HYDROCARBON UTILIZING BACTERIA IN THE RHIZOSPHERE OF *Moringa oleifera*

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ABSTRACT: The occurrence and identification of hydrocarbon utilizing bacteria found in the rhizosphere of *Moringa oleifera* (Rh) and non-rhizosphere soil (NRh) were carried out comparatively after contamination with crude oil collected from Kaduna Refining and Petrochemical Company, Kaduna. The results indicated a hydrocarbon utilizing bacterial growth range between 2.0 to 19.8 x 10^4 cfu/g from Rh and 3.4 to 14.2 x 10^4 cfu/g from NRh. Heterotrophic bacterial count ranged between 14.0 to 20.0 x 10^5 cfu/g and 10.2 to 13.0 x 10^5 cfu/g in Rh and NRh respectively. Bacteria that were capable of utilizing crude oil as their only source of carbon included the following: *Pseudomonas aeruginosa*, *Aeromonas* spp, *Bacillus mycoides*, *Arachnia propionica*, *Shigella* spp and *Kurthia* spp. Generally, *Pseudomonas aeruginosa*, *Aeromonas* spp and *Arachnia propionica* showed higher hydrocarbon degrading strengths than *Bacillus mycoides, Shigella* spp and *Kurthia* spp. The results showed that hydrocarbon utilizing bacteria occurred slightly more abundantly in Rh than in NRh.

INTRODUCTION

Since the discovery of crude oil in 1956 and its subsequent commercial production in 1958, oil spills have been a disturbing source of pollution in some parts of Nigeria. These incidents are as a result of lack of regular maintenance of pipelines and storage tanks, and sabotage. The harmful effects of oil spills in the environment are numerous: oil kills plants and animals in the estuarine zone and it also settles on beaches and kill organisms such as crabs and fish. Oil poisons algae, disrupts major food chains and decreases the yield of edible crustaceans, Nwilo and Badejo (2005).

Occurrence and identification of hydrocarbon utilizing bacteria in the rhizosphere of *Moringa oleifera* is concerned with the use of bacteria in close association with the root of the plant to degrade hydrocarbon, with the aim of solving the problem of hydrocarbon pollution in Nigeria.

Moringa oleifera is a fast growing aesthetically pleasing small tree adapted to arid sandy conditions, growing up to four meters in height and bearing fruits within the same year it was planted. It is commonly used for food, medicine and can function as wind breaks for erosion control, Josh (1996). While it grows best in dry sandy soil, it tolerates poor soil, including coastal areas, Wikipedia (2001).

MATERIALS AND METHOD

SAMPLE COLLECTION

The crude oil was collected from KRPC, Kaduna. Five grams each of both rhizosphere and non-rhizosphere soil samples were collected from the environs of Kaduna State University. The soil was dug to a depth of 10cm in order to obtain the soil in close association with the roots of the plants. The roots were freed from their adhering soil, which was assumed to be the rhizosphere soil. Non-rhizosphere soil sample, which served as the control was collected at a distance of 20cm away from the plant. The samples were placed in polythene bags, labeled and transported to the microbiology laboratory of Kaduna State University for analysis.

ENUMERATION OF BACTERIA

One gram from each of the samples of both the rhizosphere (experimental) and non-rhizosphere (control) soils were weighed aseptically and placed in test tubes containing 9ml of distilled water. The method of Kang and Siragusa (2001) was employed to prepare dilutions of up to 10^{-4}. Aliquots (1g) were inoculated into Nutrient Agar (NA) and Oil Agar for the enumeration of total heterotrophic and crude oil utilizing bacteria respectively. The plates were incubated at room temperature ($28^{o}C \pm 2$) for 48 hours and 5 days for NA and OA respectively. The colonies that developed on the plates were counted using a colony counter and recorded as colony forming units per milliliter (cfu/g) of soil.

IDENTIFICATION OF BACTERIA

Pure cultures were obtained by sub-culturing colonies from the primary culture on NA plates and incubating overnight at $37^{o}C$. Discrete colonies were stored on nutrient agar slants. Further characterization and identification were done on the basis of microscopic examination, cultural characterization, morphological characteristics and gram staining reaction.

GRAM'S STAINING

A smear of the growth was prepared on a clean grease-free slide, which was air-dried and fixed. The slide was then stained with crystal violet solution (primary dye) for 60 seconds after which it was washed with tap water and well drained. Iodine solution (mordant) was used to flood the slide for 30 seconds after which the slide was washed with tap water. Decolorization was done with acetone (alcohol) and washed with water. The slide was then flooded with safranin (counter stain) for 60 seconds, washed and allowed to dry. It was then examined under the microscope. Organisms able to retain the primary stain's color are Gram positive while Gram negative bacteria retained the red color of the counter stain.

BIOCHEMICAL TESTS

The entire biochemical tests were carried out as described by Oyeleke and Manga (2008).

Catalase test

A drop of 3% hydrogen peroxide (H_2O_2) was placed on a glass slide. Using a wire loop, a little colony was removed from the sample and applied to the hydrogen peroxide on the slide. A positive test was indicated by bubbling and frothing which were absent in a negative test.

Indole test

The sample organism was grown in 5ml of peptone water for 24 hours, after which 3 drops of KOVAC'S indole reagent was added and shaken gently. Development of a red color in the surface layer indicated a positive reaction whereas a negative reaction retains its yellow color. Indole release is indicative of the breakdown of tryptophan, an amino acid, which is peculiar to enterobacteria.

Motility test

This test determines the presence or absence of flagella. The motility medium in tube was inoculated by making a fine stab with a loopful of the culture to depth of about 1 -2cm short of

the tube's bottom, and was incubated at 37°C for 24 to 48 hours. Motile bacteria made the medium cloudy while non-motile bacteria are indicated by restricted growth along the line of inoculation.

