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Culture on Nutritive Media and Characterization of Some Endophytic Bacteria Isolated from the Roots and Leaves of Mangrove Plants: *Avicennia germinans*, *Acrostichum aureum* and *Rhizophora mangle*

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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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Original Research Article

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ABSTRACT

Aim: The aim of this study was to isolate and characterize the culturable endophytic bacteria from the roots and leaves of three different mangrove plants found growing in same environment.
Study Design: This study employs experimental design, statistical analysis of the data and interpretation.
Place of Study: The roots and leaves of three mangrove plants; *Rhizophora mangle*, *Avicennia germinans and Acrostichum aureum* were gotten from a waterfront location in old Port Harcourt township of Rivers State, situated at Longitude 4^0 45'5.18''N and Latitude 7^0 0'58.35" E.
Methodology: Using standard Microbiological techniques, the roots and leaves were treated and endophytic bacteria isolated and subjected to morphological and biochemical tests.
Results: From the roots, the hydrocarbon utilizing bacteria counts ranged from 7.0x103cfu/g – 1.6x104cfu/g, while the that of the leaves ranged from 3.0 x105cfu/g – 8.0 x105cfu/g. The nitrogen fixing bacteria counts for roots ranged from 4.5 x103cfu/g – 1.0 x104cfu/g and that of the leaves ranged from 3.0 x105cfu/g – 6.0 x105cfu/g. Nitrifying bacteria counts for the roots ranged from

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5.0x103cfu/g – 7.0x103cfu/g, while the counts from the leaves ranged from 3.5 x 105cfu/g - 5.0 x105cfu/g. Out of the 24 isolates, 14 were from the roots, while 10 were from the leaves. Percentage of occurrence of the isolates was in this order *Bacillus* sp 33% > *Staphylococcus* sp 21%> *Klebsiella* sp 13%> *Pseudomonas* sp 9%> *Micrococcus* sp 8%> *Nitrobacter* sp 8%> *Nitrosomonas* sp 4%> *Azotobacter* sp 4%. This reveals that *Bacillus* sp occurred most in the plant samples. The result also revealed that the Red Mangrove Roots had the highest number of organisms. The endophytic bacteria isolated were further subjected to morphological and biochemical identification and they were identified as: *Pseudomonas* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Klebsiella* sp, *Azotobacter* sp, *Nitrobacter* sp. and *Nitrosomonas* sp. Five of the isolates showed a high potential to degrade crude oil in the following order *Pseudomonas* sp (H2) > *Bacillus* sp (BA) > *Klebsiella* sp (SB)> *Bacillus* sp (TG) > *Pseudomonas* sp (H1).

Conclusion: From the results, the mangrove roots and leaves contained high numbers of active indigenous bacteria most of which are known to use up crude oil as their carbon source. The mangrove roots had higher number of endopyhtic bacteria than the leaves.

Keywords: Endophytic bacteria; mangrove roots; nitrogen fixation; leaves.

1. INTRODUCTION

Nigeria has the largest mangrove forest in Africa and the third-largest in the world after India and Indonesia [1]. Mangroves are found in nine states out of the 36 states of Nigeria namely Abia, Akwa-Ibom, Bayelsa, Cross River, Delta, Edo, Imo, Ondo, and Rivers States, and they are generally referred to as the Niger Delta (James *et al.,* 2013). Notwithstanding approximately 80% of Niger Delta mangrove forest has its vegetation spread evenly in only three states: Bayelsa, Rivers and Delta States. The largest stretch of about 30 to 40 kilometers of mangroves can be found in the Niger Delta region [1]. There are over hundred species of mangroves globally, but in the Niger Delta region the species commonly found are red (*Rhizophora* species), black (*Avicennia germinans*), white (*Laguncularia racemosa*), and golden leather fern (*Acrostichum aureum*) mangroves [2].

Mangrove ecosystem is one of the richest ecosystem in the world as it offers diverse services and benefits both to humans and the ecosystem where it is found [3]. They serve as a link between terrestrial and marine ecosystem [4]. Mangrove forests improve coastal water quality and provide shelter to species of fish, crab, shrimp, and mollusks. However, if these forests are compromised by oil spills, they can no longer shield coastlines, provide habitat, or feed organisms living among their roots and branches [5]. Once there is a spill, because oil rarely moves, the oil often coats the vegetation penetrating the soil. Mangroves can suffer lethal and sublethal effects when exposed to oil, the mangrove leaves can stunt or deform and branches can defoliate or die back [5]. And because these mangroves receive their oxygen through the lenticels on the exposed roots once the root is damaged or coated with oil, respiratory capabilities of the plant will suffer, which could cause them to suffocate or die [5].

