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Original paper

Isolation and Identification of oil Degrading Bacteria from Izom NNPC Sub-station Niger State

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ABSTRACT: This study was carried out to isolate and identify oil degrading bacteria from NNPC sub-station situated at Izom. Oil contaminated soil samples was taken from three (3) different spots in Izom NNPC sub- depot and labeled S1, S2, and S3, while soil sample (control) of same mass was collected from unpolluted soil at the same station. The soil samples were analyzed using standard method. The bacterial species isolated were Pseudomonas aeruginosa, Escherichia coli, Neisseria species, Bacillus subtilis, Micrococcus species, Streptococcus species, and Klebsiella species. From, the data obtained, three isolates namely Pseudomonas species, Bacillus species and Micrococcus species showed excellent degradation activity while other species studied showed moderate and low degradation activity and hence could be exploited in bioremediation activities. The study recommended amongst others that conservationist and environmentalist should adopt the use of bacteria in addressing pollution caused by oil spillage.

Keywords: Bacteria, oil spillage, Izom NNPC

INTRODUCTION

Activities associated with petroleum exploration, development and production operations have local detrimental and significant impacts on the atmosphere, soils and sediments, surface and groundwater, marine environment, biological diversity and sustainability at terrestrial ecosystems in the Niger Delta Discharge of petroleum hydrocarbon and petroleum-derived waste, streams have caused environmental pollution, adverse human health effects, and detrimental impact on regional economy, socio-economy problem and degradation of host communities in the 9 oil-producing states in the Niger Delta region (Achife et al., 2021). Recently, anthropogenic practices such as industrial activities, petroleum and petroleum derivatives (such as gasoline, diesel, and kerosene spills), and incomplete combustion of fossil fuels have caused an accumulation of petroleum hydrocarbons in the environment (Adebusoye et al., 2007). In fact, petroleum and derivatives have a major ecological impact on contaminated marine and terrestrial ecosystems. Many important processes influence the destination of hydrocarbons in the environment. Among these are sorption, volatilization, abiotic transformation (chemical or photochemical), and biotransformation. Biodegradation of oil contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contamination, has been established as one of the efficient, economic, and versatile and environmentally sound treatment.

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides (Venosa and Holder, 2007). Different factors influencing hydrocarbon degradation have been reported by Cooney et al (Cooney et al., 1985). One of the important factors

that limit biodegradation of oil pollutants in the environment is their limited availability to micro organism diesel hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded (Barathi and Vasudevan, 2001). Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkenes> branched alkenes>small aromatics> cyclic alkenes (Al-Hawash et al., 2018; Deepika and Rajalakshmi, 2013). Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all (Atlas and Bragg, 2009).

Microbial degradation is the major and ultimate natural mechanism by which one can clean up the diesel hydrocarbon pollutants from the environment (Atlas et al., 1992). Isolation involves the separation of bacteria from hydrocarbons. The recognition of biodegraded diesel-derived aromatic hydro carbons in marine sediments was reported by Jones et al. (Jones et al., 1993). They studied the extensive biodegradation of alkyl aromatics in marine sediments which occurred priodetectable biodegradation of n-alkane profile of the crude oil and the microorganisms, namely, *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas*, *Sphingomonas,* and *Rhodococcus* were found to be involved for alkyl aromatic degradation. Microbial degradation of diesel hydrocarbons in a polluted tropical stream in Lagos, Nigeria was reported by Adebusoye et al., (2007). Nine bacterial strains, namely*, Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffi, Flavobacterium sp*, *Micrococcus roseus*, and *Corynebacterium sp*. were isolated from the polluted stream which could degrade crude oil. Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. The reported efficiency of biodegradation ranged from 6% (Jones et al 1993) to 82% [for soil fungi, 0.13% (Jones and Hodge, 1999) to 50% (Rinholt et al 1979) for soil bacteria, and 0.003% (Hollaway et al 1980) to 100% (Mulkins- Phillips and Stewart, 1974) for marine bacteria. Many scientists reported that mixed populations with over all broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil (Bartha and Bossert 1984), freshwater (Corey et al 1984), and marine environments (Atlas and Floodagate 1984). Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment.

