Isolation and Optimization of Hydrocarbon Degrading Bacteria

ABSTRACT

Aims: To isolate and characterize hydrocarbon degraders in a contaminated soil sample
Study Design: This experiment was carried out under aseptic conditions. The result was confirmed by visible spectrophotometer
Place and Duration of Study: Department of Biological Sciences, Microbiology Program, Clifford University, Ihie Campus, Owerrinta, Abia State, Nigeria, between May 2021 to July 2021
Methodology: Soil sample contaminated with hydrocarbon used in this experiment was from an automobile mechanic workshop. A ten-fold serial dilution was made for each soil sample, after which 1 ml of 10^{-5} dilutions was plated out using pour plate method and incubated at 37^0C for 48 hours. The isolates were then grown on different hydrocarbons (crude oil, fuel or spent engine oil) and the degradation efficiency was confirmed by visible spectrophotometer.
Result: The isolation of hydrocarbon-degrading bacteria in topsoil and subsoil samples of a mechanic workshop located in Isiala Ngwa North Local Government Area Junction, Abia State was carried out. Four bacterial species were isolated from the soil sample collected, the isolates were subjected to hydrocarbon degradation/utilization test of different hydrocarbons (crude oil, fuel or spent engine oil) it was observed that two of the isolates identified as *Bacillus* and *Staphylococcus* sp were able to utilize hydrocarbons in the medium more efficiently than other isolates.
Conclusion: Hydrocarbon degrading bacteria were isolated from an auto-mechanic workshop in Isiala Ngwa North, Abia State. Two of the four strains (*Bacillus* sp *and Staphylococcus* sp), have the highest potential to use different hydrocarbons (fuel, crude oil and condemn oil) as the sole carbon source. The hydrocarbon degrading properties of these strains suggest that they can be useful in bioremediation of hydrocarbon polluted sites. This may be harnessed to address the contamination problem posed by motor mechanics who carelessly dispose used motor oil in the environment and hydrocarbon contamination in general.

Keywords: Bacteria, Bioremediation, Hydrocarbon degraders, Hydrocarbons, Isolates, Soil

1. INTRODUCTION

Petroleum is a heterogenous mixture of hydrocarbons (aliphatic, alicyclic and aromatic hydrocarbons) which varies in composition and physical properties depending on the reservoir's origin. They are organic compounds containing carbon and nitrogen and are water insoluble. Microorganisms can either degrade or form hydrocarbons depending on certain metabolic pathways present in the environment [1]. Accumulation of petroleum hydrocarbons in the environment is caused by anthropogenic practices such as industrial activities, petroleum and its derivatives as well as incomplete combustion of fossil fuel. Petroleum hydrocarbons mostly encountered in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment [2].

Since the discovery of crude oil, oil spillage has been a global issue. Oil spills have posed a major threat to the environment of oil producing areas which if not effectively checked can lead to the total destruction of ecosystems [3]. There has been a wide spread contamination of aquatic and terrestrial ecological ecosystems due to increasing exploration and production practices with improper waste disposal [4]. The oil contaminating compounds in the environment are light hydrocarbons (oil, gasoline, diesel), heavy hydrocarbons (lubricants, heavy oil, crude oil), halogenated solvents, and other more complex molecules

(aromatic hydrocarbons polycyclic, PAHs, etc.[5] Petroleum oil spill or contamination usually occurs during production, storage, transportation, refining, processing, blowout accidents during oilfield development, leakage from oil pipelines and storage tanks, oil tankers, oil well waxing and overhauls of refineries and petrochemical production equipment [6].

The problems of hydrocarbon contamination are known to be widespread in oil producing areas, and are of public health concern. When coupled with an insufficient ability to deal with oil-contaminated environments, especially in extreme or unique environments such as Polar Regions, deep sea areas, deserts, and wetlands, there is a constant threat of contamination wherever oil is exploited. Although oil pollution is difficult to treat, petroleum hydrocarbon-degrading bacteria have evolved as a result of existing in close proximity to naturally occurring petroleum hydrocarbons in the environment. Such organisms are candidates for the treatment of oil pollutants [7].