Due to illegal bunkering activities in the Niger Delta region, oil spills have become a case of concern as the mangrove ecosystem is extremely susceptible to oil spills [6-8]. Today, mangrove forests and swamps are among the most threatened habitats in the Niger Delta Crude oil that has been released via spillage into the Niger Delta environment within the last 50years has been estimated to be about 13 million barrels or more, making it to the list of top five world's most adversely damaged environment by petroleum [9]. Most of these spills are not cleaned or not thoroughly cleaned thereby creating wastelands in some of these areas.

Various groups of bacteria are found in the mangrove ecosystem [10] and there they perform several activities which include photosynthesis, nitrogen fixation, and methanogenesis [11]. Endophytic bacteria can be defined as those that can be isolated from healthy, superficially disinfected plant tissues and are not known to cause any damage to the host plant rather are beneficial to them [12,13]. Bacterial endophytes play an important role in the metabolic activities of their host plants, as they produce important chemical compounds that can trigger several biochemical pathways in the host plant [3]. These organisms get protection and nutrients from their host plant, while providing enzymes,

antibiotics, alkaloids and other metabolites that enables the plant to tolerate unfavourable environmental conditions, such as pollution [13]. Endophytic organisms also provides nutrients by nitrogen fixation and have potential to enhance the removal or reduce pollutants like benzene, pesticide, toluene, ethylbenzene and phosphorus solubilization [14].

2. MATERIALS AND METHODS

2.1 Sample Collection

The three mangrove plant samples (roots and leaves) were collected from the Bonny Jetty waterfront (Okrika) in Old Port Harcourt Township, Rivers State, Nigeria. The location is situated at Longitude 4^0 $45^{'}10.13976^{''}$N and Latitude 7^0 $1^{'}4.13012^{''}$ E at Bonny water front. The roots and leaves collected were separately placed in sterile polyethylene bags, transported aseptically first to the Department of Plant Science & Biotechnology Laboratory to be identified before being transferred to the Microbiology Laboratory, all of Rivers State University, Port Harcourt for immediate processing.

2.2 Isolation of Organisms

The organisms were isolated from the roots and leaves of the mangrove plants. They were treated to obtain only the endophytic bacteria using the following processes. First, the plants were washed under running water thoroughly to remove surface adhering debris. Then, they were cut into small pieces washed in sterile distilled water for 5minutes, surface-sterilized with 70% ethanol for 1 minute, 3% sodium hypochlorite (NaOCl solution) for 3minutes and then rinsed 6 times in sterile distilled water in different containers. Afterwards, they were grounded separately with a sterile mortar and pestle to make plant slurries. Serial dilution of 1g of the plant slurry was used for dilution (up to 10^{-4}). An aliquot of 0.1ml of the several dilutions were