Several bacteria are even known to feed exclusively on hydrocarbons (Broojmans et al 2009). Floodgate (Flodgate, 1984) listed 25 genera of hydrocarbon degrading bacteria and 25 genera of hydrocarbon degrading fungi which were isolated from marine environment. In earlier days, the extent to which bacteria, yeast, and filamentous fungi participate in the biodegradation of diesel hydrocarbons was the subject of limited study, but appeared to be a function of the ecosystem and local environmental conditions. Crude petroleum oil from diesel contaminated soil from North East India was reported by Das and Chandran, (2011). *Acinetobacter sp. was* found to be capable of utilizing n-alkanes of chain length C10–C40 as a sole source of carbon (Das and Chandran, 2011). Bacterial genera, namely*, Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholderia*, and *Mycobacterium* isolated from diesel contaminated soil proved to be the potential organisms for hydrocarbon degradation [42]. The degradation of polyaromatic hydrocarbons by *Sphingomonas* as was reported by Daugulis and McCracken [43]. Fungal genera, namely, *Amorphoteca, Neosartorya, Talaromyces*, and Graphium and yeast genera, namely, *Candida, Yarrowia,* and *Pichia* were isolated from diesel contaminated soil and proved to be the potential organisms for hydrocarbon degradation [Chailan et al 2004]. Singh also reported a group of terrestrial fungi, namely, *Aspergillus, Cephalosporium* and *Pencillium* which were also found to be the potential degrader of crude oil hydrocarbons. The yeast species, namely, *Candida lipolytica, Rhodoorula mucilaginosa*, *Geotrichumsp,* and *Trichosporon mucoides* isolated from contaminated water were noted to degrade petroleum compounds. Though algae and protozoa are the important members of the microbial community in both aquatic and terrestrial ecosystems, reports are scanty regarding their involvement in hydrocarbon biodegradation. Walker et al isolated an alga, which was capable of utilizing crude oil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and iso-alkane as well as aromatic hydrocarbons.

Aim and objectives of the study

The aim of this study is to isolate characterize and identify oil degrading bacteria from NNPC sub-station Izom.

Specific objectives of the study are to:

i. Determine the bacterial loads of the studied samples
ii. Isolate oil degradable bacteria from NNPC sub-station in Izom
iii. Characterize and Identify oil degradable bacteria from NNPC sub-station in Izom

Literature review

Every year huge amount of petroleum products are

produced which is readily accessible to come in contact with the soil and water environment by various ways (Eneh, 2011). An average estimate of crude oils entering the environment has been found out about 6 million tons damaging both human life, wild life and environment itself (Kafilzadeh, 2011). There are several sources from where petroleum products can get entry into the environment. Some such sources include domestic wastes, underground storage tank seepage, vehicles on roads (low amount of petroleum), storage tank/tanker/pipeline spillage (high amount of petroleum product), slow seepage from natural soil reservoirs etc. (Hussein, 2012). Petroleum and hydrocarbon components in the environment are cleaned up naturally by native microbial population followed by metabolic pathways resulting in the biodegradation of the complex molecules (Al-Hawash et al., 2018). Bacteria, fungi and microalgae mainly do the job of biodegradation (Sanscartier *et al*., 2010). Rather than working a single species of bacteria or fungi in degradation of the complex molecules, combined activity of several bacterial of fungal population shows the best result due to availability of broader range of enzymatic activity in soil, water or marine environment (Spini *et al*., 2018; Sarma et al., 2010; Al-Wasify, 2014; Ojo-Omoniyi, 2018; Das and Chandran, 2011). These microorganisms utilize the carbon in hydrocarbons as their energy sources and are naturally distributed in the environment (Adeline et al., 2009).

