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Population, Morphological and Biochemical Characterization of Microorganism in Plantain Root across different Farmlands in Toru-Orua Metropolis, Bayelsa State, Nigeria

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ABSTRACT: This paper assessed the population, morphological and biochemical characterization of microorganism in plantain root across different farmlands in Toru-Orua Metropolis, Bayelsa State, Nigeria using standard methods. Data obtained show that microbial population count ranged from 1×10^7 - 9×10^7 (cfu/g) in the study area, while fungi isolated include Aspergillus fumigatus, Aspergillus niger and Aspergillus flavus. These are the largest of all microorganisms in the soil. Others were Rhizobium, Nitrobacter, Winogradkyl, Azomona argillis and Psydormonads aeruginosa. Practices that would enhance nutrition of the plants and the proliferation of bacteria and fungi around the roots of plantain are recommended such as organic matter accumulation in form of green manuring, zero tillage and non-use of chemicals and burning.

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Numerous animals and microbes live in the area around the root zone; however, it is generally accepted that microbes play a significant role in the release of nutrients, minerals, and carbon dioxide for plant development. It has been demonstrated that the endophytic and rhizospheric bacteria greatly enhance plant growth and health (Mendes *et al.*, 2011). Researchers have looked into the possible advantages of helpful microorganisms in the rhizosphere/roots of Plantains (Dita *et al.*, 2018). Numerous microbial communities are housed by the rhizosphere, which is the thin soil layer that adheres to roots. Indicators of soil fertility and land use have been developed by several soil microbiologists using microbiological analyses of the soil. In general, microorganisms are in charge of the decomposition of organic matter, including hydrocarbons, the transformation of organic substances into various forms, and the generation of humus. These various kinds of creatures are classified into five different types: bacteria, actinomycetes, fungus, algae, and protozoa (Dita *et al*., 2018). One of the most significant staple food crops for millions of people in both developed and developing nations is the plantain (Musa sapientum var. Paradisiacal Linn) (Oriola *et al*., 2017). Farmers find plantain farming appealing since it requires less labor to produce than cassava, maize, rice, and yam (Marriott and Lancaster, 2003). Therefore, it makes a substantial contribution to the food and financial security of those involved in its production and trading, especially in developing nations (Marriott and Lancester, 2003). All across the humid tropics and subtropics, plantains (Musa sapientum var. paradisiacal Linn) are grown for food. Plantains are a vital part of the diet and a major source of revenue for many small-scale farmers in West Africa. Millions of people in both industrialized and

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developing nations depend on it as one of their main sources of sustenance (Oriola *et al*., 2017). Farmers find plantain farming appealing since it requires less labor to produce than cassava, maize, rice, and yam (Marriott and Lancaster, 2003). Therefore, it makes a substantial contribution to the food and financial security of those involved in its production and trading, especially in developing nations. Due to its accessibility in Nigeria, it is consumed by both indigenous peoples and a wide range of socioeconomic strata. Consuming plantains helps obese people lose weight and meets the caloric needs of many poor nations (Mohapatra *et al*., 2010). Plantain fruits have been used to make a variety of commercial items, including chips, beer, flour, and consumable drinks (Casimir and Jayaraman, 2001). For the treatment of a wide range of human illnesses, plantain flowers, ripe fruit, unripe fruit, leaves, and stem extract and its active ingredients have been employed (Auta and Kumurya, 2015). Being a climacteric fruit, plantains experience a variety of physiochemical changes when harvested at the pre-climacteric matured green stage. These changes relate to changes in metabolic rates and biochemical responses including respiration, ripening, and senescence in the climacteric phase (Adeyemi and Oladiji, 2009). Plantains are a good choice for blood pressure regulation since they provide a variety of minerals, are high in potassium, and are low in sodium (17 mg/100g) and fat (0.1 percent) (Mohapatra *et al.*, 2010). It is frequently advised for those who are intolerant to ingest. Therefore, this paper assessed the population, morphological and biochemical characterization of microorganism in plantain root across different farmlands in Toru-Orua Metropolis, Bayelsa State, Nigeria.

MATERIALS AND METHODS

Collection of samples: The samples were taken from actively growing plantain roots and ball of the earth with the help of spade and cutlass, these samples were taken from three different farms in Toru-Orua metropolis (UAT farm, Anagabiri farm and Koroye farm). Samples were later put in the polythene bag, labeled properly and transferred to the laboratory immediately for laboratory analysis

Sterilization and disinfection of materials: The plantain root samples were thoroughly washed in running tap water. They were then surface-sterilized using 70% ethanol for 2mins and immersed in 150ml of 1.5% sodium hypochlorite for 5 mins with shaking. The samples were then rinsed thoroughly in sterile distilled water and dried in sterile paper towels. Surface sterilized samples were macerated with a sterile mortar and pestle; they were later transferred to a petri-dish and kept in the refrigerator.

