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Bacterial community of the rhizosphere of some plants in Nigeria

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Bacterial community structures in plant rhizosphere were examined with respect to plant species as to determine the relative abundance and species diversity. A total of 16 rhizosphere soil samples were collected in duplicates from four different plants which were *Amaranthus* sp., *Ocimum basilium*, *Talinum triangulare*, and *Telfeira occidentalis*. Using standard techniques, the soil samples were analyzed for temperature, pH, total viable heterotrophic bacteria count, bacteria types and their frequency of occurrence. The mean of the pH, temperature, and total heterotrophic bacteria count of the rhizospere of various plant species are as follows; 5.08 ± 0.25, 27ºC, and 3.6 × 10^4 ± 0.13 × 10^4 cfu/g soil respectively for *Amaranthus* sp., 5.45 ± 0.77, 28.5ºC, and 3.18 × 10^4 ± 0.11 × 10^4 cfu/g soil for *Ocimum basilium*, 6.14 ± 0.08, 32ºC, and 3.18 × 10^4 ± 0.11 × 10^4 cfu/g soil for *Talinum triangulare* and 4.18 ± 1.15, 26.5ºC, and 2.995 × 10^4 ± 0.43 × 10^4 cfu/g soil respectively for *Telfeira occidentalis*. Generally, the pH values were within the moderately acidic range while the temperatures were within the mesophillic range. The bacteria isolates and their frequency of isolation were *Actinomyces* sp. (12.5%), *Azobacter* sp. (9.37%), *Bacillus* sp. (18.75%), *Enterobacter* sp. (12.5%), *Erwinia* sp. (3.12%), *Pseudomonas* sp. (12.5%), *Rhizobium* sp. (15.63%) and *Xanthomonas* sp. (15.63%). The four plant species had distinct bacteria communities. Analysis of variance (ANOVA) using paired t-test showed that there is a significant difference in the occurrence of bacteria between the rhizospere of *Amaranthus* sp., and *Talinum triangulare*, and between *Ocimum basilium* and *Talinum triangulare* at $p \leq 0.05$. *Actinomyces* species are beneficial soil dwelling bacteria critical in the decomposition of organic matter in humus formation and they are responsible for the earthy aroma which is associated with healthy soil. The *Azobacter* sp. and *Rhizobium* sp. in rhizosphere are beneficial being responsible for nitrogen fixation in plants. *Pseudomonas* sp. have anti-fungal activity that inhibit some plant pathogens and produce compounds that promote plant growth while *Erwinia* sp. and *Enterobacter* sp. are plant pathogens. *Bacillus* sp. and *Enterobacter* sp. are human pathogens which are causative agents of anthrax, food poisoning and urinary and wound infections. Managing soil bacterial community would improve soil quality, the quality of plant species and the control of plant species. The application of bacteria communities or inoculants as bio-fertilizer and phyto-stimulator should be encouraged as to improve crop yield.

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Introduction

Soil is a complex structure created by the influence to geology, topography, climate, time and anthropogenic activities (Lennart *et al*., 1998). As with air and water, human life could not be sustained without access to soil, since it is the source of food. In addition to producing food, a good quality soil also acts as an environmental filter for cleaning air and water. The economic well being of most of the nations on earth depends greatly on arable soil and how well their productivity is maintained (Lennart *et al*., 1998).

Soil quality encompasses not only the capacity of a soil for crop productivity, but also food safety for animal and human health. Doran and Parkin (2004) defined soil quality as "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environment quality and promote plant and animal health".

In a balanced soil, plants grow in an active and vibrant environment. The mineral content of the soil and its physical structure are important for their well-being, but it is the life in the earth that powers its cycle and provides its fertility. Without the activities of soil microorganisms that includes bacteria and fungi, organic materials would accumulate and litter the soil surface, and there would be no nutrient for plants.