Degree of biodegradation depends on some factors like type of microorganisms, composition of hydrocarbon, environmental conditions, bioavailability of the hydrocarbon etc. (Sanscartier *et al*., 2010).Active degrading out to investigate their capability of degradation (Tazaki, 2018; Das and Chandran, 2011; Ekanem, 2017). Biodegradation of petroleum products finally end up with the production of CO2, H2O and biomass with the complex and combined metabolic/enzymatic reaction of various microorganisms (Islam et al., 2013). This is a step by step process proceeding from minor organic molecule change with intact core structure, fragmentation while it is still readily identifiable as a petroleum product and finally complete mineralization into inorganic substances or end products (Islam et al., 2013). There are many harmful effects of petroleum in the environment. In a study carried out in the Niger Delta Region of Nigeria revealed that adverse effects on soil (degraded agricultural lands) and water resources (surface water pollution with difficulty in oxygen transportation in deep water), aquatic lives (reduced availability of fish), crops (destruction of crops), livestock (death of animals) and plants. As a result farm productivity and animal farm income has been reduced a lot (Ugwu, 2009; Eneh, 2011; Tabieh and Al-Horani, 2010; Al-Turki, 2010). Human health can also be hampered. Development of kidney and liver diseases, cancer, bone marrow damage etc. can result from exposure to high oil concentrations (Islam et al., 2013).

Hydrocarbon degrading bacteria

Hydrocarbon degrading bacteria can be defined as bacteria with the capability to degrade (leading to the formation of less complex intermediate compounds) and/or to mineralize (leading to the formation of water and carbon dioxide) hydrocarbons. Heterotrophic bacteria depend on carbon and electron sources from environment to be used as their energy resource. Microbial degradation of organic contaminants therefore occurs as result of microorganisms using the contaminants as carbon, energy or nutrient source for their own growth and reproduction. Besides environmental factors such as oxygen, temperature, soil physical-chemical conditions, bioavailability of the contaminant and available nutrients (Romantschuk, *et al*., 2000), the capability to degrade hydrocarbons in soil is also influenced by other factors such as a bacterial species-dependent capability; that makes every species differ in their capability to metabolize hydrocarbons (Siciliano *et al*., 1998) the bacterial ability to quickly distribute genetic information within a population and thereby to adapt to environmental changes (van Elsas, *et al*., 2003) the presence of the contaminant as selective pressure to maintain their degrading capability (van der Lelie, *et al*., 2005) catabolic genes encoding degradation enzymes (Romantschuk, *et al*., 2000).

Bioremediation

Bioremediation makes use of indigenous oil–consuming microorganisms, called petrophiles, by enhancing and fertilizing them in their natural habitats. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize then as a food source (Harder, 2004). Microorganisms degrade these compounds by using enzymes in the metabolism and can be useful in cleaning up contaminated sites (Alexander, 1999). Microbial remediation of a hydrocarbon–contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan, 2001; Mishra et al., 2001; Eriksson et al., 1999). A large number of *Pseudomonas* strains capable of degrading polyaromatic hydrocarbons (PAHs) have been isolated from soil and aquifers. Other petroleum hydrocarbon degraders include *Yokenella* spp.,*Alcaligenes* spp., *Roseomonas* spp., *Stenotrophomonas* spp., *Acinetobacter* spp., *Flavobacter* spp., *Corynebacterium* spp., *Streptococcus* spp.,

Providencia spp., *Sphingobacterium* spp., *Capnocytophaga* spp., *Moraxella* spp., and *Bacillus* spp. (Rusansky et al., 1987; Antai, 1990; Bhattacharya et al., 2003).

