

Isolation and Characterization of Bacteria from Selected Forest Soil and Water Hyacinth (*eichhornia crassipes*) Compost for Effective Soil Amendments

Roseline .N. Akwukwaegbu^{1*} Herbert .O. Stanley¹, Gideon .O. Abu¹ and Ijeoma F. Vincent-Akpu²

¹ Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt 500004, Rivers State, Nigeria

² Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Port Harcourt 500004, Rivers State, Nigeria

* Correspondence: Roseline_Akwukwaegbu@uniport.edu.ng

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Abstract: A forest is a sizable tract of agricultural land that has been left untouched for several decades and is covered with trees and plants. Water hyacinth and harboring river were collected from new Calabar River in River state Nigeria. Microbiological analyses of the samples were determined using standard techniques. Bacteria population of the soil and water hyacinth compost ranged from $100.66 \pm 2.07 \times 10^9$ CFU/g to $56.00 \pm 8.88 \times 10^8$ CFU/g. The hydrocarbon utilizing bacterial (HUB) counts range from $55.33 \pm 5.50 \times 10^8$ to $31.0 \pm 3.11 \times 10^8$ CFU/g respectively. *Klebsiella* sp., *Escherichia* sp., *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp and *Serratia* sp. with *Bacillus* sp. and *Pseudomonas* sp. as the predominate genera. This study finds that soil from forests and water hyacinth is a good source of microbes that contain organic soil amendment of various groups of nitrogen-fixing and phosphate-solubilizing bacteria. These bacteria can be harvested as biomass or prepared into microbial suspension. To assess their effectiveness in enhancing soil quality and integrity for productive agriculture, this study suggests using the isolates from water hyacinth (*Eichhornia crassipes*) compost and forest soil either separately or in combination as soil amendment

Key words: forest, genera, water hyacinth compost, and microbe

INTRODUCTION

A forest is a large area of agricultural land covered with trees and other vegetation that has not been used for many years. Another way to define it is as a part of the earth with a high tree cover or a large area of tree-covered terrain that provides habitat for wild animals. In contrast to natural forests, which are created and managed by humans, all forests are either man-made or indigenous. The most common life form in this complex biological ecosystem is the tree. In 2019, Britannica et al. Organic amendments have been discovered as a substitute for chemical fertilizers in ecologically friendly agriculture, increasing crop yields and enhancing soil fertility. By offering more environmentally acceptable nutrient sources, it has grown to be an essential component of the integrated fertilizer delivery system and has the potential to boost agricultural output (Wu et al., 2005). They improve root development by releasing hormones that promote growth. Microorganisms convert complex nutrients into basic ones that plants can use. They are the result of one or more species of microorganisms that can mobilize nutritionally significant elements from non-useful to useable form through biological processes like nitrogen fixation, phosphate solubilization, removal of contaminants that support plant growth, and biodegradation in soil. Living microbial inoculants of bacteria, algae, or fungi, either separately or in combination, are known as biofertilizer

A soil amendment is a material that can improve the physical and/or chemical properties of soil to make it more favorable for plant growth. Examples of organic additions derived from living organisms include wood chips, grass clippings, straw, compost, manure, biosolids, sawdust, and wood ash (Davis & Wilson 2005). However, the focus of this review is on compost and farmyard manure. In general, plant and animal matter that has fully decomposed through a particular process initiated and supervised by humans is referred to as compost (FiBL, 2012). The average nutritional concentrations of compost are 0.95% N, 0.58% P₂O₅, and 0.95% K₂O, albeit they vary based on the type of compost (farm and town compost). According to Sankaranarayanan (2004), well-decomposed FYM typically contains 0.5% N, 0.2% P₂O₅, and 0.5% K₂O. FiBL (2012) asserts that the availability of potassium and phosphorus from FYM is comparable to that of chemical fertilizers. In contrast, farmyard manure is a more or less decomposed blend of straw and litter used as bedding material, along with livestock feces and urine, primarily from cattle, as well as potential leftovers from the cattle's feed.

Agriculture is the second most significant economic sector in Nigeria, after petroleum. For agriculture to be viable and for food security to be realized, soil integrity and quality must be maintained. Soil fertility refers to a soil's capacity to sustain plant growth by providing essential nutrients and a favorable physicochemical and biological environment as a growing medium. Biofertilizer, which contains living microorganisms, aids in plant growth by boosting the availability, supply, or uptake of primary nutrients to the host through natural processes like nitrogen fixation, phosphorus solubilization, and stimulating plant growth along with the synthesis of growth-promoting substances (FAO, 2020).