Bacteria and fungi play key roles in maintaining a healthy soil. They act as decomposers that convert energy in soil organic matter into forms useful to the rest of the organisms in the soil food web. Saprotrophs, well represented by fungi and bacteria, extract soluble nutrients (Singer and Munns, 2000). Bacteria also form partnerships with plants (mutualists). The most well known are the nitrogen fixing bacteria. Bacteria are also plant pathogens which include *Zymomonas* and *Erwinia* species and species of *Agrobacterium* that cause gall formation in plants. They are also lithotrophs or chemoautotrophs that carry out biogeochemical transformations (Duineveld, 2001).

Soil microorganisms including bacteria are critical in creating and maintaining good soils structure by proper aeration and formation of humus and particle aggregate (Nolin *et al.,* 1999). Stable aggregates improve water infiltration and the soil water holding capacity. Soil texture, in combination with variations in structure and moisture levels, can drastically affect the aeration status, thus influencing the distribution of physiological groups in the microbial community. In a diverse bacterial community, many organisms will compete with disease causing organisms in roots and on above ground surfaces. These result in disease suppression in plants (Gupta and Germida, 2000; Larink *et al*., 2001). Bacteria are more competitive when easy to metabolize substrates are present. This includes fresh, young plant residue and the compounds found near living roots. Plants produce certain types of root

exudates to encourage the growth of protective bacteria. These materials create a unique environment called the rhizosphere for soil microorganisms (Willey, *et al.,* 2008). Bacteria and other microorganisms are highly concentrated in the rhizosphere (Green *et al.,* 2006).

The rhizosphere is a key soil habitat where the numerous interactions taking place between plant root and soil microorganisms determines growth conditions for both the plant and the microorganisms in the rhizosphere. Rhizosphere microorganisms increase their numbers when substrates from the plants become available; thus composition and function also change. In addition, rhizosphere microorganisms serve as labile sources of nutrients which are continuously shunted into growth cycles of macro and microphytes (Ehrlich and Roughgarden, 1997) creating a soil microbial loop and thereby playing critical roles in organic matter synthesis and degradation (Stenberg *et al.,* 1998; Willey *et al*., 2008). Consequently, soils that maintain a high level of microbial biomass are capable not only of storing more nutrients, but also of cycling more nutrients through the system (Domseh *et al*., 1999).

The diversity of organisms may depend on the type of plant growing on the soil and the proximity of the organism to the plant root itself. The number and activities of organisms decreases with increasing distance from the root (Lennart *et al*., 1998). A wide range of microbes in the rhizosphere can promote plant growth. Plant growth-promoting rhizobacteria include the genera *pseudomonas* and *Achromobacter.* A critical process that occurs on the surface of the plant, and particularly in the root zone, is associative nitrogen fixation; a process carried out by representatives of the genera *Azotobacter*, *Azospirillum* and *Acetobacter.* Evidence suggests that their major contribution may not be nitrogen fixation but the production of growth–promoting hormones that increase root hair development (Willey *et al*., 2008).

Soil quality can improve or deteriorate depending on influencing factors. (Lennart *et al*., 1998) suggested strategy for an integrated evaluation of soil quality. The different indications used are such that they reflect biological, chemical and physical components of the soil. The use of microbial variables as indicators of soil quality has often been recommended (Torstensson, 2007). Wick and Kuline (2007) noted that soil microbial parameters may also be helpful as indicators of changes in soil quality. This was supported by Kennedy and Papendick (1999) who stated that minor differences in quality factors may be early warning signals of soil degradation and can be used as indicators, so that degrading effects can be prevented and soil building practices can be implemented.

In Nigeria, there is little or no literature on the bacterial community structures in plant rhizosphere with respect to plant species. Thus there is a need to

conduct such a study. The objective of this study therefore is to examine the bacterial community structures in plant rhizosphere with respect to plant species as to determine the relative abundance and species diversity of the rhizoshere bacteria. This was achieved by the isolation and identification of bacteria species associated with rhizosphere of different plant species. The benefits of soil dwelling bacteria, the roles they play in soil environment and the bacteria species that are pathogenic to plants and humans are also highlighted.