Enzymes participating in degradation of hydrocarbons

Cytochrome alkenes hydroxylases constitute a super family of ubiquitous Heme- thiolate Monooxygenases which play an important role in the microbial degradation o foil, chlorinated hydrocarbons, fuel additives, and many other compounds. Depending on the chain length, enzyme systems are required to introduce oxygen in the substrate to initiate biodegradation. Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 forms that may contribute as an ensemble of is forms to the metabolic conversion of given substrate. In microorganisms such P450 multiplicity can only be found in few species Zimmer et al., (1998). Cytochrome P450 enzyme systems was found to be involved in biodegradation of petroleum hydrocarbons**.** The capability of several yeast species to use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon and energy is mediated by the existence of multiple microsomal Cytochrome P450 forms. These cytochrome P450 enzymes had been isolated from yeast species such as *Candida maltosa, Candida tropicalis*, and *Candida apicola* . The diversity of alkane oxygenases systems in prokaryotes and eukaryotes hat are actively participating in the degradation of alkanes under aerobic conditions like Cytochrome P450 enzymes, integral membrane di- iron alkane hydroxylases (e.g., alkB), soluble di-iron methane Mono oxygenases, and membrane bound copper containing methane Mono oxygenases have been discussed by Van Beilen and Funhoff (2005).

Uptake of hydrocarbons by biosurfactants

Biosurfactants are heterogeneous group of surface-active chemical compounds produced by a wide variety of microorganisms. Surfactants enhance solubilization and removal of contaminants. Biodegradation is also enhanced by surfactants due to increased bioavailability of pollutants. Bioremediation of oil sludge using biosurfactants has been reported by Chandra et al (2013). *Pseudomonas* are the best-known bacteria capable of utilizing hydrocarbons as carbon and energy sources producing biosurfactants. Among Pseudomonads*, P. aeruginosa* is widely studied for the production of glycol lipid type biosurfactants. However, glycol lipid type biosurfactant are also reported from some other species like *P. putida* and *P. chlororaphis*. Biosurfactants increase the oil surface area and that amount of oil is actually available for bacteria to utilize it. Biosurfactant can act as emulsifying agents by decreasing the surface tension and forming micelles. The micro droplets encapsulated in the hydrophobic microbial cell surface are taken inside and degraded.

Although capability in degrading hydrocarbons spread across wide range of bacterial species, but in general, bacterial genera *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Achromobacter*,*Micrococcus*, *Mycobacterium*, and *Nocardia* have reported as the most active bacteria in the degradation of hydrocarbons in soil (Frick *et al*., 1999), whereas *Cellulomonas, Clavibacter, Curtobacterium, Pseudomonas* and *Microbacterium*have been suggested as the most promising endophytic bacteria (Ryan *et al*., 2008). Endophytic bacteria are defined as those bacteria that colonize the internal tissue of the plant showing no negative effects on their host (Ryan, 2008).

Bacterial pathways for the degradation of hydrocarbons contaminants have been the subject of intense study and suggest several important physiological events as key factors that lead to the efficient catabolism of these compounds, which are: bioavailability, or the amount of a substance that is physiochemically accessible to microorganisms; chemotaxis, or the directed movement of motile organisms towards or away from chemicals in the environment; and transport mechanisms for the intracellular accumulation of aromatic molecules (Parales, *et al*., 2008). Degradation of hydrocarbon by bacteria takes place through complex sequence of reduction-oxidation reactions, which are catalyzed by enzymes. Aliphatic hydrocarbons are oxidized by several alkane hydroxylase enzyme systems including cytochrome P450 enzymes, an integral membrane mono or diiron alkane hydroxylase (i.e., alkane monooxygenase), a soluble di-iron methane mono oxygenase (sMMO) and a membrane-bound copper-containing (possibly iron-containing) methane monooxygenase (pMMO) (van Beilen and Funhoff, 2005). For the reaction to take place,the compound must pass through the bacteria's cell membrane so the organism's electrontransport system can be used for energy storage. Frequently, the bacteria are attached to alkanedroplets, which make it more available to bacterial attack, however, the enhanced bioavailability can cause a toxicity problem for the bacteria. In addition, soil moisture lower than 50% and pH above 8.5 appear to inhibit hydrocarbon degradation (Cookson, 1995). The degradation of hydrocarbons can be divided into aerobic and anaerobic metabolic modes.