Materials and Reagents: Nutrient Agar (NA), Mackconkey Agar (MA), sabouroaud Dextrose Agar (SDA), Bunsen burner, weighing balance, cotton wool, wire loop, incubator, conical flask, Beaker, pipettes, petri-dishes, Test tubes, Test tubes rack, autoclave, microscope, oil immersion, plantain root sample and volumetric flask.

Serial Dilution and Inoculation: Serial dilution and inoculation was carried out for each sample, making normal saline diluted in distilled water according to the manufacture's instruction. 9ml of Normal saline was introduced into 10-fold test tubes for serial dilution and sterilized before introducing the samples, 21 test tubes were used, using separate sterile pipettes, 1 gram of root sample was weighed out into the first test tube properly mixed. using a different sterile pipette, 1ml from the first test tube was pipette into the second test tube already containing 9 ml of diluted normal saline, this continued following the same procedure till the last dilution (the last test tube). Using the spread plate method for all media preparation and pour plate method for SDA, 1ml of each sample unit from the test tubes was pipetted into the sterile Petri dishes containing solidified Agars that were measured autoclaved and allowed to cool, media used was as Sabouraud Dextrose Agar [SDA], Nutrient Agar [NA], MacConkey Agar [MA].

After introducing the diluted samples from each test tube, Antibiotics was added to the SDA to inhibit the growth of Bactria on the SDA plate and the plate was incubated for 5 days, other plates were incubated at 37°c for the 24hr. After incubation, there was growth on the MacConkey Agar plates. Growth were found on the NA and SDA plate, the representative colonies on the Nutrient Agar [NA] plates were subculture on fresh nutrients agar to run further tests and identify the growths on the plates using H_2O_2. Production of effervescence (bubbles) in 5-10 seconds is a positive test.

Identification of Isolates: Identification of isolates was based on culture, morphological and biochemical characteristics following standard methods, biochemical test and characterization of isolate.

Gram Staining Technique: Colonies from different pure culture plates were emulsified into a drop of distilled water on a slide and a thin preparation was made. The smear was allowed to air dry, covered with crystal violet stain for 60sec and was rapidly washed off with clean water. The smear was then decolorized

with alcohol and washed off rapidly, counter stained with seferanine for 60sec and washed off and examined microscopically under the x100 objective lens.

Morphological Identification of Fungi: The plates were examined for the morphological characteristics of the fungal colonies. The macroscopic observation was aimed at determining the size, shape growth, and colour of the plate. This was done with a hand.

Microscopic examination of fungal isolates: The examination and microscopic examination of fungal isolates require the observation of microscopic features such as shape, size of hyphae, the shape of sporangia, conidia, conidiophores, and spores. Using a flamed inoculating needle, the edge of each colony is picked and slides of the different colonies are made, a drop of lacto phenol cotton blue stain is added to the slides and covered with cover slip and examine under the microscope using x100 and x400 magnification starting from third day of the culture. The microscopic characteristics observed were recorded accordingly.

Lacto phenol cotton blue staining technique: Lacto phenol cotton blue wet mount which is most widely used in the preparation of slides for microscopic examination of fungi. A drop of 70% ethanol was placed on a clean microscopic glass slide. The test fungal isolate was then immersed in the drop of alcohol, drops of lacto phenol cotton blue were added and the wet preparation was covered with a glass cover slip. The wet preparation was examined using low power objective and thereafter, 40x objective.

Biochemical test: Oxidase test: We add 0.2 ml of 1% α-naphthol, then add 0.3 ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents). We shake vigorously to ensure mixing and thorough oxygenation of the culture. We used a cotton board, dipped into the solution and picked a part of the colony, we observed for color changes on the area with the picked organism, Microorganisms are oxidase positive when the color changes to deep blue within 15 to 30 seconds and Negative if there is no color change.

Catalase Test: Approximately 2-3 ml of freshly prepared 3% H_2O_2 hydrogen peroxide is taken in a test tube. A portion of the young bacterial colony is picked up using a sterile glass capillary, plastic or wooden stick and immersed into the tube contain.