Fig. 1. Rhizophora mangle – Red Mangrove leaves and roots

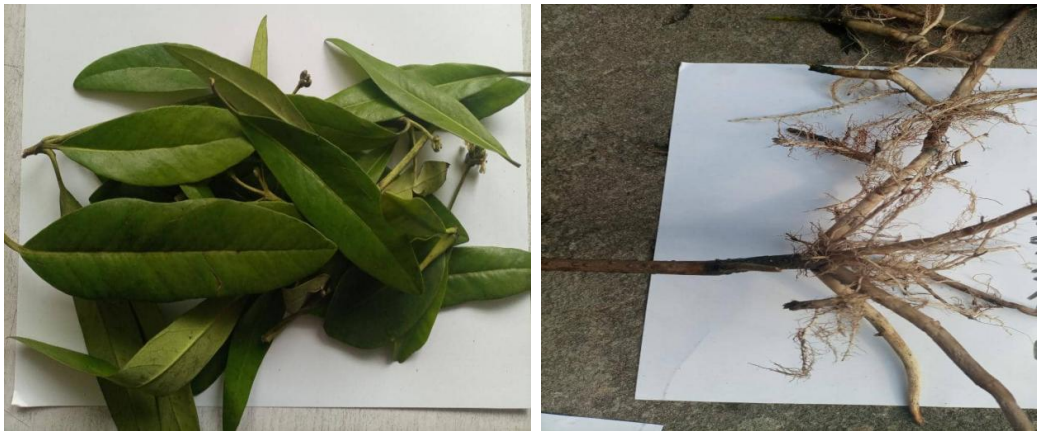

Fig. 2. Acrostichum aureum – golden leather fern leaves and roots

Fig. 3. Avicennia germinans – Black Mangrove leaves and roots

inoculated aseptically unto the properly dried media; Burk's N-Free Agar, Winogradsky Agar (for both *Nitrosomonas* and *Nitrobacter*), Mineral Salt Agar and Nutrient Agar. Using vapour phase transfer method, filter paper was dipped into crude oil and placed on the cover of the plate containing Mineral Salt Agar. The inoculated plates were incubated at 37^{o}C for 24hours to 7days. After incubation, bacterial colonies were differentiated and counted based on their morphological characteristics. Individual colonies were picked randomly and sub-cultured by streaking them onto nutrient Agar plates using the streak plate technique and incubated at 37ºC. Pure cultures were stored in slants and refrigerated at 4°C until required for use.

The isolates were screened to determine those that had the ability to degrade crude oil in contaminated water.

2.3 Characteristics and Identification of Bacterial Isolates

The bacterial isolates were characterized on the basis of their colonial morphology, use of selective media, cultural and biochemical characteristics. References were made to Bergey's Manual of Determinative Bacteriology [15] for identification of bacteria. Pure cultures of bacteria were each subcultured onto freshly prepared Nutrient Agar plates, incubated at 37°C for 24hours and these served as pure stock cultures for the morphological features such as shape, size, colour, edge, texture and elevation of the colony, motility and gram stain which were observed visually with hand lens and the the various biochemical test which includes; gram reaction, motility, methyl red, Voges Proskauer, catalase, oxidase, indole, citrate utilization and sugar fermentation tests, starch hydrolysis, Nitrate and hydrogen sulphide test.

3. RESULTS AND DISCUSSION

3.1 Isolation of the Test Organisms

The three plant samples used were identified in the Department of Plant Science & Biotechnology Laboratory as: *Avicennia germinans (*Black mangrove*),* (*Acrostichum aureum* (Golden leather fern mangrove) and *Rhizophora mangle* (Red mangrove). The results of the enumeration of the roots and leaves of the three different plant samples used for this study are presented in Table 1.

Results of Total Heterotrophic Bacterial mean counts for Black Mangrove Roots was 1.9x10^{5}CFU/g, Golden leather fern roots had a mean count of 2.7x10^{5}CFU/g while the Red Mangrove Roots had a mean count of 3.1x10^{5}CFU/g. For the leaves, Black Mangrove was 1.2x10^{5}CFU/g, Golden leather fern had a mean count of 2.2x10^{5}CFU/g while the Red Mangrove had a mean count of 2.0x10^{5}CFU/g.

Results of average Hydrocarbon Utilizing Bacterial counts for roots ranged from 7.0x10^{3}CFU/g to 1.6x10^{4}CFU/g with the sample Red Mangrove recording the highest counts and the Black Mangrove recording the lowest counts. While for the leaves, the counts ranged from 3.0x10^{3}CFU/g to 8.0x10^{3}CFU/g with the sample Black Mangrove recording the lowest count and the Red Mangrove recording the highest bacterial counts.

Table 1. Total Bacterial Counts from Mangrove Plants Samples

Sample Code (CFU/g)	Total Heterotrophic Bacteria (cfu/g)	Hydrocarbon Utilizing Bacteria (cfu/g)	Nitrogen Fixing Bacteria (cfu/g)	Nitrifying Bacteria (cfu/g)
BMR	1.9x 10^5±0.007	7.0 x10^3±0.007	1.0 x10^4±0.007	7.0 x10^3±0.21
BML	1.2x 10^5±0.07	3.0 x10^3±0.07	6.0x10^3±0.70	3.5 x10^3±0.007
RMR	3.1 x10^5±0.007	1.6 x10^4±0.00	7.0 x10^3±0.007	7.0 x10^3±0.006
RML	2.0 x10^5±0.00	8.0 x10^3±0.00	3.0± x10^3±0.00	5.0 x10^3±0.14
GLFR	2.7 x10^5±0.007	1.2 x10^4±0.007	4.5 x10^3±0.007	6.0 x10^3±0.007
GLFL	2.2 x10^5±0.07	5.0 x10^3±0.07	5.0 x10^3±0.07	5.0 x10^3±0.007