Empirical studies

Akeredolu and Akinnibosun (2017) aimed to evaluate the physicochemical properties and presence of bacteria

from soil samples contaminated with different petroleum products which are petrol (PMS), diesel (AGO), pure kerosene (DPK) and mixed kerosene (DPK) obtained from the tank farm of NNPC/PPMC Depot in Benin City, Nigeria. The mean count for heterotrophic bacterial ranged from 1.83x104±0.09 cfu/g soil contaminated with mixed DPK to 5.93x104±0.02 cfu/g for soil contaminated with PMS. Nine (9) bacterial isolates were characterized and identified; *Bacillus subtilis*, *Micrococcus varians*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Alcaligenes* sp., *Corynebacterium* sp., *Bacillus* sp., *Arthrobacter* sp., *Pseudomonas* sp. *Bacillus* sp. and *Pseudomonas* sp. were the most occurring bacteria isolates in the four soil samples. From the physico-chemical analysis, the pH value ranged from 6.50-6.70, electrical conductivity ranged from 14.20mhos/cm-32.50mhos/cm, moisture content from 10.20%-14.50%, carbon from 1.38%-2.97%, nitrogen from 2.37%-8.19%, 0.396%-0.950%, phosphate 1.95%-5.52% and total hydrocarbon from 125mg/kg-3,050mg/kg These results revealed that the presence of these bacteria under the stated soil condition can enhance bioremediation of petroleum contaminated soil.

Lima *et al.,* (2018) isolated and characterization of hydrocarbon-degrading bacteria from gas station leaking-contaminated groundwater in the Southern Amazon, Brazil. Twenty-three hydrocarbon-degrading bacteria strains were isolated from gas station leaking-contaminated groundwater located in the Southern Amazon, Brazil. Based on hydrocarbon (diesel, hexadecane, benzene, toluene and xylene) degradation ability, two strains were selected for further study. The amplification and sequencing of the 16S rRNA gene showed that these two strains belonged to the genus Bacillus (Bacillus sp. L26 and Bacillus sp. L30). GC-MSanalysis showed that strain L30 was the most effective in degrading n-alkane (C10-C27) from diesel after 7 days ofcultivation in mineral medium. Both strains produced biosurfactants and showed emulsification activity, especially thestrain L30. Alkane hydroxylase gene (group III), which is important for alkane biodegradation, was present in strains. As a result, this study indicated that these bacteria could have promising applications in hydrocarbon bioremediation.

Achife *et al.,* (2021) aimed at determining the microbiological quality of soil and water sources in communities around petroleum products depot in Suleja, Nigeria. Soil and water samples were collected from petroleum depot and the five communities around the petroleum products depot and a control site. Microorganisms in the soil and water samples were enumerated by spread inoculation on general purpose media and selective media. Bacterial and fungal isolates were tested for their potential to utilize petroleum products in a Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil) as a source of carbon and energy. The utilization rate was determined by spectrophotometry. The capacities of selected bacterial and fungal isolates to mineralize crude oil were further tested in minimal salt medium. The bacteria isolated were Staphylococcus aureus, Streptococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli. The microbial isolates were not evenly distributed in the six experimental and control plots. Soil samples had higher aerobic heterotrophic bacterial counts than the water samples. Crude oil was most utilized by the microbial isolates. Bacterial isolates from genera Pseudomonas and Bacillus had the highest capacity in utilizing the petroleum products. Among the fungal species, Aspergillus niger and Penicillium notatum exhibited greater capacity to utilize the petroleum products. Present study revealed isolates capable of utilizing the various petroleum products which can be useful in oil spill bioremediation in the tropical environments.

Ebakota *et al.,* (2017) isolated and characterized hydrocarbon-degrading bacteria in top and subsoil of selected Mechanic Workshops in Benin City Metropolis, Nigeria. The isolation of hydrocarbon-degrading bacteria in topsoil and subsoil samples of selected mechanic workshops located in the Benin metropolis was carried out. The highest and lowest bacterial counts in topsoil samples were found out to be 2.94 x 106 CFU/g and 2.39 x 106 CFU/g in CENTRAL road and OGBELAKA road automobile workshops respectively. The highest and lowest bacterial counts in subsoil samples were found out to be 1.14 x 106 CFU/g and 1.09 x 106 CFU/g in OGBELAKA road and CENTRAL road automobile workshops respectively. The bacterial species isolated were Bacillus spp., Pseudomonas spp., Staphylococcus spp. and Streptococcus spp. The isolates were also subjected to hydrocarbon degradation/utilization test where it was observed that Pseudomonas spp utilized the hydrocarbon in the medium more efficiently than the other isolates. The isolates from this study are thus recommended as bacterial species for possible use in bioremediation of hydrocarbons.

