

An Ecological Survey of Microorganisms Associated with Plantain Roots (Rhizosphere)

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Abstract: Problem statement: Micro-organisms are more predominant around root zone and as such play a vital role to plant. Micro-organisms are diverse and have property modification which are beneficial to plant growth and root development. **Approach:** The lack of knowledge on the specific microorganisms associated with plantain roots in Cross River State soils (which in turn leads to an avoidable loss of crop if appropriate management methods were employed) led to the need for this study. Different ecological zones have different population of micro-organisms. The purpose of this study is to: to enumerate the rhizosphere microorganisms (bacteria and fungi) associated with plantain roots at different locations across the ecological zones of the state and to identify the rhizosphere microorganisms associated with plantain roots of different location representing the ecological zones of the state. **Results:** To ascertain this, it was necessary to isolate micro-organisms from the roots of plantain in order to determine the different populations of microorganisms in different ecological zones across Cross River State, Nigeria. The isolation of bacteria and fungi colonizing the root of plantain were determined at six locations across the state, as follows: Obanliku, Boki, Etung, Obubra Biase and Odukpani Local Government Area. The activity growing roots of plantain were removed with the attached suckers and transferred to the laboratory for microbial analysis. Serial dilution method was used to determine the population of bacteria and fungi present in the root samples collected. Also, staining reaction as well as biochemical test were carried out to identify the types of bacteria present and their biochemical reactions. **Conclusion/Recommendations:** The result showed that several types of bacteria and fungi were present around the roots of plantain. The types of bacteria and fungi are listed below; Bacteria: *Micrococcus*, *Rhizobium*, *Azomonas-agilis*, *Pseudomonads aeruginosa*, *Nitrobacter winogradskyl*; Fungi: *Rhizopus*, *Aspergillus niger* and *Nigrospora*. Many of these bacteria and fungi species are known organic matter decomposers, nitrogen fixers and contributors to plant nutrition. The use of appropriate cultural methods could enhance good nutrition around plantain crops.

Key words: Plantain roots, ecological survey of microorganisms, rhizosphere

INTRODUCTION

The environment around root zone is populated by many microorganisms and animals; though it is generally believed that microorganisms play an important role in the release of nutrient/minerals and carbon dioxide for plant growth. Many soil microbiologists have used microbial analysis of soil as an index of soil fertility and land use. Microorganisms are generally responsible for the breakdown of organic matter including hydrocarbons, conversion of organic components from one form to another and the production of humus. These groups of organisms are diverse and as such are thought of as being of five distinct types: bacteria, actinomycetes, fungi, algae and protozoa. The organisms form a robust community capable of surviving and functioning under extremes of temperatures, water availability, pH, energy and salt concentration, exchange of nutrients and microorganisms Ezomoh and Ojealare, 2002.

The rhizosphere: According to (Alexander, 1977), is the portion of the soil under the direct influence of roots of plants. It is the region of the soil through which any soil organism influencing the plant via the root must pass or transmit the effect and through which all plant nutrients passed. Many interactions of the soil occur here. The extent of this zone depends on the activities of the soil, but generally influence of the root extends for only few millimeters.

Rhizosphere microorganisms distribution fluctuate with textual changes and are more numerous in silty or silty clay horizons than in the intervening sand or coarse horizons. Early work on soil microbiology was almost limited to the study of bacteria alone, which was involved in the transformation of sulfur and nitrogen compounds. According to (Paul and Clark, 1996), this provides explanation for some chemical changes which were known to take place in the rhizosphere and as such related to soil fertility. Bacteria growth is stimulated by

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nutrients such as amino acids and vitamins released from the root tissue. Fungi populations which are incorporated into the rhizosphere soil are difficult to derive, due to inability to separate the active fungi from inactive one. There are other rhizosphere inhibiting organisms whose population tend not to react to influx of decomposable organic matter and usually not affected by root growth. These include actinomycetes, protozoa and algal population. Unlike algal propagule which derived its energy supply from sole energy, actinomycetes derive its own energy from decomposable soil organism matter components protozoa propagule are limited by the distribution and density of prey population (Fogel, 1988).

Microorganisms associated with root of crops: These organisms have property modification which are beneficial to plant growth and root development. According to (Barbar and Iyeh, 1977), they range from more complex to the prove production of growth-promoting substances to the clearly documented gains of symbiotic root associations involved in nitrogen fixation.