MATERIALS AND METHODS

Sample Collection

Soil samples were collected randomly from various forests (Etche, Ogbogoro, and Choba) in Rivers state.

Water hyacinth compost was obtained from new Calabar River in Rivers state and proceeds for composting procedures according to Abu et al., 2024

Microbiological Analysis

Media Preparation

To isolate complete heterotrophic bacteria, nutrient agar was produced in accordance with the manufacturer's instructions. According to Eze and Okpokwasili (2011), mineral salt agar was made with the following ingredients in one liter of aqueous solution: MgSO_4 0.42g, KCl 0.28g, k_2HPO_4 1.25g, KH_2PO_4 0.83g, NaNO_3 0.42g, NaCl 10.0g, and agar 15.0g.

Enumeration of Bacterial Population in Water Hyacinth compost

The microorganisms in the soil and water hyacinth compost were counted. Countable colonies of pure cultures of isolate strains on Nutrient Agar were obtained from the materials using serial dilution and plating procedures. To promote growth and proliferation, isolated isolates were cultivated on Nutrient Agar (NA) plates in a controlled laboratory environment. Nutrient Agar (NA) plates were used to count all of the heterotrophic bacteria present in biocompost samples. Following the proper sample dilutions, 0.1 ml aliquots were plated on NA plates and incubated for one to two days at 37.0C. Colonies were tallied at the conclusion of the incubation period.

Estimation of total hydrocarbon utilizing bacteria in soil and WHC

By plating aliquots (0.1 ml) of samples diluted 10-2, 10-3, and 10-4 on mineral salts agar, which contained (gl-1): NaCl, 10.00; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.42; KCl, 0.29; KH_2PO_4 , 0.83; $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 1.25; NaNO_3 , 0.42; Agar, 15.0 and deionized or distilled water to the 1000 ml mark (pH, 7.2), mean counts of total hydrocarbon utilizers were determined. The medium was autoclaved for 15 minutes at 1210C and 15 psi of pressure to sterilize it. The culture was supplied with crude petroleum in the vapour phase, which was the only source of energy and carbon. To do this, inoculation plates were inverted over sterile filter paper that had been saturated with the sterile crude oil included in Petri dish covers. Every plate was incubated for 24 to 48 hours at 37.0°C.

Sub-culturing, purification, and preservation of isolates

Pure cultures were isolated from discrete colonies by subculturing on nutrient agar and incubated for 24 hours at 37°C. The plate cultures with the isolated bacterial colonies that had grown on them were located inside the laminar airflow. One colony was selected to be used in the inoculating loop. After that, the culture plate was once more sealed. The new plate was streaked with the single colony in a zigzag pattern. To minimize the risk of contamination, the plate's cover was minimized.

Identification of Isolates

An oil immersion objective (x100 magnification) was used to view the isolates, and their morphology and colony characteristics were examined macroscopically. Microscopical analysis was done using Gram staining. Indole, catalase, citrate, motility, urease, starch hydrolase, and sugar fermentation were among the biochemical tests that were carried out. The isolates were identified using Bergey's Manual of Systematic Bacteriology (Buchman & Gibbon, 2011)..

RESULTS**The cultural, morphological and biochemical properties**

The cultural and morphological characteristics of bacteria isolated from the soil and water hyacinth. The sizes ranged from 0.1 to 0.9 (cm); the surface showed a variety of smooth, shiny, rough and dry characteristics; the opacity were opaque or transparent in nature; the isolates were circular, irregular and round and the edge were either entire or serrated in nature.

The isolates were identified as species of *Klebsiella*, *Escherichia*, *Staphylococcus*, *Bacillus*, *Pseudomonas* and *Serratia*.

Table 3.1A: Cultural and morphology test of bacteria isolated from the soil forest soil and WHC