Key: BMR – Black Mangrove Roots, BML – Black Mangrove Leaves, RMR – Red Mangrove Roots, RML – Red Mangrove Leaves, GLR – White Mangrove Roots, GLL – White Mangrove Leaves

Table 2. Morphology and biochemical characteristics of the bacterial Isolates

Isolates	Gram Sain	Shape	Colour	Elevation	Translucent	CAT	OXI	CIT	MOT	IND	MR	VP	GLU	LAC	SUC	MAN	STH	NITRATE	H2S	Probable organisms
H1	-ve	Rod	Yellow	Flat	Opaque	+	+	+	+	-	-	-	-	-	-	-	-	+	-	*Pseudomonas* sp
H2	-ve	Rod	Yellow	Flat	Opaque	+	+	+	+	-	-	-	-	-	-	-	-	+	-	*Pseudomonas* sp
H3	+ve	Bacilli	Cream	Raised	Opaque	+	-	+	-	-	+	+	A	-	-	-	-	-	-	*Micrococcu*s sp
H5	-ve	Rod	White	Raised	Translucent	+	+	-	+	+	-	+	AG	+	AG	A	-	+	+	*Azotobactcer* sp
T A	+ve	Cocci	Yellow	Smooth	Translucent	+	+	-	-	-	+	+	A	-	AG	A	+	+	-	*Staphylococcus* sp
T B	+ve	Cocci	Yellow	Smooth	Translucent	+	+	+	-	-	+	-	A	-	A	A	-	+	-	*Staphylococcus* sp
T C	+ve	Rod	Cream	Raised	Opaque	+	+	-	-	-	-	+	AG	-	AG	A	+	+	-	*Micrococcu*s sp
T D	+ve	Rod	Cream	Raised	Opaque	+	+	-	+	-	+	+	AG	-	AG	A	+	+	-	*Bacillus* sp
T E	+ve	Cocci	Yellow	Smooth	Translucent	+	-	+	-	-	+	+	AG	-	-	A	-	+	-	*Staphylococcus* sp

Isolates	Gram Sain	Shape	Colour	Elevation	Translucent	CAT	OXI	CIT	MOT	IND	MR	VP	GLU	LAC	SUC	MAN	STH	NITRATE	H2S	Probable organisms
T F	+ve	Cocci	Yellow	Smooth	Translucent	+	+	-	-	-	+	+	A	-	AG	A	+	+	-	*Staphylococcus* sp
T G	+ve	Rods	Cream	Raised	Opaque	+	+	-	-	-	-	+	AG	-	AG	A	+	+	-	*Bacillus* sp
KA	+ve	Cocci	Yellow	Smooth	Translucent	+	-	-	-	-	+	-	A	-	A	-	+	+	-	*Staphylococcus* sp
K B	+ve	Rods	Cream	Raised	Opaque	+	-	-	+	-	+	+	AG	A	AG	A	+	+	-	*Bacillus* sp
K C	+ve	Rod	Cream	Raised	Opaque	+	+	-	+	-	+	+	AG	-	AG	A	+	+	-	*Bacillus* sp
K D	-ve	Rod	Cream	Raised	Translucent	+	+	+	+	-	+	+	AG	-	AG	A	+	+	-	*Bacillus sp*
K E	-ve	Rod	Cream	Flat	Opaque	+	+	-	+	-	-	-	A	-	A	-	-	-	+	*Nitrosomonas* sp
B A	-ve	Rod	Cream	Raised	Translucent	+	+	+	+	-	+	+	AG	-	AG	A	+	+	-	*Bacillus sp*
B B	+ve	Cocci	Gray	Flat	Opaque	+	+	-	+	-	-	-	-	-	-	-	-	-	-	*Nitrobacter* sp
B C	-ve	Rod	Gray	Flat	Opaque	+	+	-	-	-	-	-	AG	-	-	-	-	-	-	*Nitrobacter* sp
B D	-ve	Rod	Cream	Flat	Opaque	+	-	+	+	-	-	+	A	-	A	A	-	+	-	*Klebsiella* sp
B E	-ve	Rod	Cream	Raised	Translucent	+	+	+	+	-	+	+	AG	-	AG	A	+	+	-	*Bacillus sp*
S A	-ve	Rod	Cream	Flat	Opaque	+	-	+	+	-	-	+	A	-	A	A	-	+	-	*Klebsiella* sp
S B	-ve	Rod	Cream	Flat	Opaque	+	-	+	+	-	+	+	A	-	A	A	-	+	-	*Klebsiella* sp
S C	-ve	Rod	Cream	Raised	Translucent	+	+	+	+	-	+	+	AG	-	AG	A	+	+	-	*Bacillus sp*