Knudsen (1991) said that varieties of microbes have been demonstrated to produce plant growth hormones, such as cytokines. The substances could be proposed to stimulate root tissue development, thereby increasing the capacity of the root system to provide nutrients and water required for above ground biomass functions (Carrillo and Vazquez, 1992). Microbes associated with plant roots play other vital role in the soil, such as development of stable soil structure, making rhizosphere environment conducive for plant, through the influence or activity or fungal mycelia production in association of soil particle with root tissue. Microbial association also led to the improvement of soil aeration, water infiltration and root penetration.

Different species of aerobic bacteria are associated with root of crops (Barbar and Iyeh, 1977). Such bacteria include those of the members of the genera *pseudomonas*. These are organisms that are beneficial to roots of plant.

Objective of the study:

- To enumerate the rhizosphere microorganisms (bacteria and fungi) associated with plantain roots at different locations across the ecological zones of the state
- To identify the rhizosphere microorganisms associated with plantain roots of different location representing the ecological zones of the state

MATERIALS AND METHODS

Study area: Samples were collected from six different locations across Cross River State as highlighted below: Kayang in Boki local government, which lies on latitude 6°43' and longitude 9°24'east. The soil type is sandy, clay and loamy soil (Lateritic soils), falls within the forest vegetation.

Bendeghe-Ekim in Etung Local Government lies within latitude 5°50' North of the equator and longitude 8°31' east of the Greenwich meridian. It is located in the central part of the state. It falls within the tropical rainforest vegetation with loamy clayey type of soil. The area is made up of natural forest.

The Ochon in Obubra Local Government is found within latitude 5°45' north and 6°18' north and longitude 7°55' east in the central part of the Cross River State. The environment lies between the basement complex rock. Most of the soils in the area are derived from granitic breakdown. It is a tropical rainforest zone.

Ibogo in Biase Local Government Area falls within the rainforest zone and its soils are basaltic soils.

Odukpani Local Government lies within latitude 4°27' north and longitude 2°20'E, it is a rainforest zone.

Abiesang and Lishukwel in Obanliku Local Government are bounded in the north by Benue State and in the east by cameroun. The soil is lateritic soil, reddish at the top of the hill due to mountain and brownish at the base.

Sample collection and preparation: Actively growing plantain was removed with roots and ball of earth with the help of spade and cutlass. The samples were latter put in the polythene bag, labeled properly and transferred to the laboratory immediately for analysis.

Laboratory analysis:

Preparation of media for the microbial analysis: Roots of plantain were used to prepare the extract. Roots were cut off, the tissue removed washed and autoclaved for bacteria and PDA was also prepared. It was made selective for fungi by addition of streptomycin at 1 mg mL⁻¹. Roots were cut off into 100 mL⁻¹ beaker, shake thoroughly, a ten-fold serial dilution up to 10⁻⁴, 10⁻⁵ and 10⁻⁶ were made and transferred into Root Extract (REA) in Petri dishes and root extract agar was added and swarp for even distribution, these were done for bacteria.

For fungi, PDA was introduced into Petri dishes and then 1ml aliquot at 10⁻³ was added and spread with a bent rod in a dectopic environment, then wrapped with masking tape. Inoculated plates were then incubated at ambient temperature (30°C) for 24 h for bacteria and 72 h for fungi.

In gram staining methyls violet added for 60 sec, then decolourise with 70% alcohol for 10-20 sec and washed with water for 2 sec. Finally, safranine added for 20 sec, then washed with water for 2 sec and blot dry. After which, it was mounted on a microscope to identify the type of bacteria present, using shapes and colour as characteristic. Some of the identified bacteria were gram-ve, some were rods in chains, some rods in cluster and some single.

These were done to identify the type of organisms present and the biochemical reaction in the roots of plant.

Bacteria: The prepared sample (Alequote) at the dilution forming unit (10^{-6}) was used to inoculate for bacteria. The extract used was Root Extract Agar (REA). The alequote was pipetted into Petri dish and root extract agar added REA incubated for 24 hs and bacteria colonies were counted.

Fungi: Fungi was inoculated at 10^{-3} , 1ml of the alequote was introduced in a Petri dish containing PDA and incubated for 72 h to grow and the type of fungi present were identified and the population counted.

Plating and counting of the organisms: Both bacteria and fungi were incubated with bacteria at 10^{-6} and fungi at 10^{-3} . the alequote were dropped into agar in Petri dishes, wrapped very well to avoid contamination, incubated and bacterial colonies counted using colony counter and fungal growth identified, as well fungi population counted.

Biochemical test for the identification of the organisms present: Colonies of bacteria were subcultured in another Petri dish using nutrient agar and incubated for 24 h. A drop of water was dropped on a slide using syringe and needle and then bacteria growth or pure culture scooped and mixed with water on the slide and smeared on a flame to dry.