Attempts to remediate hydrocarbon polluted sites using physiochemical methods had been in existence but due to other implications their application were discouraged. Bioremediation using microorganisms capable of degrading hydrocarbon is now believed to more promising and effective. Bacteria have been screened and utilized to degrade waste products produced by the food, agricultural, chemical and pharmaceutical industries. In recent years, the use of bacteria to deal with environmental pollutants has become a promising technology because of its low cost and eco-friendly nature [8]. Many microorganisms have the ability to utilize hydrocarbons as sole sources of carbon as energy for metabolic activities .and these micro organisms are widely distributed in the nature. The microbial utilization of hydrocarbons depends on the chemical nature of the compounds within the petroleum mixture [9]. The remediation of petroleum hydrocarbon pollution has attracted much attention thus there is continuous development and improvement of microbial remediation technology [10], [11]. The ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as evidence that those organisms are the active degraders in that environment; likewise hydrocarbon micro seepage is a widely distributed natural phenomenon in the geochemical carbon cycle. This study therefore was designed to isolate and characterize hydrocarbon degrading bacteria from a contaminated soil, also to confirm the ability of the isolates to degrade wide range of hydrocarbons. The abilities of the isolates to degrade hydrocarbons are investigated through their growth in the different hydrocarbons used.

2. MATERIALS AND METHODS

2.1. The Study Site

Topsoil and subsoil samples were collected from Chima & Sons auto-mechanic workshop in Isiala Ngwa North Local Government Area, Abia State.

2.2. Sample Collection

Three (3) soil samples were collected using a sterile sample bottles at three different points in the automobile mechanic workshops. The topsoil sample was collected at the surface of the soil, ensuring that the soil particles clogged to the spatula were scraped off after collection. The procedure for collection of topsoil samples was repeated for subsoil samples. The soil samples were arranged in a box and transported to the laboratory for microbial analysis.

2.3. Sterilization of Glass Wares and Other Materials:

All the glass wares used were washed, dried and sterilized in a hot-air oven at a temperature of 160 ^{0}C for 1 hour. The area (bench) where the work was done was properly swabbed with cotton wool and methylated spirit. The wire loop was also sterilized by flaming before and after use, using a spirit lamp.

2.4. Preparation of Culture Media:

Sterile distilled water was used for serial dilution and Nutrient Agar (NA) was used for culturing. In preparing the media, 8.4 g of nutrient agar was dissolved in 300 ml of distilled water. The media were autoclaved at 121 ^{0}C for 15 minutes

2.5. Microbiological Analysis:

Under an aseptic condition, topsoil sample collected was grounded to break soil clogs using a sterile mortar and pestle. This was repeated for the subsoil samples. A ten-fold serial dilution was made for each soil sample, after which 1 ml of 10^{-5} dilutions was plated using pour plate method and incubated at 37 ^{0}C for 48 hrs

2.5.1. Microbial Count and Pure Culture Isolation:

Total viable counts of bacteria were determined by enumerating the colony forming units (CFU) after incubation for 48 hrs. Pure cultures of bacterial isolates were obtained by sub-culturing on the nutrient agar plates and pure cultures were then transferred to agar slants for further biochemical tests

2.6. Characterization and Identification of the Isolates:

Bacterial isolates were characterized by morphological and biochemical characteristics. The biochemical tests carried out on each bacterial isolates were: Catalase, Oxidase, Citrate utilization, Coagulase, Indole, Sugar fermentation test and hydrogen sulfide test.

2.6.1. Hydrocarbon Degradation Test:

100 ml of Mineral Salt Medium (MSM) was prepared into four conical flasks, supplemented with 1 ml of hydrocarbon (crude oil, fuel or spent engine oil). The media was inoculated with a loop full of the bacterial isolates and conical flasks were placed in a shaker. The absorbance of the culture was also measured using a spectrophotometer. Serial dilution and pour plate was done on the cultures in the conical flask every two days to enumerate the number of colonies that thrived and/or can survive the MSM - hydrocarbon mixture.