For the isolation of Nitrogen fixing bacteria, the Burks N-Free medium was used, the results of average Bacterial counts for roots ranged from $4.5x10^3$CFU/g to $1.0x10^4$CFU/g with the sample Black Mangrove recording the highest counts and the Golden Leather Fern recording the lowest counts. While for the leaves, the counts ranged from $3.0x10^3$CFU/g to $6.0x10^3$CFU/g with the sample Red Mangrove recording the lowest counts and the Black Mangrove recording the highest bacterial counts.

For the isolation of nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) the Winogradsky medium was used, the results of average Bacterial counts for roots ranged from $6.0x10^3$CFU/g to $7.0x10^3$CFU/g with the sample Black Mangrove and Red Mangrove recording the highest counts and the Golden Leather Fern recording the lowest counts. While for the leaves, the counts ranged from $3.5x10^3$CFU/g to $5.0x10^3$CFU/g with the sample Black Mangrove recording the lowest count and the Red Mangrove recording the highest bacterial counts.

3.2 Characterization and Identification of Bacterial Isolates

The results of morphological and biochemical characteristics of the bacterial isolates are presented in Table 2. From the morphological features such as shape, size, colour, elevation, etc the colony of the bacterial isolates were identified macroscopically and under a hand lens and from the biochemical characteristics such as motility test, gram stain, catalase test, oxidase test, indole test, methyl red test, Voges-proskauer test, sugar fermentation test, starch hydrolysis and citrate utilization test the following probable organisms identified include; *Pseudomonas* sp*, Micrococcus* sp*, Nitrobacter* sp*, Nitrosomonas* sp*, Staphylococcus* sp*, Azotobacter* sp*, Klebsiella* sp *and Bacillus* sp.

The various organisms isolated from the roots and leaves of the three plant samples are found in Table 3. *Bacillus* sp had the highest occurrence followed by *Staphylococcus* sp the Klebsiella sp then *Pseudomonas*, *Micrococcus* and *Nitrobacter* sp while *Nitrosomonas* sp and *Azotobacter* sp was least. Also the table shows where the various isolates where isolated from *Azotobacter* sp and *Bacillus* sp were isolated from the Black Mangrove Leaves, *Staphylococcus* sp, *Bacillus* sp and *Nitrobacter* sp was isolated from the roots of the Black Mangrove, from the Red Mangrove leaves, *Staphylococcus* sp, *Micrococcus* and *Bacillus* sp was isolated, *Staphylococcus* sp, *Pseudomonas* sp, *Klebsiella* sp, *Nitrobacter* sp, *Nitrosomonas* and *Bacillus* sp were isolated from the roots of the Red Mangrove, for the Golden Leather Fern roots, the following were isolated, *Staphylococcus* sp, *Klebsiella* sp and *Bacillus* sp and for the Golden Leather Fern leaves, *Pseudomonas* sp, *Micrococcus* sp and *Bacillus* sp were isolated.

Also the Red Mangrove roots had the highest count of organisms while the Black Mangrove Leaves had the lowest.

The results of the mangrove roots counted on all plates from each media, the Total Heterotrophic Bacterial (THB) counts from the roots was highest in the Red Mangrove followed by the Golden Leather Fern and the Black Mangrove roots recorded the lowest bacterial counts. For the hydrocarbon degraders in the roots, the bacterial counts were highest in the Red Mangrove (RM) as they had the highest growth of organisms followed by the Golden Leather Fern (GLF) while the Black Mangrove (BM) had the lowest counts of the hydrocarbon degraders.