MATERIALS AND METHODS

Study area

This study was carried out in Izom community. Izom community is located along Minna-Suleja road in Gurara local government area of Niger State Nigeria. It covered about 954 $km^2$of land and geographically located on latitude of 9.24' 35"E and longitude of 7.01' 73"N. NNPC sub- depot station in Izom was established in 1989 with sole responsibility of pipeline and products marketing activities.

Sample collection

Oil contaminated soil samples was taken from three (3) different spots in Izom NNPC sub- depot and labeled S1, S2, and S3, while soil sample (control) of same mass was collected from unpolluted soil at the same Izom. The soil samples were collected above ground to a depth of 15cm beneath the surface using small shovel and were put into new sterile polyethylene bags with aseptic conditions. The samples were made in three replicates. Collected samples were taken back to the Biology laboratory as soon as possible and stored at 4^0C until further processing.

Isolation of bacterial cultures

All glasswares to be used were thoroughly washed and sterilized using an autoclave at 121ºC for 15 minutes (Venosa and Holder, (2007). For the sample preparation, 9ml of distilled water was dispensed into 6 sterile test tubes with the first test tube containing 1g of soil samples. For the serial dilution, 1ml from the first test tube was transferred into the second test tube containing only 9ml of distilled water. 1ml was again transferred into the third test tube after thoroughly shaking the process continuous up to the last test tube to produce 10^1 to 10^6. For the media preparation and sterilization, Plate Count on nutrient Agar and MacConkey Agar were utilized. After sterilization at 121ºC for 15 minutes, the media was allowed to cool at 45^0C and poured into sterile petri dishes in an aseptic condition after 1ml of serially diluted microbial mixture from each test tube was aseptically transferred into the sterile petri dishes respectively and was incubated at a temperature of 37^0C for 24hours, after which they were examined for bacterial growth. The number of colonies on each plate were counted and recorded. Distinct isolated colonies were selected for further study to determine their capability to degrade hydrocarbon compounds.

Isolation of petroleum degrading bacteria

For the isolation of petroleum degrading bacteria, a mineral base medium composed of 0.8 g K2 HPO4 , 0.2 g KH2 PO4 , 0.05 g CaSO4 2H2 O, 0.5 g MgSO4 H2 O, 0.09 g FeSO4 7H2 O and 1 g (NH4)2 SO4 was used for isolation of native bacteria from oil contaminated samples. This medium was freshly prepared for each test. Each pure culture isolated from soil earlier was streaked on mineral salt agar plate. After which the plates were incubated at 37˚c for 48 -72 hours. By this period of time only petroleum degrading bacteria remained on the surface of the plates.

Identification of bacterial isolates

Bacteria which grew on mineral salt agar plate were selected for gram staining, microscopic and biochemical identification. Triple sugariron agar test (TSI), catalase, oxidase, indole production test, nitrate reduction test, methyl red test (MR), voges proskauertest (VP) and citrate utilization test were performed and based on the test results the bacterial isolates were identified.

Gram staining

Clean and grease free slide was taken and a drop of saline was placed on the slide. The organism to be studied is placed on the saline and made a smear and heat fixed. Crystal violet was added in drop to the sample and allows it for one minute and washed the stain in running tap water. Gram' siodine was added which is mordant and allowed for one minute and washed in tap water. Rinse the sample with ethanol for30 seconds and gently washed it in water. Safranin was added to the slide and allowed for 1 minute and then washed it off. The slide was allowed to air dry and viewed under microscope using oil immersion objective lence.

Catalase test

18-24 hours of culture sample was placed in a clean slide. One drop of hydrogen peroxide solution was added and emulsified. Observation of air bubbles in the slide represents the positive result no bubbles is negative result.