3. RESULTS AND DISCUSSIONS:

3.1. Isolation and Screening of Hydrocarbon Degrading Bacteria

Four species of bacteria were isolated on Nutrient broth media and only two bacterial species were able to utilize hydrocarbon while other species that were unable to degrade hydrocarbon are known as heterotrophs which feeds on the byproduct of the hydrocarbon degraders. These two species were identified as *Bacillus* sp. and *Staphylococcus* sp. The result of the biochemical tests is as shown in Table 1

Table 1: Biochemical Test for Bacterial Isolates

								SFT				
Sample	Cultural Characteristics	Gram Reaction	Catalase	Coagulase	Oxidase	Indole	Citrate	S	B	G	H_2S	Possible Bacteria Species (sp.)
A	Milky, sticky, flat, rough, opaque	Positive Rod	+	-	-	-	+	-	Y	-	-	*Bacillus* sp.
B	Yellow, slimy, shinny, raised	Positive Cocci	+	+	-	-	+	-	Y	-	-	*Staphylococcus* sp.
C	Milky, rhizoid, flat	Positive Rod	+	-	-	-	+	-	Y	-	-	*Bacillus* sp.
D	Circular, yellow, raised	Positive Cocci	+	+	-	-	+	-	Y	-	-	*Staphylococcus* sp.

Key: SFT – Sugar Fermentation Test, S – Slope colour, B – Butt colour, G – Gas production, Y – Yellow, R – Red, H_2S – Hydrogen sulfide, + - Positive, - - Negative

Soil samples laden with hydrocarbons from Chima and Sons automobile mechanic repair workshop harbored bacteria of possible biodegradation importance. *Bacillus* and *Staphylococcus* spp. isolated were found to be able to utilize hydrocarbon as energy source. *Bacillus* spp. and *Staphylococcus* spp. degraded the hydrocarbon in fuel, crude oil and condemned oil (Figures 1 – 6). Several authors have reported excellent degradation of oil by pure cultures. The majority of previous work on hydrocarbon degrading bacteria in some environments had been conducted under enrichment conditions and over 500 species have been recognized as capable of hydrocarbon degradation [12]. *Bacillus* and *Staphylococcus* spp were among the bacterial isolates able to utilize spent lubricating oil as reported by Ekanem and Ogunjobi [4]. Onifade and Abubakar [13] reported that *Bacillus* sp were the predominant isolates from a crude oil polluted soil, this may due to the ability of the organism to produce spores which may shield them from the toxic effects of the hydrocarbons. *Bacillus* has been shown to degrade aliphatic and aromatic hydrocarbons up to 82.41% and 81.56% respectively [14]. *Bacillus* was isolated from spent engine oil contaminated soil and was proven to degrade the hydrocarbon [15] *Staphylococcus* spp also has been identified as having the capability to degrade petroleum [16]

It is, however evident from the outcome of this study that hydrocarbon polluted soil harbor degrading microorganisms and can possibly be used for bioremediation of hydrocarbon polluted soil.