Urease test

A urea agar slant was inoculated with the culture and incubated for 24 to 72 hours. The development of a bright pink or red color indicated a positive reaction. This test is important in differentiating enterobacteria.

Citrate utilization test

In order to identify an organism that is able to use citrate as a carbon source, its culture was inoculated into a Simmon citrate agar slant and incubated for 24 - 72 hours. The development of a blue color indicated a positive reaction.

Methyl red - Voges Proskauer test

5mls of MR-VP broth was inoculated with the test organism and incubated for 48 – 72 hours at 35°C after which, 1ml of the broth was transferred into a small serological tube and 2 – 3 drops of methyl red was added. A red color on addition of the indicator signified a positive test while a yellow color signified a negative test. To the rest of the broth in the original tube, 5 drops of 40% potassium hydroxide were added followed by 15 drops of naphtol in ethanol. The tube was shaken and the cap loosened and the tube was placed in a sloping position. A red color at the liquid-air interface within 1 hour indicated a VP negative test.

Triple sugar iron test

This test is based on the fermentation of the sugars, glucose and lactose; and the production of gas and hydrogen sulphide. A sterilized wire loop was used to stab the butt of the TSI medium with the test organism 2 – 3 times. The same wire loop was also used to streak the slant which was then capped loosely and incubated at 37°C. The result was interpreted and recorded accordingly.

Screen test of Isolates for ability to utilize Hydrocarbon

Bacterial isolates were tested for their ability to utilize petroleum hydrocarbon using the turbidity method as described by Nweke and Okpokwasili (2011). The bacterial isolates were cultured in nutrient broth (NB) and incubated at 28°C for 24 hours. 0.1ml of the NB grown culture was inoculated into separate test tubes containing 10ml of sterile mineral salts medium and 1% (v/v) each of hydrocarbons (crude oil) according to the methods of Ijah (1998). Control tubes were incubated at room temperature under a stationary condition and monitored for 7 days. The growth of the inocula was determined by visual observation of the oil medium turbidity as compared with the uninoculated control tubes.

RESULTS AND DISCUSSION

The results obtained as shown in Table 1 revealed that one gram of soil from the rhizosphere of *Moringa oleifera* has a higher bacterial count range (14.0 – 20.0 x 10^{5} cfu/g) than non-rhizosphere soil sample of the same quantity (10.2 – 13.0 x 10^{5} cfu/g). This could be as a result of higher nutritional composition in the rhizosphere as compared to bulk soil, which corresponds with the report of Bornerer (2011) that because of organic substances, there is a higher microbial biomass and activity in the rhizosphere than in bulk soil.

Table 2 contains results of the total count of hydrocarbon utilizing bacteria. The counts ranged from 2.0 – 19.8 x 10^{4} cfu/g and 3.4 – 14.2 x 10^{4} cfu/g for rhizosphere and non-rhizosphere soil samples respectively. This indicates that both soils contained bacterial isolates that were able to grow in the presence of complex hydrocarbon substance (crude oil) but the rhizosphere soil had a higher concentration of bacteria capable of degrading hydrocarbon. The isolates selected from the rhizosphere of *Moringa oleifera* were *Pseudomonas aeruginosa*, *Shigella* spp, *Aeromonas*

spp, *Arachia propionica*, *Bacillus mycoides* and *Kurthia* spp (Table 3).

Table 1: Heterotrophic Bacterial Count (x 10^5 cfu/g)

Sample Number	Rh	NRh
1	14.4	10.8
2	20.0	13.0
3	14.0	12.0
4	19.2	10.2
Mean	16.9	11.5
Range	14.0 – 20.0	10.2 – 13.0

Key:
Rh – Rhizosphere soil
NRh – Non-rhizosphere soil

Table 2: Hydrocarbon Utilizing Bacterial Count (x 10^4 cfu/g)

Sample Number	Rh	NRh
1	2.0	3.4
2	9.0	7.4
3	19.8	13.4
4	16.2	14.2
Mean	11.75	9.6
Range	2.0 – 19.8	3.4 – 14.2

Key:
Rh – Rhizosphere soil
NRh – Non-rhizosphere soil

Table 3: Degree of Hydrocarbon Utilization by Isolates from the Rhizosphere of *Moringa oleifera* after 7 Days

Bacteria Name	Degree of Hydrocarbon Utilization
Pseudomonas aeruginosa	***
Aeromonas spp	***
Bacillus mycoides	**
Shigella spp	***
Arachnia propionica	***
Kurthia spp	**

Keys:
*** - Very high degrading strength
** - Moderate degrading strength

The ability of *Pseudomonas* spp and *Bacillus* spp to degrade hydrocarbon in this research supports the report of Benka-Coker & Ekundayo (1996) and Rahman *et* al (2003) that both *Pseudomonas aeruginosa* and *Bacillus* spp have hydrocarbon degrading strength but *Pseudomonas aeruginosa* had a higher strength than *Bacillus* spp in the degradation. The abilities of *Aeromonas* spp and *Arachnia propionica* to degrade hydrocarbon agrees with the work of Ibrahim *et. al* (2008). There is however, little information on the hydrocarbon degrading abilities of *Shigella* and *Kurthia* species.

CONCLUSION AND RECOMMENDATION

The result of this study showed that hydrocarbon utilizing bacteria of different degrading strengths can be found in the rhizosphere of *Moringa oleifera*, which has the ability to grow

even under harsh conditions. It is therefore recommendable that *Moringa oleifera* be planted in hydrocarbon polluted areas. It is also necessary to carry out further investigations on the possibility of genetic modifications on the hydrocarbon utilizing bacteria native to the rhizosphere of *Moringa oleifera* with the aim of increasing their degrading strengths.

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