Indole test

Tube of tryptone broth was inoculated with a small amount of a pure culture and Incubated at 37°C for 24 to 48 hours. To test for indole production, 5 drops of Kovác's reagent was directly added to the tube. Formation of a pink to red color ("cherry red ring") in the reagent layer on top of the medium within seconds of adding the reagent shows positive reaction. The reagent layer remained yellow or be slightly cloudy shows negative reaction.

Methyl red test

MR broth media was prepared and sterilized. The sterile tubes were taken and the broth was poured. Test organisms were inoculated and the tubes were kept in the incubator for 24 hours. After 24 hours the MR indicator was added to the tubes, and the colour change was observed which indicate positive result.

Starch hydrolysis test

Inoculated plates of starch agar with assigned bacteria was incubated at 37 ºC for 24-48 hours. A small amount of Gram's Iodine was dripped on the plate around the inoculated area and a small amount in uninoculated area. A clear zone was observed around the inoculum was compared with the inoculated area with the uninoculated area, and record the results.

Glucose utilization test

0.1mL Inoculum of each of the bacteria was transferred into broth of glucose and incubated the broth at37 ºC for 24-48 hours in a rotary shaker. Results are observed for the broth and compared to the uninoculated.

Oil degrading ability of bacterial isolates

The bacterial isolates that exhibited excellent growth on mineral salt agar were selected. A loopful of culture was inoculated into 100ml sterile nutrient broth. The flasks were incubated and shaken at 200 rpm, for 12h at 30°C. One ml volumes of the culture broth from each of the identified isolates were mixed to prepare mixed bacterial consortium. Individual bacterial cultures and bacterial consortium (1.0 %) were transferred to 250 ml conical flasks, each containing 100 ml of sterile defined mineral salts medium with 1% petroleum oil. An uninoculated control was also studied concurrently. The flasks were incubated at 30°C and shaken at 200 rpm for 8 days. At 2-day intervals, an estimation of bacterial growth was done going by the turbidity formed.

RESULTS

Bacterial load count

Plate count on nutrient agar and McConkey agar, bacterial load counts were higher in uncontaminated soil than the oil contaminated soil (Table 1).

Microbial Isolates

For the contaminated soil samples, a total of eight (7) bacteria species were isolated. These include Pseudomonas aeriginosa, Escherichia coli, Neisseria Streptococcus species, Bacillus subtilis, Micrococcus Streptococcus species, Streptococcus Streptococcus species, and Klebsiella Streptococcus species. However for the uncontaminated (control), soil samples (6) six bacteria species were isolated which includes Pseudomonas aeriginosa, Escherichia coli, Neisseria Streptococcus species, Bacillus subtilis, Micrococcus Streptococcus species, and Streptococcus species (Table 2).

Oil degradation

The bacterial isolates tested for their ability to grow in mineral salts medium (MSM) and degrade with oil as carbon source. Three (3) Pseudomonas species , Bacillus species and Micrococcus species) out of seven (7) isolates showed excellent growth while others showed low to moderate growth respectively (Table 3).

DISCUSSION

Bacterial plate count on nnutrient agar and McConkey agar, bacterial load counts were higher in uncontaminated soil than the oil contaminated soil. This is could be indicative that oil contaminated soils are acidic for bacteria. This is likely due to the microbial biodegradation activities going on in the soil samples. According to (Brajesh et al., 2013), bacteria can survive at different ranges of pH values but the degradation of hydrocarbons tends to favor near neutral pH of 6.5-8.0. However, the degradability declines as the pH shifts farther from neutral (Khan et al., 2011). This shows that more degradation is taking place in the contaminated soil than the uncontaminated soil. Uncontaminated soil may be having lowest temperature which allow more bacteria to live. This is likely due to the lower rate of biodegradation because elevation in temperatures decreases the viscosity of hydrocarbons, which results into greater rates of diffusion. This allows the petroleum pollutants to be more accessible for the microbes in the contaminated soil (Atlas, 2018).