Indole Test: Peptone water solution was measured and diluted following the manufacturer instruction, pure bacterial culture was grown in sterile tryptone broth over-night, and 1.0ml of chloroform was added to the broth and shaken gently. After this, 5v drops of P-Dimenthylaminbenzaldehyde [KPVAC'S reagent] was added to the broth culture, Red color on the surface layer of the broth indicates positive while negative result appeared yellow on the surface of the broth.

RESULTS AND DISCUSSION

The populations of microorganisms that exist around the roots of plantain in the various locations across Toru-Orua metropolis of Bayelsa, Nigeria are different due to difference in ecological locations. Roots samples collected in UAT, Angalabiri and Koroye farm, contain high population of bacteria compared to other Area in Toru-Orua (Table 1). The fungal population follows the same trend. Characterization/identification of microbial isolates (morphological and biochemical characteristics), that is, morphological characteristics of isolates are shown in Tables 2 and 3. Five bacteria were isolated; they are micrococcus which are gram negative cocci in chain, oxidase negative, and indole negative (Table 5).

Biochemical Tests: The results of the Biochemical tests carried out are shown in Fig. 1, Fig. 2 and Fig. 3, and Table 4. Five bacteria were isolated; they are micrococcus which are gram negative cocci in chain, oxidase negative, and indole negative.

Table 1. Population of microorganisms found in plantain root across different Farm lands in Toru-Orua Metropolis

Location	Population count (cfu/g)	Fungi (cfu/g)
UAT farm	1×10^7, 3×10^7	2×10^4, 1×10^4, 1×10^4
Angalabiri farm	5×10^7, 9×10^7	
Koroye farm	4×10^7, 4×10^7, 5×10^7	

Table 2. Morphological characteristics of microbial isolates (Mackconkey Agar Plate)

Mc (sample)	Size	Shape	Edges	Elevation	Colour	Texture	Opacity
Sample A 10^{-5} (1)	2 mm	Round	Entire	Raised	Creamy brown	Smooth and shiny	Opaque
Sample A 10^{-7}(2)	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Sample C 10^{-7} (3)	1.5 cm	Irregular	Irregular	Flat	Yellowish brown	Rough	Transparent
Sample C 10^{-5}	2 mm	Round	Entire	Raised	Creamy brown	Smooth and shiny	Opaque
Sample B 10^{-5}	1.5 cm	Round	Entire	Raised	Red	Smooth and shiny	transparent
Sample B 10^{-7}(TNC)	1.5 cm	irregular	Entire	Raised	Brownish white	Rough	Opaque

Table 3. Morphological characteristics of microbial isolates (Nutrient Agar Plate)

Nutrient Agar (sample)	Size	Shape	Edges	Elevation	Colour	Texture	Opacity
Sample A 10^{-5} (1)	2 mm	irregular	lobate	umbonate	Yellowish white	Rough and shiny	Transparent
Sample A 10^{-7}(2)	2 mm	Irregular	lobate	Raised	Creamy brown	Smooth and shiny	Opaque
Sample C 10^{-7} (TNC)	1.5 cm	Round	lobate	umbonate	Yellowish white	Rough and shiny	Transparent
Sample C 10^{-5}	2 mm	irregular	Entire	Raised	Yellowish white	Rough	Opaque
Sample B 10^{-5}	2 mm	Round	Entire	Raised	Creamy brown	Smooth and shiny	Transparent
Sample B 10^{-7}(TNC)	2 mm	irregular	Rough	Raised	Yellowish white	Rough and smooth	Opaque