| ID | Colour | Size(cm) | Elevation | Surface | Edge | Form | Opacity | Organism suspected |
|------|--------|----------|-----------|------------|-----------|-----------|-------------|---------------------------|
| PA1 | White | 0.3 | Flat | SS | Entire | circular | Opaque | <i>Pseudomonas</i> sp. |
| PA2 | White | 0.1 | Flat | SS | Entire | Round | Opaque | <i>Pseudomonas</i> sp. |
| PA3 | Grey | 0.3 | Flat | SS | Serrated | Round | Transparent | <i>Klebsiella</i> sp. |
| PA4 | White | 0.3 | Flat | SS | Entire | Round | Transparent | <i>Bacillus</i> sp. |
| PA5 | White | 0.4 | Flat | Rough | Serrated | Round | Transparent | <i>Escherichia</i> sp. |
| PA6 | White | 0.3 | Flat | SS | Entire | Round | Opaque | <i>Bacillus</i> sp. |
| PA7 | White | 0.2 | Flat | SS | Entire | Irregular | Opaque | <i>Bacillus</i> sp. |
| PA8 | Cream | 0.4 | Unbonated | Smooth/dry | Serrated | Irregular | Opaque | <i>Serratia</i> sp. |
| PA10 | White | 0.1 | Flat | SS | Entire | Round | Transparent | <i>Staphylococcus</i> sp. |
| PA11 | white | 0.3 | Flat | SS | Entire | Irregular | Opaque | <i>Bacillus</i> sp. |
| PA12 | White | 0.1 | Flat | SS | Entire | Round | Transparent | <i>Pseudomonas</i> sp. |
| PA13 | White | 0.2 | Flat | Dry | Serrated | Round | Opaque | <i>Pseudomonas</i> sp. |
| PA14 | Cream | 0.3 | Flat | Dry /rough | Irregular | Round | Opaque | <i>Serratia</i> sp. |
| PA15 | White | 0.4 | Flat | Dry /rough | Serrated | Irregular | Opaque | <i>Bacillus</i> sp. |
| PA17 | Cream | 0.3 | Unbonated | Smooth/dry | Entire | circular | Opaque | <i>Serratia</i> sp. |
| PA18 | White | 0.4 | Flat | Smooth/dry | Entire | Round | Opaque | <i>Bacillus</i> sp. |
| PA19 | Green | 0.4 | Raised | Rough/dry | Serrated | Irregular | Opaque | <i>Pseudomonas</i> sp. |
| PA20 | Brown | 0.2 | Flat | SS | Entire | Round | Transparent | <i>Bacillus</i> sp. |

Key: SS = Smooth and shiny:
PA = Isolates code

Table 3.1b Biochemical test of bacteria isolated from the soil forest soil and WHC

| Isolate ID | Catalase | Oxidase | Citrate | Glucose | Lactose | Indole | MR | VP | Sucrose | TSIA | H ₂ S | Gram-stain | Starch hydrolysis | Motility | cmc hydrolysis |
|------------|----------|---------|---------|---------|---------|--------|----|----|---------|------|------------------|------------|-------------------|----------|----------------|
| PA1 | + | - | - | A | A | - | + | - | A | AB | - | -rod | - | - | - |
| PA3 | + | - | - | A | - | - | + | + | - | AB | - | +rod | + | + | + |
| PA4 | + | - | - | A | A | - | + | - | A | AA | - | +rod | - | + | - |
| PA5 | + | - | + | A | A | - | - | + | A | AA | - | -rod | + | + | + |
| PA8 | + | - | - | A | A | - | - | - | - | AB | - | +cocci | - | + | - |
| PA10 | + | - | - | A | A | - | - | - | - | AB | - | +cocci | - | + | - |

Microbiological Properties of Forest soil and water hyacinth compost

The Total Heterotrophic Bacterial Count (THBC) of the soil and water hyacinth compost samples is shown in Table 3.2. The counts range from $100.66 \pm 2.07 \times 10^9$ CFU/g to $56.00 \pm 8.88 \times 10^8$ CFU/g. The hydrocarbon utilizing bacterial (HUB) counts range from $55.33 \pm 5.50 \times 10^8$ to $31.0 \pm 3.11 \times 10^8$ CFU/g

Table 3.2: Microbial population on forest soil and water hyacinth compost

| Samples | THBC (CFU/g) | THUB (CFU/g) |
|-----------------|-------------------------------|------------------------------|
| Control soil | $75.00 \pm 1.30 \times 10^9$ | $35.20 \pm 6.08 \times 10^9$ |
| Etche forest | $69.33 \pm 3.50 \times 10^8$ | $32.33 \pm 1.52 \times 10^8$ |
| Ogbogoro forest | $100.66 \pm 2.07 \times 10^9$ | $55.33 \pm 5.50 \times 10^8$ |
| Choba soil | $68.00 \pm 8.88 \times 10^8$ | $43.66 \pm 3.21 \times 10^8$ |
| WHC | $56.00 \pm 8.88 \times 10^8$ | $31.0 \pm 3.11 \times 10^8$ |

Values are mean \pm standard deviation (M \pm S.D) of triplicate determinations (n=3)

Values bearing the same superscript letter (a) is significantly different ($P < 0.05$) when compared to control

Bacterial species found in forest soil and WHC

Bacterial species found in forest soil and WHC is shown in table 3.2 *Bacillus* sp and *Pseudomonas* sp seem to be the most predominate genera common in the samples is agree with

the work of (Abu et al., 2024) who discover that *Bacillus* sp and *Pseudomonas* sp are genera that have bioferilizing potential.