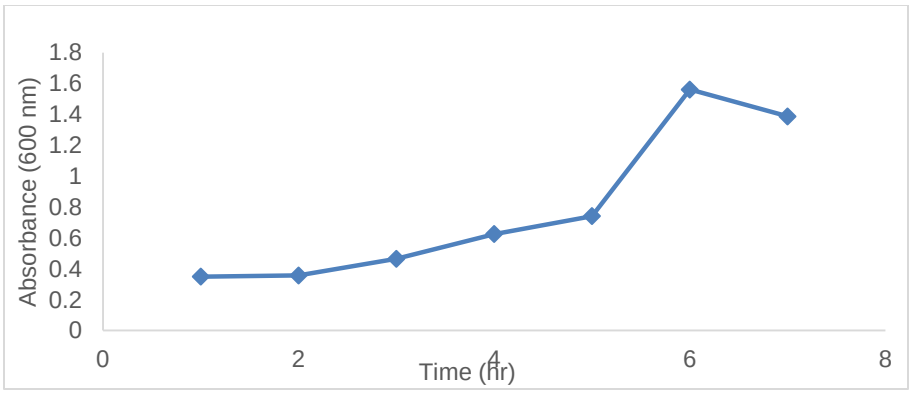

Figure 1: Growth Profile of *Bacillus* sp on fuel

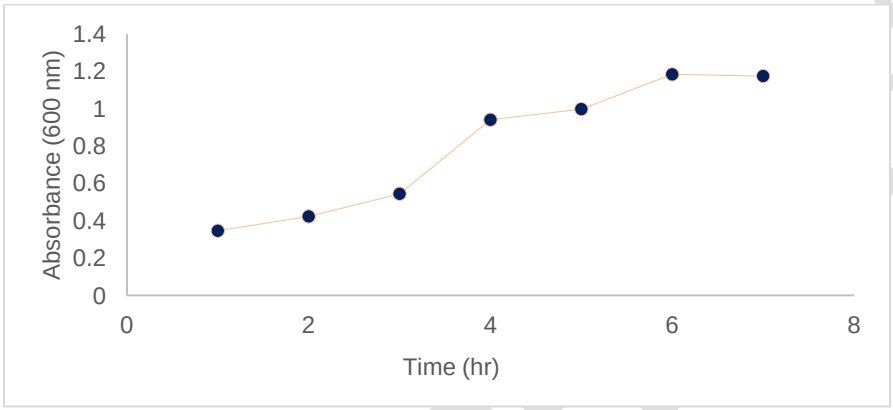

Figure 2: Growth profile of *Staphylococus* sp. on Fuel

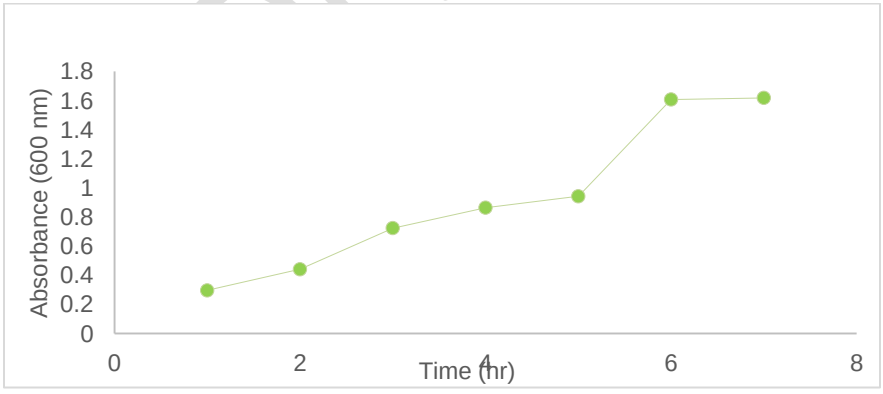

Figure 3: Growth Profile of *Bacillus* sp. on Crude Oil

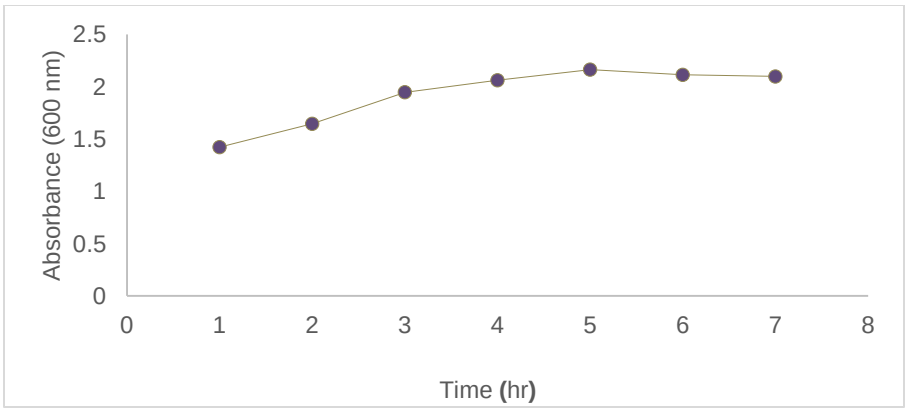

Figure 4: Growth Profile of *Staphylococcus* sp. on Crude Oil

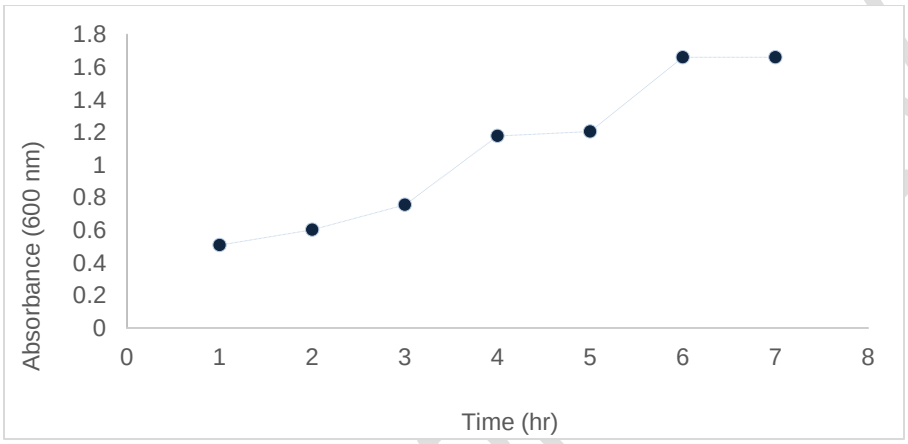

Figure 5**:** Growth Profile of *Bacillus* sp on Spent Engine Oil

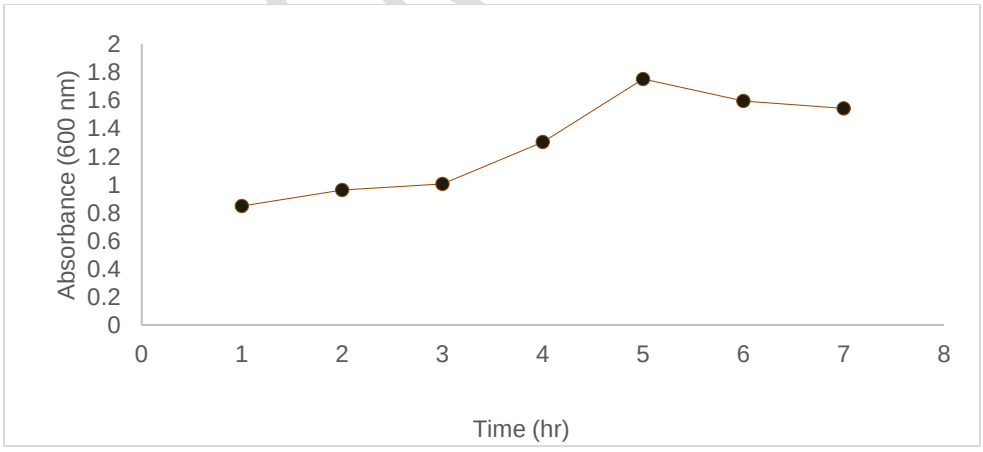

Figure 6: Growth Profile of *Staphylococcus* sp on Spent Engine Oil

CONCLUSION

The results of this study indicated that from the hydrocarbon degrading bacteria isolated from Chima & Sons auto-mechanic workshop in Isiala Ngwa North Local Government Area, Abia State, two strains (*Bacillus* spp and *Staphylococcus* spp), have the highest potential to use different hydrocarbons (fuel, crude oil and spent engine oil) as the sole carbon source. The hydrocarbon degrading properties of these strains suggest that they can be useful in bioremediation of hydrocarbon polluted sites

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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