains. Ichor Vinothini have are implicated *Pseudomonas putida* in the biodegradation of petroleum hydrocarbon. Their claim is consistent with the result obtained from this study. The isolated *Pseudomonas putida* strain LB22 was able to degrade PAH [36].

The bacterial isolates used in this study were found to all have plasmids. Plasmid carriage may provide genetic control for adherence to, or emulsification of, hydrocarbons, ultimately resulting in removal via biodegradative pathway(s) Okpokwasili [37]. However, there is dearth of information on the isolation of degradative plasmids in *Shewanella* spp. Many researchers have isolated and identified degradative plasmids in *Pseudomonas putida*. Worsey, Franklin isolated TOL plasmid pWWO from *Pseudomonas putida* [38,39].

In both cured and uncured treatments the consortium was able to efficiently degrade the crude oil. Although uncured treatment had slightly more percentage of the crude oil degraded, 96.98% loss of the PAH against 95.31% loss of the PAH in the cured treatments. This means that the removal of plasmid from the consortium did not result in the loss of biodegradation potential but however led to slightly decreased degradation. This result is supported by the study carried out by Akpe who observed that loss of plasmids by *Klebsiella pneumoniae* and *Serratia marscencens* did not lead to complete loss of their degradative abilities. It only resulted in reduction in their degradation potential [12].

However, many workers have also shown that plasmids carrying genes influencing degradation of hydrocarbons abound in bacterial populations. Because these bacteria are widely distributed in chronically

polluted estuarine environments, plasmid carriage may provide genetic control for adherence to, or emulsification of, hydrocarbons, ultimately resulting in removal via biodegradative pathway(s). The work by Okpokwasili showed that plasmid DNA was found to be associated with clarification of phenanthrene-degrading *Flavobacterium* species [37].

Conclusion

This study showed that the aerobic heterotrophic bacterial consortium used were able to degrade petroleum hydrocarbon with or without the presence of their degradative plasmid. Although the percentage degradation in the uncured was slightly higher over the 56-day period monitored, there was no significant difference observed in the rate of hydrocarbon degradation at the end of the monitored period. The consortium of aerobic heterotrophic bacteria used in the study were identified as *Shewanella* sp. BAB 3791, *Shewanella algae* MARS14, *Shewanella* sp. ECSMB56, *Vibrio alginolyticus* CSMCRI-1113, *Pseudomonas putida* LB22, *Bacillus cereus* NB4, and *Bacillus pumilus* BF17 using their 16S rRNA sequence analysis. The model developed from the work can be effectively used in predicting PAH and TPH concentration over time.