Materials and methods

Description of the study area and sampling

Rhizosphere soil samples were collected from four different plant species in a farm located opposite the day care centre in the Rivers State University of Science and Technology, Nkpolu-Oroworukwo in Port Harcourt, Nigeria. The farm measures 224,000 m^2 in size. The common names and scientific names of the plants are Green plant *(Amaranthus* sp.), scent leaf plant (*Ocimum basilium*), water leaf plant (*Talinum triangulare*) and fruited pumpkin – "*Ugu*" (*Telfeira occidentalis*). These plants were located at the north, east, west, and south end of the farm respectively.

Collection and processing of samples

Rhizospere samples of each of the plants were collected in duplicates at each sampling station. At each sampling site, the designated plant was uprooted with the hand and shaken to remove the loose soil. The soil sample that adhered to the roots was thereafter collected into sterile black polythene bags and labeled appropriately. The pH values were obtained using a pH meter while the temperature values were obtained using a mercury thermometer. Eight samples, two from each sampling station were collected on each visit and transported to the laboratory. The samples were air dried and sieved through 2.0 mm pore sieve to obtain fine soil particles. One gram of each air-dried fine soil sample was mixed in a test tube containing 9 ml of sterile distilled water using a sterile spatula, after which it was rigorously agitated. This solution constitutes the original 10^{-1} dilution of propagules in the sample. The sampling was carried out bi-weekly for a period of three months (October to December 2008).

Cultivation and enumeration of Bacteria in the samples

One gram (1 g) of each sample of fine soil was thoroughly shaken in 9ml of normal saline. An aliquot (1.0 ml) was transferred into the next tube serially

in one- tenth step wise to 10^{-4} dilution from the dilutions of 10^{-3} and 10^{-4} of each soil sample 0.l ml aliquot was transferred aseptically onto freshly prepared Nutrient agar plates and spread with a sterile bent glass rod (Obire *et al.,* 2002). The dilutions for 10^{-3} and 10^{-4} were plated out for bacteria because the dilution of 10^{-1} gave a confluent growth. Cultured plates were inverted and incubated at 28^{o}C for 24 hours after which the plates were examined for growth. The discrete colonies which develop were counted and the average counts for duplicate culture plates were recorded as total viable aerobic heterotrophic bacteria in the sample.

Isolation, characterization and identification of bacteria

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the culture plates onto freshly prepared Nutrient agar plates which were incubated at 28^{o}C for 24 hours. Discrete bacteria colonies which developed were sub-cultured into small bottles which contained glycerol solution and incubated at 28^{o}C for 24 hours. These served as pure stock cultures for subsequent characterization tests. The pure isolates obtained were subjected to various characterization procedures. Cultural characteristics (which include pattern of growth, pigmentation and appearance of isolates on nutrient agar plates) were observed after 24 hours of incubation at 28^{o}C. Gram staining and biochemical reactions exhibited by the isolates in test methods adopted in accordance with those described by Cruickshank *et al*. (1975). The following standard characterization tests were performed: Gram's staining reaction, oxidase test, catalase test, coagulase test, methyl red test, indole test, Voges Proskauer test and carbohydrate fermentation test. The bacteria were identified on the basis of their cultural, morphological, and physiological characteristics. Further identification was made by comparison of their cultural, morphological and physiological characteristics with those of known taxa and with reference to Holt (1997).

Results

The mean of the pH, temperature, and total heterotrophic bacteria count recorded for rhizospere of the various plant species are as follows; 5.08 ± 0.25, 27^{o}C, and 3.6 × 10^{4} ± 0.13 × 10^{4} cfu/g soil respectively for *Amaranthus* sp, 5.45 ± 0.77, 28.5^{o}C, and 3.18 × 10^{4} ± 0.11 × 10^{4} cfu/g soil for *Ocimum basilium*, 6.14 ± 0.08, 32^{o}C, and 3.18 × 10^{4} ± 0.11 × 10^{4} cfu/g soil for *Talinum triangulare* and 4.18 ± 1.15, 26.5°C, and 2.995 × 10^{4} ± 0.43 × 10^{4} cfu/g soil respectively for *Telfeira occidentalis*.