Table 3.3 Abundant of genera found in forest soil and WHC

| Etche forest | Ogbogoro forest | Choba soil | WHC |
|------------------------|------------------------|-------------------------|---------------------------|
| <i>Bacillus</i> sp. | <i>Bacillus</i> sp. | <i>Bacillus</i> sp. | <i>Bacillus</i> sp. |
| <i>Pseudomonas</i> sp. | <i>Pseudomonas</i> sp. | <i>Pseudomonas</i> sp. | <i>Pseudomonas</i> sp |
| <i>Serratia</i> sp. | <i>Alcaligenes</i> sp. | <i>Alcaligenes</i> sp. | <i>Staphylococcus</i> sp. |
| <i>Klebsiella</i> sp | <i>Escherichia</i> sp. | <i>Escherichia</i> sp. | |
| <i>Erwinia</i> sp. | <i>Klebsiella</i> sp | <i>Streptococcus</i> sp | |

DISCUSSION

The morphological characteristics and microscopic features of bacteria isolates from the study site which include. As shown in table 3.1 *Acinetobacter species*, , *Klebsiella species*, , *Escherichia species* *Staphylococcus*, *species*, *Bacillus species*, *Pseudomonas species* and *Serratia species*. However, other additional microorganisms were Isolated in this study. This could be as a result of the high organic carbon content of a wetland soil and water hyacinth compost (Unanaonwi & Doubra, 2017) isolated similar microorganisms' including *Corynebacterium* and *Listeria* species which were absent in this study. From the morphological features, 6 bacteria species were suspected which include: *Klebsiella species*, *Escherichia species* *Staphylococcus*, *species*, *Bacillus species*, *Pseudomonas species* and *Serratia species* Mean values of bacteria isolates as presented in table 3.2 shows that the soil and WHC have bacterial population within a normal range and the sample are not a sterile in nature.

The total heterotrophic bacterial count (THB) of soil bacteria and WHC as shown in table 3.2indicated that the count range from $100.66 \pm 2.07 \times 10^9$ CFU/g to $56.00 \pm 8.88 \times 10^8$ CFU/g. The hydrocarbon utilizing bacterial (HUB) counts range from $55.33 \pm 5.50 \times 10^8$ to $31.0 \pm 3.11 \times 10^8$ CFU/g, this is in line with that the presence of bacterial activity increase the Microbial load in soil and WHC. The result obtained shows that the indigenous bacterial community in soil are and WHC were able to thrive and multiply themselves in the environment. A similar observation was reported by (Abu et al., 2024; Ibiene *et al* 2011 and Eze & Okpokwasili 2011)

Result from table 3.3 revealed the abundance of diverse genera of bacteria and fungi including those that have been classified as microbial biofertilizers. This finding agrees with Thomas & Sing (2019) who reported that the most important groups of microbes used in the preparation of biofertilizer are bacteria, which have symbiotic relationship with plants. The isolates *Azotobacter*, *Rhizobium*, *Bacillus* *Enterobacter*, *Klebsiella* and *Pseudomonas*, which were grouped as nitrogen

fixing, phosphate solubilizing, phosphate mobilizing and plant growth promoting rhizobacteria. This result agrees with Adesemoye (2008) who also reported similar bacteria isolates classified as PGPR in his study on soil. In his study, he reported the following genera; *Pseudomonas*, *Azotobacter* and *Klebsiella sp.* as organic amendment

CONCLUSIONS

Modern agriculture relies heavily on the use of organic amendments, which offer a substitute for chemical fertilizers and encourage plant growth and agricultural yield without endangering human or environmental health. In addition to being an annoyance in nutrient-rich water bodies, the seaweed (*Eichhornia crassipes*) sometimes known as water hyacinth, is an inexpensive substitute source of organic fertilizer that is widely available. Numerous bacterial biofertilizer species were isolated from the water hyacinth, (*Eichhornia crassipes*). In order to encourage plant growth and boost crop production for sustainable agriculture, this study finds that soil from forests and water hyacinth is a good source of microbes that contain organic soil amendment of various groups of nitrogen-fixing and phosphate-solubilizing bacteria. These bacteria can be harvested as biomass or prepared into microbial suspension. To assess their effectiveness in enhancing soil quality and integrity for productive agriculture, this study suggests using the isolates from water hyacinth (*Eichhornia crassipes*) and forest soil either separately or in combination as biofertilizer.

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