Fig. 1. Nutrient Agar Subculture plates

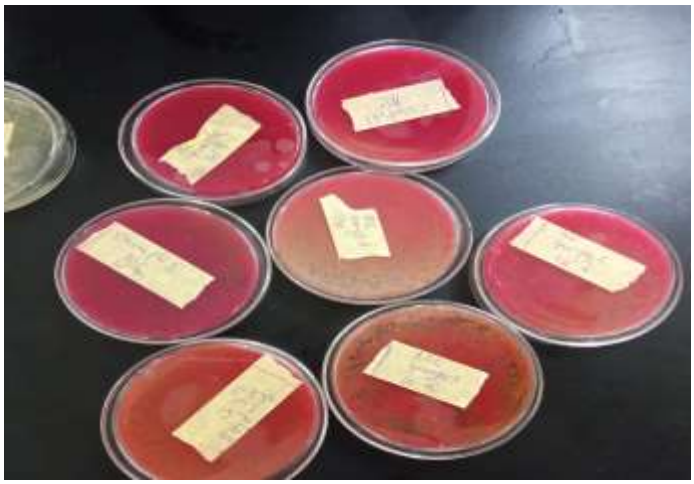

Fig. 2. MacConkey Agar Subculture plates

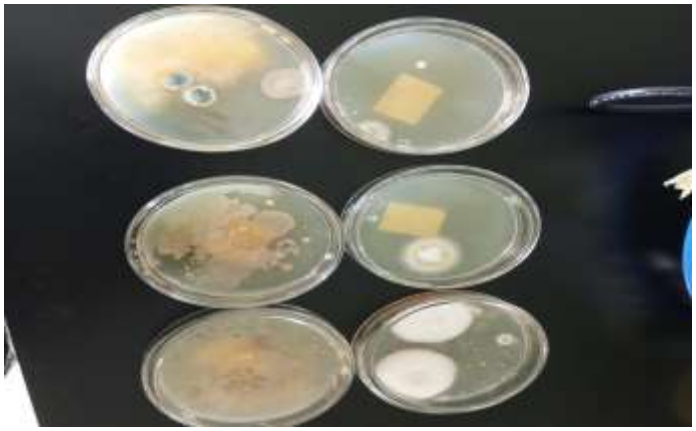

Fig. 3, Fungi plates

In the Tables, the population of bacteria was higher than fungi in all the locations. The population of the organisms in school farm and axis between Toru-Orua and Angalabiri metropolis are different from other areas within Toru-Orua. The high population of bacteria in the soil corresponds to study of (Isirimah *et al*., 2006), an unpublished undergraduate project of 2004 which stated that the population of bacteria is higher than fungi in silty or silty clay soils. This indicates the populations of bacteria and fungi and the probable isolates present in plantain roots in different ecological zones of the state. In those locations where we have higher population of microorganisms (like school farm and axis between Toru-Orua and Angalabiri), there was evidence of good nutrition in those locations. So, microorganisms in essence characteristically enhance the nutrition of plants.

Nitrobacter winogradkyi was found in roots samples collected in school farm and Toru-Orua and Angalabiri axis due to the presence of cover crops around the plantain. These varieties of fungi were also isolated. They include Aspergillus fumigatus, Aspergillus niger and Aspergillus flavus. These are the largest of all microorganisms in the soil. Others were Rhizobium, Nitrobacter, Winogradkyl, Azomona argillis and Psydormonads aeruginosa. Two different bacteria were found and isolated from samples collected at Koroye compound farm. This corresponds to (Isirimah *et al*., 2006) which state that different types of bacteria are found around the rhizosphere environment.

Table 4. Identification of Fungal isolates

S/NO	Morphology	Microscopic examination with lactophenol	Fungi identified
1	Characterized by green echimulate conidia 2.5 to 3µm in diameter, produce in chains basipetally from greenish phialides, 6 to 8 by 2 to 3µm in size.	2.5-8µm wide, septate hyaline acute angle branching tree or fan like branching stipes may resemble hyphae of zygomycetes	Aspergillus fumigates
2	Black colouration in front and creamish in reverse view.	Aseptate hyphae with rough head of pigment	Aspergillus flavus
3	Colonies with loose white to yellow mycelium, rapidly turning dark brown and eventually black on the development of conidia.	Vesicle were light, yellow brown. Phialides growing radially along the periphery of vesicles. Primary phialides and secondary phialides are bogh brown	Aspergillus niger

Table 5. Index/Biochemical tests

Isolate	Gram reaction	Catalase test	Oxidase test	Indole	Most Probable organism
UAT farm	-ve (colli in chains)	-ve	-ve	-ve	Micrococcus
Koroye farm	-ve (Rod in chains)	-ve	-ve	-ve	Nitrobacter
Angalabiri farm	-ve (Stout rods in clusters)	-ve	-ve	-ve	Rhizobium
Isolate	**Gram reaction**	**Catalase test**	**Oxidase test**	**Indole**	**Most Probable organism**
UAT farm	-ve (Rod in chains)	-ve	-ve	-ve	Azomonas agilis
Koroye farm	-ve (Rod in chains)	-ve	-ve	-ve	Pseudomonas aeruginosa
Angalabiri farm	-ve (Rod in chains)	-ve	-ve	-ve	Micrococcus

Conclusion: Isolation and identification methods of microorganisms in the roots of plantain were presented in this paper. It was revealed in the paper that the populations of bacteria and fungi and the probable isolates present in plantain roots are differ in the different ecological zones of the case study state. In those locations, where we have higher population of microorganisms (like UAT farm and Angalabiri farm), there was evidence of good nutrition in those locations. So, microorganisms in essence characteristically enhance the nutrition of plants.

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