References

1. Mandri T, Lin J (2007) Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. Afr J Biotechnol 6: 23-27.
2. Salleh AB, Ghazil FM, Raham RNZA, Basri M (2003) Bioremediation of petroleum hydrocarbon pollution. Indian J Biotechnol 2: 411–425.
3. Eze VC, Okpokwasili GC (2010) Microbial and other related changes in Niger Delta River sediment receiving industrial effluents. Cont J Microbiol 4: 15-24.
4. Wilson SC, Jones KC (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ Pollut 81: 229-249.
5. Juhasz AL, Naidu R (2000) Bioremediation of high molecular weight polycyclic aromatic hyrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int Biodeter Biodegr 45: 57–88.
6. Sayler GS, Hooper SW, Layton AC, King JM (1990) Catabolic plasmids of environmental and ecological significance. Microb Ecol 19: 1-20.
7. Foght JM, Fedorak PM, Westlake DW (1990) Mineralization of [14C] hexadecane and [14C]phenanthrene in crude oil: specificity among bacterial isolates. Can J Microbiol 36: 169-175.
8. Lal B, Khanna S (1996) Degradation of crude oil by Acinetobacter calcoaceticus and Alcaligenes odorans. J Appl Bacteriol 81: 355-362.
9. Efroymson RA, Alexander M (1991) Biodegradation by an arthrobacter species of hydrocarbons partitioned into an organic solvent. Appl Environ Microbiol 57: 1441-1447.
10. Malachowsky KJ, Phelps TJ, Teboli AB, Minnikin DE, White DC (1994) Aerobic mineralization of trichloroethylene, vinyl chloride, and aromatic compounds by rhodococcus species. Appl Environ Microbiol 60: 542-548.
11. Focht DD (1998) Performance of biodegradative microorganisms in soil; xenobiotic chemicals as unexploited metabolic niches, In: Environmental Biotechnology (ed). Plenum Press, New York.
12. Akpe, Romanus A, Ekundayo, Omolola A, Esumeh, et al. (2013) Degradation of Crude Oil by Bacteria: A Role for Plasmid-Borne Genes. Global Journal of Science Frontier Research 13: 1-7.
13. Ruel KC, Joseleau JP (2008) Development of immunomicroscopic methods of bioremediation, France.
14. Jalal A, Rashid N, Rasool N, Akhtar M (2009) Gene cloning and characterization of a xylanase from a newly isolated Bacillus subtilis strain R5. J Biosci Bioeng 107: 360-365.
15. Ehrenfeld EE, Clewell DB (1987) Transfer functions of the Streptococcus faecalis plasmid pAD1: organization of plasmid DNA encoding response to sex pheromone. J Bacteriol 169: 3473-3481.
16. Kraft R, Tardiff J, Krauter KS, Leinwand LA (1988) Using mini-prep plasmid DNA for sequencing double stranded templates with Sequenase. Biotechniques 6: 544-546, 549.
17. Silhavy TJ, Berman ML, Enquist LW (1984) Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
18. Holt JG, Krieg NR, Sneath PHA (1994) Bergey's Manual of Determinative Bacteriology (9th Edn.). Lippincott Williams and Wilkins.
19. MacFaddin JF (2000) Biochemical Tests for Identification of Medical Bacteria (3rded.), Philadelphia: Lippincott Williams and Wilkins.
20. Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) Brock Biology of Microorganisms. 13th ed. Benjamin Cummings California, USA.
21. Yamada R, Tanaka T, Ohnishi Y, Suematsu K, Minami M, et al. (2000) Identification of 142 single nucleotide polymorphisms in 41 candidate genes for rheumatoid arthritis in the Japanese population. Hum Genet 106: 293-297.
22. Katsura K, Kawasaki H, Potacharoen W, Saono S, Seki T, et al. (2001) Asaia siamensis sp. nov., an acetic acid bacterium in the alpha-proteobacteria. Int J Syst Evol Microbiol 51: 559-563.
23. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725-2729.
24. Onifade AK, Abubakar FA (2007) Characterization of Hydrocarbon-Degrading Microorganisms Isolated from Crude Oil Contaminated Soil and Remediation of the Soil by Enhanced Natural Attenuation. Res J Biol Sci 2: 36-40.
25. Chikere CB, Ekwuabu CB (2014) Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. Afr J Environ Sci Technol 8: 401-406.
26. Ichor T, Okerentugba PO, Okpokwasili GC (2014) Biodegradation of Total Petroleum Hydrocarbon by Aerobic Heterotrophic Bacteria Isolated from Crude Oil Contaminated Brackish Waters of Bodo Creek. J Biorem Biodegrad 5: 236-241.
27. Said WA, Lewis DL (1991) Quantitative assessment of the effects of metals on microbial degradation of organic chemicals. Appl Environ Microbiol 57: 1498-1503.
28. Head IM, Jones DM, Röling WF (2006) Marine microorganisms make a meal of oil. Nat Rev Microbiol 4: 173-182.
29. Pucci GN, Acuña AJ, Tonin NL, Tiedemann MC, Pucci OH (2012) Diversity of culturable marine bacteria on the coastline of the central area of San Jorge Gulf, Argentina. Revista de Biología Marina y Oceanografía 47: 367-371.
30. Okpokwasili GC, Nnorom EE (1990) Microbial degradation of petroleum hydrocarbon by brackish water isolate. In: Akpata TVI, Okoli DUU (eds.), Nigeria Wetlands 138–146. Nigeria MAB Committee Ibadan.
31. Odokuma LO, Dickson AA (2003) Bioremediation of a crude oil Polluted tropical rain forest soil. Glob J Environ Sci 2: 29-40.
32. Okerentugba PO, Ezeronye OU (2003) Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Afr J Biotechnol 2: 288-292.
33. Ibiene AA, Stanley HO, Immanuel OM (2013) Biodegradation of polyethylene by Bacillus sp. Indigenous to the Niger delta mangrove swamp. Nig J Biotechnol 26: 68-79.
34. Borah D, Yadav RNS (2014) Biodegradation of Complex Hydrocarbon by a Novel Bacillus cereus strain. Environ. Sci. Technol 7: 176-184.
35. Patowary K, Saikia RR, Kalita MC, Deka S (2015) Degradation of polyaromatic hydrocarbons employing biosurfactant-producing Bacillus pumilus KSs_2. Ann Microbiol 65: 225-234.
36. Vinothini C, Sudhakar S, Ravikumar R (2015) Biodegradation of petroleum and crude oil by Pseudomonas putida and Bacillus cereus. Int J Curr Microbiol App Sci 4: 318-329.
37. Okpokwasili GC, Somerville CC, Sullivan M, Grimes DJ, Colwell RR (1986) Plasmid-mediated degradation of hydrocarbons by estuarine bacteria. Oil Chem Pollut 3: 117-129.
38. Worsey MJ, Franklin FC, Williams PA (1978) Regulation of the degradative pathway enzymes coded for by the TOL plasmid (pWWO) from Pseudomonas putida mt-2. J Bacteriol 134: 757-764.
39. Franklin FC, Bagdasarian M, Bagdasarian MM, Timmis KN (1981) Molecular and functional analysis of the TOL plasmid pWWO from Pseudomonas putida and cloning of genes for the entire regulated aromatic ring meta cleavage pathway. Proc Natl Acad Sci U S A 78: 7458-7462.