The bacteria isolated from the rhizospere of the plants and their frequency of isolation during the present study were *Actinomyces* sp. (12.5%), *Azobacter* sp. (9.37%), *Bacillus* sp. (18.75%), *Enterobacter* sp. (12.5%), *Erwinia* sp. (3.12%), *Pseudomonas* sp. (12.5%), *Rhizobium* sp. (15.63%) and *Xanthomonas* sp. (15.63%). There were variations in the occurrence of the bacterial types in the various rhizospere. The percentage occurrence of bacteria in the rhizospere of the different plant species is as shown in Fig. 1.

The four plant species had distinct bacteria communities. Analysis of variance (ANOVA) using paired t-test showed that there is a significant difference in the occurrence of bacteria between the rhizospere of *Amaranthus* species and *Talinum triangulare*, and between *Ocimum basilium* and *Talinum triangulare* at $p \leq 0.05$.

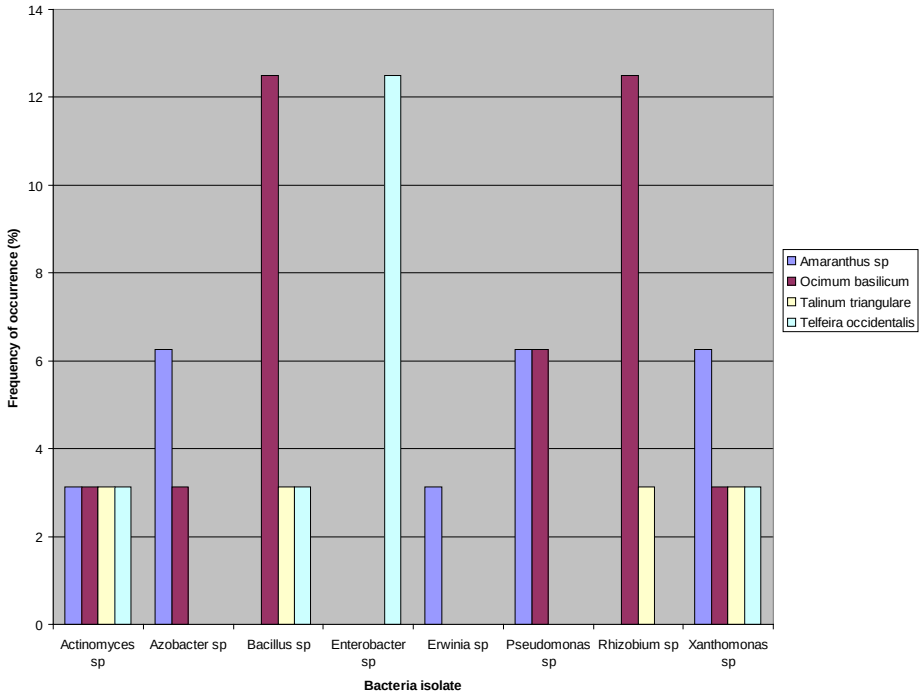

Fig. 1. Occurrence of the bacteria (%) in the rhizosphere of the various plant species.

Discussion

The present investigation has revealed the pH, temperature and aerobic heterotrophic bacteria population of the rhizosphere of different plant species (*Amaranthus* sp, *Ocimum basilium*, *Talinum triangulare*, and *Telfeira occidentalis*). Generally, the pH values were within the moderately acidic range while the temperatures were within the mesophillic range. However, the

rhizosphere of *Telfeira occidentalis* was the most acidic and this must have accounted for the lowest population of bacteria recorded. The rhizosphere of *Ocimum basilium* and *Talinum triangulare* recorded the same bacteria population though they had different pH and temperature values. The rhizospere of *Amaranthus* sp. had a pH value within range that was recorded by *Ocimum basilium* and *Talinum triangulare* but with a lower temperature, recorded the highest bacterial population. These shows that environmental factors such as temperature and acidity have influence on the bacteria population of rhizosphere of plants (Lennart *et al*., 1998).