RESULTS

The Table 1 below presents the population of micro-organisms that exist around the roots of plantain in the various locations across cross river state of Nigeria the population counts of bacteria and fungi are different due to difference in ecological locations. Roots samples collected in Biase, Obubra, Etung and Obanliku 2 (Lishikwel) contain high population of bacteria compared to other local government areas. The fungal population follow the same trend.

Five bacteria were isolated; they are micrococcus which are gram-ve cocci in chain, oxidase-ve, coagulase-ve, indole-ve, motility-ve and sucrose and glucose A.

Table 1: Population of microorganisms found in plantain roots across different ecological zones of cross river state

Location	Population count bacteria (cfu/g)	Fungi (cfu/g)
Boki	04×10^6	3×10^3
Obanliku 1	04×10^6	3×10^3
2	18×10^6	3×10^3
Etung	14×10^6	2×10^3
Obubra	13×10^6	6×10^3
Biase 1	15×10^6	3×10^3
2	25×10^6	4×10^3
Odukpani	06×10^6	2×10^3

It was isolated in Kayang in Boki local government area. Others were Rhizobium, Nitrobacter, Winogradkyl, Azomona argillis and Psydormonads aeruginosa. Two different bacteria were found isolated from samples collected at Obanliku and Biase. This corresponds to (Isirimah *et al.*, 2006) which state that different types of bacteria are found around the rhizosphere environment.

Nitrobacter winogradkyl was found in roots samples collected in Etung and Obubra due to the presence of cover crops around the plantain. These varieties of fungi were also isolated.

They include Rhizopus, Aspergillus niger and nigrosora. These are the largest of all microorganisms in the soil.

Of all the bacteria isolated from roots of plantain across different ecological zones of Cross River State, micrococcus were 21×10^6 cfu g⁻¹ in Boki, 15×10^6 in Biase and 18×10^6 in Lishikwel (Obanliku) and Pseudomonas 15×10^5 in Odukpani. For fungi, rhizopus at 3×10^3 in Boki and 6×10^6 in Biase, Aspergillus 3×10^3 cfu g⁻¹ in Etung, 2×10^3 in Obubra, 3×10^3 cfu g⁻¹ in Biase, 4×10^3 cfu g⁻¹ in Odukpani and Nigrospora 2×10^3 cfu g⁻¹.

DISCUSSION

From Table 1, the population of bacteria was higher than fungi in all the locations. The population of the organisms in Biase and Lishikwel in Obanliku are different from other local government areas.

The high population of bacteria in the soil corresponds to study off (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.

These 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 Biase and Obanliku), there was evidence of good nutrition in those locations. So microorganisms in essence characteristically enhance the nutrition of plants.

Table 2: Index biochemical test

Isolate	Gram reaction	Lactose	Mitol	Glucose	Sucrose	Citrate	Methyl red	VIP	Catalase	Oxidase	Cogulase	Indole	Motility	Probable isolate	Population count
Kayang (Boki LGA)	Gram-ve colli In chains	A	-	A	A	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Micrococcus	
Obanliku (Abiesang)	Gram-ve stout rods in clusters	--	A	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Rhizobium	
Etung (Benege-Ekim)	Gram-ve rod in chains	A	-ve	-ve	-ve	-ve	-ve	--	-ve	-ve	-ve	-ve	-ve	Nitrobacter winogradkyl	
Obubra (Ochon)	Gram-ve rod in chains	A	-ve	-ve	-ve	-ve	-ve	--	-ve	-ve	-ve	-ve	-ve	Nitrobacter winogradkyl	
Biase A (Ibogo) chains	Gram-ve rod in chains	A	--	A	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Micrococcus	
B	Gram-ve rod in chains	-VE	-ve	A	A	--	--	--	--	--	--	--	--	Azomonas agillis	
Odukpani	Gram-ve rod in chains	--	--	A	A	--	--	--	--	--	--	--	--	Pseudomonads aeruginosa	
Obanliku (Lishukwel)	Gram-ve rod in Chain	-VE	-ve	A	A	--	--	--	--	--	--	--	--	Azomonous agillis	

Table 2 shows the result of various tests to identify the bacteria and fungi, to classify the bacteria and fungi in their different groups following the standard methods. Those organisms present were representative of the common organisms found around the root (rhizosphere) of plants, especially plantain. They represent the organisms that enhance the nutrition of plantain.

CONCLUSION

Practices that would enhance the proliferation of bacteria and fungi around the root of plantain should be encouraged. The practices include organic matter accumulation in form of green manuring, zero tillage and non-use of chemicals and burning would encourage the proliferation of microbes as well as nutrition of the plants.

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