Table 3. Total number of Isolates from each plant sample

Isolates	BML	BMR	RML	RMR	GLFL	GLFR	SUM
Pseudomonas sp	-	2	1	1	1	1	4
Staphylococcus sp	2	1	1	2	1	1	8
Klebsiella sp	-	1	-	1	-	2	4
Micrococcus sp	-	-	1	1	1	-	3
Bacillus sp	2	1	2	3	2	1	11
Nitrosomonas sp	-	-	-	2	-	1	3
Nitrobacter sp	-	2	-	2	-	-	4
Azotobacter sp	1	-	-	-	-	-	1

Key: BMR – Black Mangrove Roots, BML – Black Mangrove Leaves, RMR – Red Mangrove Roots, RML – Red Mangrove Leaves, GLR – White Mangrove Roots, GLL – White Mangrove Leaves

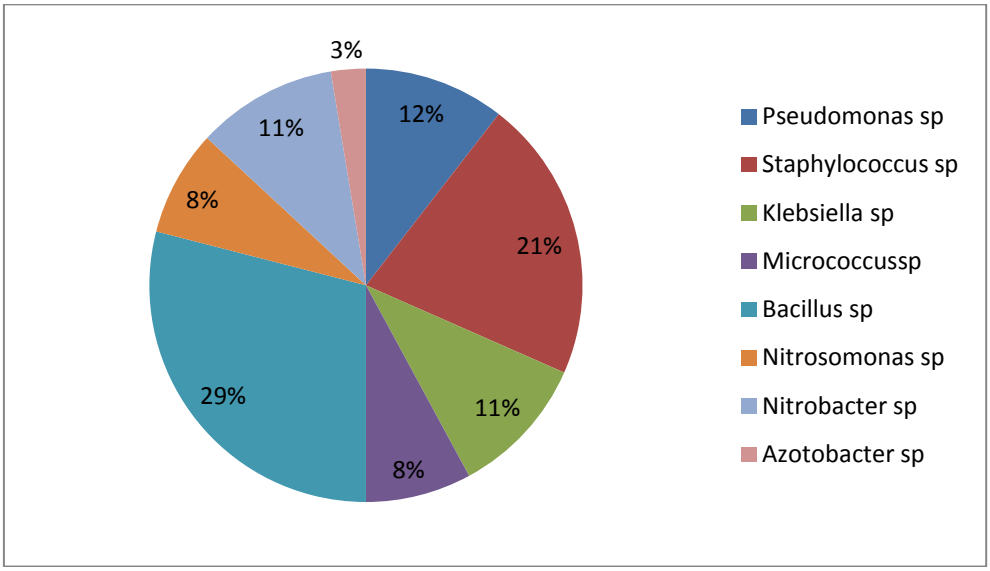

Fig. 4. Percentage occurence

Results for the Nitrogen Fixing Bacteria showed that the roots of the Black Mangrove recorded the highest counts and that of the Golden Leather Fern recorded the lowest bacterial counts. For the Nitrying Bacteria results, the roots of the Black Mangrove recorded the highest bacterial counts while the Golden Leather Fern roots revealed the lowest bacterial counts.

From the results for the mangrove leaves counted on all plates from each media, the Total Heterotrophic Bacterial (THB) counts were highest in the Golden Leather Fern followed by the Red Mangrove and the Black Mangrove contained the lowest counts of bacteria; for the Hydrocarbon Utilizing Bacteria (HUB), the leaves of the Red Mangrove recorded the highest bacterial counts while the Black Mangrove recorded the lowest counts; for the Nitrogen fixing bacteria, the leaves of the Black Mangrove revealed highest bacterial counts while the leaves of the Red Mangrove recorded the lowest count; lastly for the nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) the leaves of the Red Mangrove recorded highest bacterial counts and the Black Mangrove recorded the lowest count.

Important economic and environmental functions about endophytes isolated from mangrove plants have been reported by several researchers [16-18]. The Red Mangrove had highest count of organisms this indicates that the red mangrove is well adapted to the environment and has the ability to support and aid the sustenance of varying species of bacteria [19].

Morphological and biochemical characteristics of the isolates were carried out to identify the probable organisms isolated from the mangrove plants. In the present study the plants yielded a total of 49 bacterial isolates (23 from the roots and 15 from the leaves). But based on visible morphological differences 24 isolates were selected for further study. They were studied further and the probable organisms identified include; 4 *Pseudomonas* sp*, 3 Micrococcus* sp*, 4 Nitrobacter* sp*,1 Nitrosomonas* sp*,* 8 *Staphylococcus* sp*, 1 Azotobacter* sp*,* 4 *Klebsiella* sp and 11 *Bacillus* sp. Of the thirty eight isolates, 23 (*Pseudomonas* sp, *Klebsiella* sp, *Bacillus* sp, *Staphylococcus* sp, *Nitrobater* sp, *Nitrosomonas* sp) were from the roots of the mangrove while 15 (*Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Micrococcus* sp, *Azotobacter* sp) were from the leaves.

