



Isolation and Optimization of Hydrocarbon – Degrading Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 Programme, Clifford University, Ihie Campus, Owerinta, 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 °C for 48 hrs. 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

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highest potential to use different hydrocarbons (fuel, crude oil and condemn oil) as the sole carbon source. These strains' hydrocarbon – degrading abilities suggest that they could be effective in bioremediation of hydrocarbon – polluted locations. This could be used to combat contamination caused by motor mechanics who carelessly dispose of old motor oil in the environment, as well as general hydrocarbon contamination.

Keywords: Bacteria; bioremediation; hydrocarbons; degraders; hydrocarbon; isolates; soil.

1. INTRODUCTION

Petroleum is a heterogeneous combination of hydrocarbons (aliphatic, alicyclic, and aromatic hydrocarbons) with different compositions and physical properties depending on the origin of the reservoir. They are carbon and nitrogen-based organic molecules that are water insoluble. Microorganisms can either degrade or form hydrocarbons depending on certain metabolic pathways present in the environment [1]. Anthropogenic behaviours such as industrial operations, petroleum and its derivatives, and incomplete fossil fuel combustion all contribute to the accumulation of petroleum hydrocarbons in the environment. Because of their energetic and carbon needs for growth and reproduction, as well as the need to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment, indigenous bacteria degrade or metabolize petroleum hydrocarbons most commonly encountered in the environment [2].

Oil spills has been a global issue since the discovery of crude oil. Oil spills have posed a significant hazard to the environment in oil – producing countries, and if not successfully controlled, can result in ecosystem damage [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]. Light hydrocarbons (oil, gasoline, diesel), heavy hydrocarbons (lubricants, heavy oil, crude oil), halogenated solvents, and other more complex molecules (aromatic hydrocarbons polycyclic, PAHs, etc.) are oil polluting chemicals in the environment [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].

Hydrocarbon pollution is known to be ubiquitous in oil-producing locations, and it poses a public health risk. There is a persistent concern of contamination wherever oil is used when it is combined with an insufficient ability to cope with oil-contaminated surroundings, especially in extreme or unique conditions such as Polar Regions, deep marine areas, deserts, and wetlands. Despite the difficulty of treating oil pollution, microorganisms that degrade petroleum hydrocarbons have evolved as a result of living in close contact to naturally occurring petroleum hydrocarbons in the environment. These creatures could be used to clean up oil pollution [7].

There have been attempts to use physiochemical approaches to rehabilitate hydrocarbon-polluted locations, but their use was discouraged due to additional concerns. Bioremediation with hydrocarbon – degrading microorganisms is currently thought to be more promising and successful. Bacteria have been screened and used in the culinary, agricultural, chemical, and pharmaceutical industries to breakdown waste products. Because of its low cost and eco-friendly nature, the use of bacteria to deal with environmental toxins has become a promising technology in recent years [8]. Many microorganisms have the ability to utilize hydrocarbons as sole sources of carbon as energy for metabolic activities .and these microorganisms 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 extract large numbers of specific oil – degrading microorganisms from an environment is sometimes considered as proof that those organisms are the active degraders in that environment; similarly, hydrocarbon micro seepage is a prevalent natural phenomenon in the geochemical carbon cycle. As a result, the goal of this work was to isolate and characterize

hydrocarbon – degrading bacteria from polluted soil, as well as to confirm the isolates' capacity to digest a broad variety of hydrocarbons. The isolates' ability to degrade hydrocarbons is examined by observing their development in the various 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 Nigeria.

2.2 Sample Collection

Three (3) soil samples were collected using 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 °C for 1 hr. 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 °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 °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 loopful of the bacterial isolates and the conical flasks were placed in a shaker. The absorbance of the culture was also measured using a spectrophotometer. Serial dilution and pour plate were 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 were cultured on MSM which was incorporated with different hydrocarbons namely fuel, diesel, spent engine oil and crude oil, only two bacterial species were able to utilize hydrocarbon while other species that were unable to degrade hydrocarbons are known as heterotrophs which feeds on the byproduct of the hydrocarbon – degraders. The growths of the organisms on MSM with hydrocarbons are as shown in Plates 1 – 4.

