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Biodegradation of crude petroleum by bacterial consortia from oil-contaminated soils in Ota, Ogun State, South-Western, Nigeria

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HIGHLIGHTS

- Biodegradation of crude and processed oils using indigenous isolates was evaluated.
- Four major bacteria strains were able to utilize petroleum as energy source.
- Both aliphatic and aromatic components of crude petroleum were reduced differently.
- There is evidence that petroleum degradation capabilities could be plasmid encoded.

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ABSTRACT

Pollution from petroleum products is of public health concern because of its attendant health and environmental impacts. Biodegradation of Bonny light crude petroleum and other selected hydrocarbons by bacteria isolated from soils of three different mechanic workshops in Ota, Ogun State were studied. Species of bacteria were isolated by the enrichment of the soil samples in minimal salt medium supplemented with 1% (v/v) crude petroleum as the sole carbon and energy source. The petroleum utilizing bacteria belonging to the genera *Bacillus* sp. SB4, *Pseudomonas* sp. SC8, *Serratia* sp. SC11, and *Acinetobacter* sp. SC12 were screened and subjected for oil degradation using turbidity and total viable count, reduction in pH and residual oil concentration as indices. Gas Chromatographic (GC) analysis was used to analyze the component and percentage of the petroleum utilized. Plasmid curing and profiling were done to determine whether the ability to utilize carbon is plasmid or chromosomally encoded. Results revealed that four bacteria strains were able to utilize petroleum as energy source. The GC fingerprints showed that both the aliphatic and aromatic components of crude petroleum were reduced to varying degree but the nonadecane C19 could not be reduced. Strain SC11 could not reduce anthracene, pyrene, benzo(a)pyrene and chrysene components. Strain SB4 depleted 24 to 57% aliphatics and 20 to 42% aromatics and strain SC8 depleted 38 to 67% aliphatics and 30 to 79% aromatics while strain SC11 depleted 12 to 46% aliphatics and 13 to 29% aromatics. All organisms harbored plasmid which could suggest that petroleum degradation capabilities could be plasmid encoded. This indicates that the petroleum utilizing bacteria are could be used bioremediation of the petroleum polluted environment.

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1. Introduction

Crude oil composes of complex Polycyclic aromatic hydrocarbons (PAHs) and several other organic pollutants with great potentials too cause serious hazards in the spate of environmental catastrophes like uncontrolled drilling, oil spills, soil and water pollution etc. (Xu and Lu, 2010; Ting et al., 2011; Roy et al., 2014). The most widely used primary source of energy is the hydrocarbons; this is because they are source of large amounts of energy. Hydrocarbons molecules that make up crude petroleum and other oil components are highly toxic to microorganisms, plants, animals and humans (Haritash and Kaushik, 2009; Nie et al., 2012). Oil pollution results from routine normal operations of crude oil exploration, exploitation, refining, and transportation (Wu et al., 2010; Nwankwegu and Onwosi, 2017). Whenever crude oil is released into the environment; it causes enormous pollution which can be deliberate or by accidental. The issues often cause a lot of problems to both the living and non-living components of the environments, more so that some hydrocarbon components have been identified to belong to a family of substances that can cause cancer and other central nervous system disorders. There are other ways through which petroleum also penetrates into the environments; these could be through waste disposal, accidental spills, leakage tankers, oil bunkering, vandalization of oil pipes and losses during storage (Chen et al., 2015). The fact that petroleum product dominates the Nigerian's economy also creates a lot of conditions for exposure of large amounts of these toxins into environments (Nishant et al., 2016). Bonny light Crude petroleum is mainly composed of different components of hydrocarbon molecules, which are mainly alkanes from C1 to C30 aliphatics, C6–C8 aromatics, cyclohexanes, and other compounds containing nitrogen, oxygen and sulphur (Nishant et al., 2016). Major components in the petroleum pollutants are degradable, and they will become utilized from the environment as microorganisms use them for source of carbon and energy (Nwinyi and Olawore, 2017).

Though physicochemical treatment of oil contaminants is popular, use of bioremediation is a better alternative due to its numerous benefits in terms of efficiency and economics (Janbandhu and Fulekar, 2011; Suja et al., 2014). Biodegradation of crude petroleum by natural strains of bacteria represents one of the main processes of eliminating environmental pollution from the environment (Mona et al., 2016). Bacteria from genera *Arthrobacter, Bacillus Pseudomonas, Acinetobacter, Flavobacterium, Rhodococcus* Mycobacterium isolated from soil and other environmental samples are well-known PAH-degraders (Suja et al., 2014). All these microorganisms have been noted to have the ability to utilize and use petroleum resources as source of energy as they successfully mineralized different PAHs. Several bacteria species and strains have been identified to be capable of utilizing PAHs as a carbon source (Ting et al., 2011), it has been proposed that better results may be obtained by using mixed bacterial culture or bacterial–fungal consortium (Silva et al., 2009a,b; Xu and Lu, 2010). In real life situation, use of pure strain in biodegradation of contaminated soils is not representative of the real characteristics of environmental microorganisms. This is more so because the success of the bioremediation process is a function of the cooperative metabolic activities of microbial consortia (Hesnawi and Mogadami, 2013; Roy et al., 2014; Yenn et al., 2014). One major advantage of using microbial consortia is the possession of multiple metabolic capabilities which enhances increased efficiency of the bioremediation process (Zhang et al., 2010; Adekunle and Adeniyi, 2015)

In the light of the above, the isolation and proper characterization of bacteria surviving in oil-contaminated soils and their succession pattern is a gateway to achieving success in any bioremediation process (Zhang et al., 2010). The best method of decontamination of the environment polluted with petroleum components is an application of methods based mainly on metabolic activity of microorganisms (Groudev et al., 2014). This ability to actively utilize particular components of petroleum oil is expressed by many microorganisms which could be plasmid encoded. The biodegrading ability of any microorganism is a function of its genetic makeup while the various arrays of chemical reactions involved in the microbial metabolic pathways are enzyme mediated. Several previous researches focusing on the characterization of bacterial enzymes and the various pathways responsible for the degradation of hydrocarbon has been carried out. Prominent examples are the characterization of alkane mono-oxygenase (alkB) and cytochrome P450 families which are known to be actively involved in petroleum degradation pathways (van Beilen et al., 2006; van Beilen and Funhoff, 2007). Also, the genome sequences of several hydrocarbon-degrading bacteria have been documented in previous studies. Such bacteria include *Alcanivorax borkumensis* SK2, *Geobacillus thermodenitrificans* NG80-2, *Desulfatibacillum alkenivorans* AK-01, *Polymorphum gilvum* SL003B-26A1T and *Pseudomonas aeruginosa* N002; Callaghan et al., 2012; Nie et al., 2012). Numerous potential genes and pathways in relation to hydrocarbon degradation have also been obtained from complete genome sequencing. This research therefore was aimed at (i) isolation and identification of petroleum utilizing or degrading bacteria strains from oil contaminated soil using basic microbiological techniques, (ii) screening of the isolated bacterial strains for degradation of petroleum and other hydrocarbon substrates, (iii) determination of the components of the crude petroleum that could be utilized or degraded by the bacterial isolate and evaluation of their percentage degradation and (iv) determination of the role of plasmid in the biodegradation process. This is necessary as a means of cleaning up the over three decades old constantly polluted soils and which have not been given any research attention prior to this study.

2. Materials and methods

2.1. Collection of soil samples

Ten soil samples each were randomly collected in duplicates from three locations (A, B and C) heavily contaminated with petroleum products as a result of constant disposal of waste oils for over three decades. Collection of soil samples were