From the results of the isolation done by Rahman et al, [20], 8 genera of isolated bacteria consist of Klebsiella, Pantoea, 3 Vibrio, 2 Enterobacter, Pseudomonas, Virgibacillus, Staphylococcus, and 8 Bacillus isolates were isolated from the leaves of mangrove leaves. The results of this study were also different from Feliatra [21] research. The research found 7 genera, namely Neisseria, Plesiomonas, Yersinia, Corynebacterium, Bacillus, Staphylococcus and Acinobacter. While that of [22] resulted in 5 bacterial species from leaves and none from the roots of mangrove plants. Ntabo et al 2018 also

reported isolating Forty-two bacterial isolates (twenty three from the leaves and nineteen from the roots) from the leaves and roots of six mangrove plants. From the leaves 8 *Bacillus* species, 4 *Streptomyces, Staphylococcus,* Pseudochrobactrum*, Klebsiella, Achromobacter, Alcaliigens,* 3 *Myroides,* 2 *Serratia* and from the roots; 13 *Bacillus, 2 Myroides, 2 Streptomyces, Pseudomonas, Staphylococcus*. There appears to be significant variation in the number and types of indigenous bacteria isolated from diverse host plant species. These endophytes vary from one plant species to another and their diversity depends on the climatic condition of a particular region and age of the plant [23]. Several factors may explain these differences, including host specificity, geographical distribution, plant age, and tissue type [23].

Several other researchers [24,25] all reported isolation of several endophytes from mangrove plants which all have several enzymatic capabilities with mostly *Bacillus* sp. showing strong enzymatic production. Our data corroborate the results obtained by [26] Ando et al. (2001) who isolated a large number of *Bacillus* sp. from mangrove sediments in Japan and reported the possible ability of these isolates to degrade organic pollutant compounds by fermentation. Similarly, the endophytic strain *B. amyloliquefaciens* (RS261) is a biological agent isolated from the leaf of *R. stylosa* [27].

Endophytic bacteria often produce metabolites similar to those produced by their host plants [28-31]. Many of these endophytes have shown ability to synthezise bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proven useful for drug discovery [32].

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From this study, all identified isolates corresponded to genera commonly isolated and identified from the endopyhtes of plants. It was observed that all mangrove plants harbor bacterial endophytes with various enzymatic activities [23]. Based on the assessed microbial populations, the present study suggests that mangrove ecosystems harbour diverse functional microbial groups that may potentially be directed to biotechnological approaches for either ecological restoration or agricultural purposes [26,27]. Some endohytic bacteria can be used as biofertilizers because they fix Nitrogen and this influences plant growth through the production of phytohormones, siderophores, they can induce systemic tolerance by producing 1-aminocyclopropane-1-carboxylase deaminase and also induce systemic resistance and antagonistic activities [25]. *Klebseilla pneumonia* was able to fix N_2 and produce indole acetic acid (IAA) hormone [33]. These Nitrogen-fixing endophytic bacteria have edge over its rhizospheric counterparts because, they make the fixed nitrogen available directly to the plants and they face less competition as they are sheltered inside plant tissues [25]. Endophytic bacteria are known to have several potential applications in medicine and in other sectors of biotechnology. This study when compared to the report by [23], it is evident that beneficial microorganisms that may play a significant role in the C, N, or P cycles are in the mangrove ecosystem, as well as potential degraders of petroleum hydrocarbon [34]. Abiye et al., 2022 from their research were able to prove that various endophytic bacteria (*Pseudomonas aeruginosa* (MN314747), *Brevibacillus brevis*, *Bacillus amyloliquefaciens*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa*, *Nitrobacter* sp, *Staphylococcus aureus*) isolated from mangrove plants can be used to clean up oil spills in hydrocarbon polluted environment.

4. CONCLUSION

The results revealed that there are high numbers of active indigenous bacteria in the roots, most of which are known to possess catabolic abilities to use up pollutants. The these mangrove roots may harbour bacterial genera that may play important role in nitrogen fixation, medicine and can also be enhanced to bring about bioremediation in polluted environment. This study has several culturable functional groups of bacteria that might be directed to further biotechnological approaches. Therefore, further studies should be carried out to determine the potential application of these isolates in bioremediation, growth promotion, enzyme production and generally in biotechnology.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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