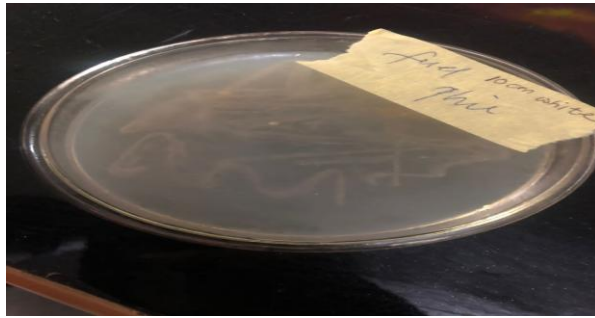


Plate 1. Organisms growing on Minimal Salt Medium supplemented with Fuel as the carbon source



Plate 2. Organisms growing on Minimal Salt Medium supplemented with Diesel as the carbon source



Plate 3. Organisms growing on Minimal Salt Medium supplemented with Crude oil as the carbon source



Plate 4. Organisms growing on Minimal Salt Medium supplemented with Spent engine oil as the carbon source

Table 1. Biochemical Test for Bacterial Isolates

Sample	Cultural Characteristics	Gram Reaction	Catalase	Coagulase	Oxidase	Indole	Citrate	SFT			Possible Bacterial Species (sp)	
								S	B	G		H ₂ S
A	Milky, sticky, flat, rough, opaque	Positive Rod	+	-	-	-	+	-	Y	-	-	<i>Bacillus</i> sp
B	Yellow, slimy, shinny, raised	Positive Cocci	+	+	-	-	+	-	Y	-	-	<i>Staphylococcus</i> sp
C	Milky, rhizoid, flat	Positive Rod	+	-	-	-	+	-	Y	-	-	<i>Bacillus</i> sp
D	Circular, yellow, raised	Positive Cocci	+	+	-	-	+	-	Y	-	-	<i>Staphylococcus</i> sp

Key: SFT means Sugar Fermentation Test, S – Slope colour, B – Butt colour, G – Gas production, Y – Yellow, R – Red, H₂S – Hydrogen sulfide, + - Positive, - - Negative

These two species were identified as *Bacillus* sp. and *Staphylococcus* sp. The result of the biochemical tests is as shown in Table 1.

Soil samples laden with hydrocarbons from Chima and Sons automobile mechanic repair workshop harbored bacteria of possible biodegradation importance. *Bacillus* and *Staphylococcus* sp. isolated were found to be able to utilize hydrocarbon as energy source. *Bacillus* sp. and *Staphylococcus* sp. 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* sp 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* sp also has been identified as having the capability to degrade petroleum [16]. Two strains belonging to the genus *Bacillus* (*Bacillus* sp. L26 and *Bacillus* sp. L30) showed hydrocarbon degradation ability on diesel, hexadecane, toluene and xylene [17].