For the contaminated soil samples, a total of eight (7) bacteria species were isolated. These include Pseudomonas aeriginosa, Escherichia coli, Neisseria Streptococcus species, Bacillus subtilis, Micrococcus Streptococcus species, Streptococcus Streptococcus species, and Klebsiella Streptococcus species. However for the uncontaminated (control), soil samples (6) six bacteria species were isolated which includes: Pseudomonas aeriginosa, Escherichia coli, Neisseria Streptococcus species, Bacillus subtilis, Micrococcus Streptococcus species, Streptococcus species. This result is in line with the work of Ugoh and Moneke (2011) who reported that the bacterial isolates from the soil contaminated with petroleum products from the different sites showed Pseudomonas sp., Bacillus sp. and Klebsiella spp. having highest percentage occurrence frequency of 60%.On the other hand, only Pseudomonas aeriginosa had highest growth on the uncontaminated soil. Also, Micrococcus sppand Bacillus subtilis were lowest in occurrence on the contaminated soils (Table 2),

Table 1: Bacterial load count of oil contaminated soil and uncontaminated soil of NNPC sub depot Izom

Samples	**Oil contaminated soil** (cfu/ml)	**Uncontaminated oil** (cfu/ml)
S1	18.8×10^5	22.8×10^5
S2	14.6×10^5	20.5×10^5
S3	11.8×10^5	19.8×10^5

Table 2: Bacterial isolated from oil contaminated soil and uncontaminated soil of NNPC sub depot Izom

Isolates No.	Oil contaminated soil	Uncontaminated oil
1	*Pseudomonas aeriginosa*	*Pseudomonas aeriginosa*
2	*Escherichia coli*	*Escherichia coli*
3	*Bacillus subtilis*	*Bacillus subtilis*
4	*Neisseria species*	*Neisseria species*
5	*Micrococcus species*	*Micrococcus species*
6	*Streptococcus species*	*Streptococcus species*
7	*Klebsiella species*	-

Table 3: Distribution of bacterial showing different levels of degradation ability of oil.

Isolates No.	Bacterial isolates	Level of degradation
1	*Pseudomonas aeriginosa*	**+++**
2	*Escherichia coli*	**+**
3	*Bacillus subtilis*	**+++**
4	*Neisseria species*	**++**
5	*Micrococcus species*	**+++**
6	*Streptococcus species*	**+**
7	*Klebsiella species*	**++**

+++ = Excellent growth, ++ = Moderate growth and + = Low growth

while Bacillus subtilis, Micrococcus spp. and Streptococcus spp. were the lowest occurring bacterial species on the uncontaminated soils. This is consistent with the work of Alexander (2014) who reported that Pseudomonas spp., Escherichia coli and Klebsiella spp. were found to utilize the diesel soil samples in the soil environment in Keffi, Nasarawa State. Three isolates namely Pseudomonas species, Bacillus species and Micrococcus species showed excellent growth while others species studied showed moderate and low growth on oil degradation activity. Microorganisms are known to attack specific compounds present in crude oil that is a complex mixture of saturates, aromatics and polar compounds (Barathi S, N Vasudevan, 2001). An effective degradation of crude oil would require simultaneous action of several metabolically versatile microorganisms with faourable environmental conditions such as pH, temperature and availability of nutrients. The necessity for seeding with complementary hydrocarbon degrading bacteria arises from the rationale that indigenous microbial populations may not be capable of degrading a wide range of potential substrates in a complex mixture such as crude oil.

It seems that a natural microbial community includes a variety of microorganisms that can degrade, alone or together, most crude oil components, but that some of their degrading ability would not be expressed in a single batch culture owing to unfavorable physiological conditions.

Conclusion

The result obtained in this study shows seven bacteria were isolated from the petroleum contaminated soil while six were isolated from uncontaminated soil. Species strains found in the contaminated soil are indigenous and effective in the utilization of crude oil as the sole carbon and energy. The study provides information that would lead to selection of bacterial species that could be employed for bioremediation in environments polluted with used engine oil.

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