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

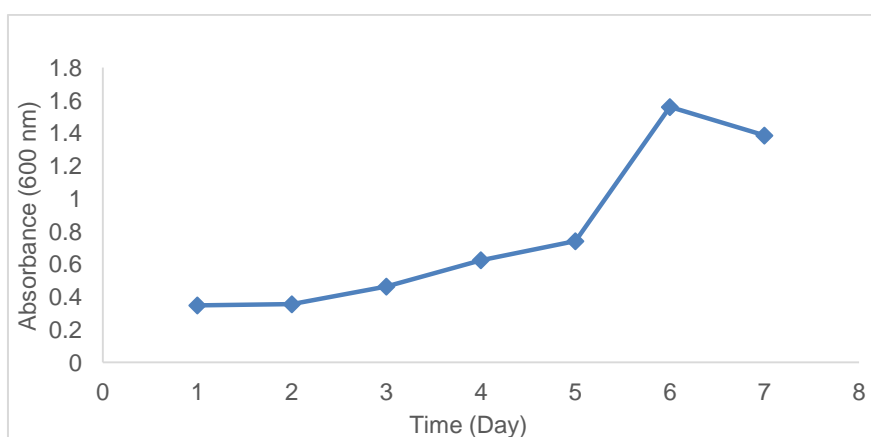


Fig. 1. Growth Profile of *Bacillus* sp on fuel at various time intervals

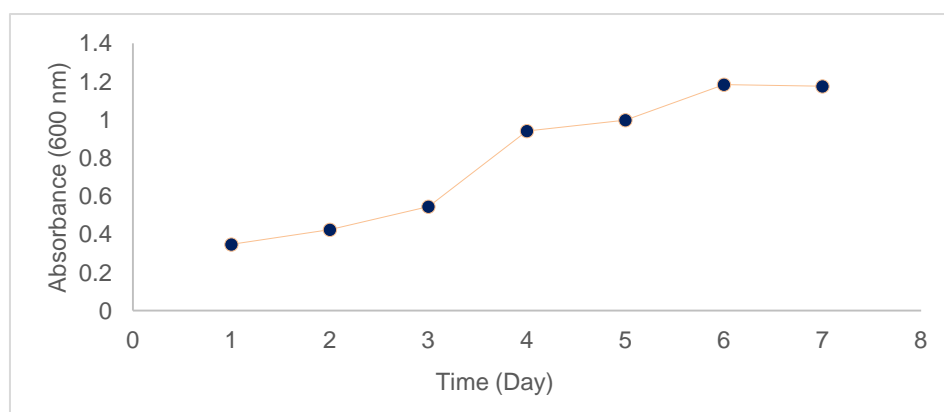


Fig. 2. Growth profile of *Staphylococcus* sp. on Fuel at various time intervals

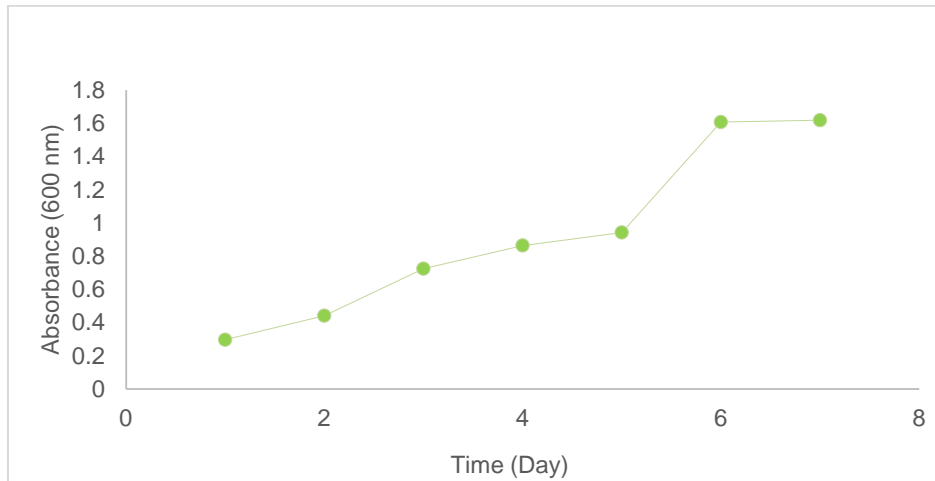


Fig. 3. Growth Profile of *Bacillus* sp. on Crude Oil at various time intervals

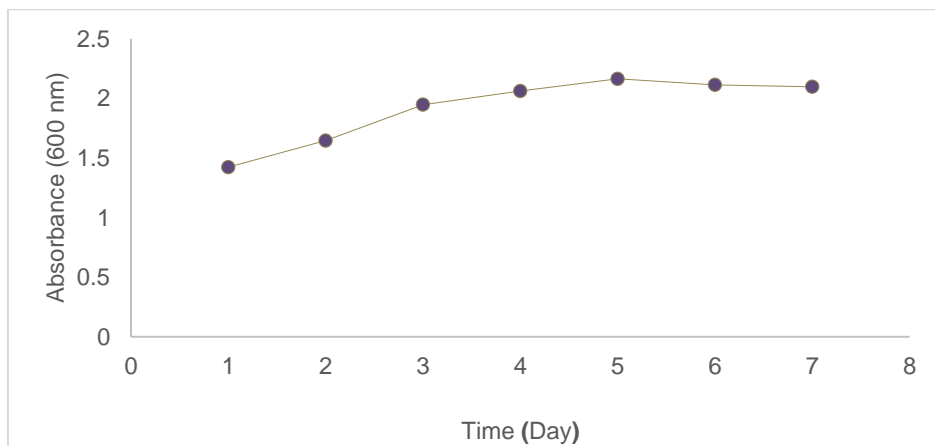


Fig. 4. Growth Profile of *Staphylococcus* sp. on Crude Oil at various time intervals

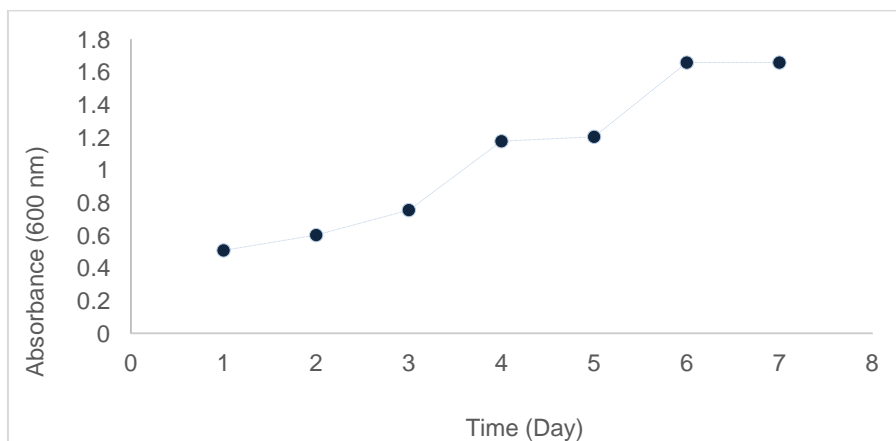


Fig. 5. Growth Profile of *Bacillus* sp. on Spent Engine Oil at various time intervals

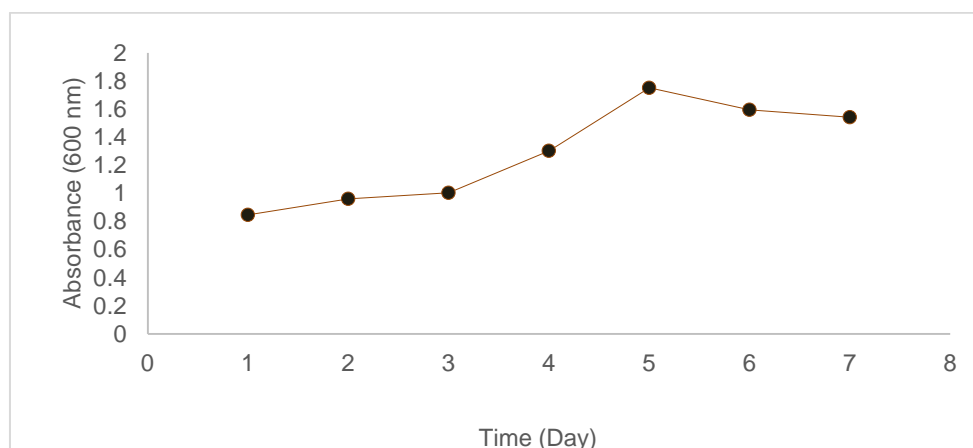


Fig. 6. Growth Profile of *Staphylococcus* sp on Spent Engine Oil at various time intervals

4. CONCLUSION

The findings showed that two strains (*Bacillus* sp and *Staphylococcus* sp) of hydrocarbon – degrading bacteria isolated from Chima & Sons auto-mechanic workshop in Isiala Ngwa North Local Government Area, Abia State, have the greatest potential to use different hydrocarbons (fuel, crude oil, and spent engine oil) as the sole carbon source. These strains' hydrocarbon – degrading abilities suggest that they could be effective in bioremediation of hydrocarbon – polluted areas.

DISCLAIMER

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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