The investigation also revealed the types of aerobic heterotrophic bacteria present in the rhizosphere and has shed light on the bacterial community status of the soil as influenced by the presence of the roots of different species of plant. The four plant species had distinct bacteria communities and there was a significant difference in the occurrence of bacteria between the rhizosphere of *Amaranthus* species and *Talinum triangulare*, and between *Ocimum basilium* and *Talinum triangulare*. Although *Actinomyces* and *Xanthomonas* species were isolated from the rhizosphere of all the plant species studied, *Erwinia* was isolated only from *Amarantus* sp., while *Enterobacter* was isolated only from *Telfeira occidentalis*. *Azobacter* and *Pseudomonas* species were isolated from *Amaranthus* sp., and *Ocimum basilium* while Rhizobium sp., was isolated from the rhizosphere of *Ocimum basilium* and *Talinum triangulare*. Except from the rhizosphere of *Amaranthus*, *Bacillus* species was isolated from all the other plant species. This shows that environmental factors and plant species have influence on the bacterial community structure or the diversity of bacteria species occurring in the rhizosphere.

Green *et al*. (2006) stated that bacteria are especially concentrated in the rhizosophere which is the narrow region next to the root exudates to encourage the growth of protective bacteria. *Actinomyces* sp. was isolated during this study are critical in the decomposition of organic matter and in humus formation and their presence is responsible for the 'sweet earthy aroma', which is associated with a good healthy soil, *Azobacter* are free living nitrogen fixing bacteria but evidence suggests that its major contribution is the production of growth-promoting hormones that increase root hair development. It is known that *Azospirillum* induces he proliferation of plant root hairs which can result in improved nutrient uptake (Wick and Kuline, 2007). The roots of plants are involved in the uptake of mineral nutrients and water for plant growth, but they also release a wide range of organic compounds in the surrounding soil. *Erwinia* sp. and *Enterobacter* sp. are plant pathogens in that they could cause gall formation in plants. *Pseudomonas* sp. and *Xanthomonas* sp. are bacteria that promote plant growth; *Pseudomonas fluorescens* is known to possess anti-

fungal activity that inhibits some plant pathogens. *Rhizobium* sp., which was also isolated, forms nodules on plant roots, they live freely in soil but when they approach the plant root they are assumed to be invaders.

The study also reported the presence of *Bacillus* sp. and *Enterobacter* sp. that are potential pathogens for humans and animals. *Bacillus* sp. can be responsible for diseases such as Anthrax, food poisoning and meningitis. *Enterobacter* sp. is responsible for urinary tract and would infections (Willey *et al.,* 2008). The bacteria isolated in this study are either decomposers, mutualists (*Azobacter*) or plant pathogens (*Erwinia and Enterobacter*) causing gall formation in plants. The potential pathogens to human health which were isolated are *Bacillus* and *Enterobacter.*

A wide range of microbes in the rhizosphere can promote plant growth, orchestrated by their ability to communicate with plants using complex chemical signals. Some of these chemical signal compounds include auxins, gibberellins, glycolipids, and cytokinins, and are beginning to be fully appreciated in terms of their biotechnological potential. Plant growth-promoting rhizobacteria include the genera *Pseudomonas* and *Achromobacter* (Willey *et al*., 2008). These can be added to the plant, even in the seed stage, if the bacteria have the required surface attachment proteins. The genes that control the eression of these attachment proteins are of great interest of agricultural biotechnologists.

These plant growth enhancing bacteria occur naturally in soils, but not always in high enough numbers to have a dramatic effect. In the future, farmers may be able to inoculate seeds with anti-fungal bacteria such as *Pseudomonas* species to ensure that the bacteria reduce pathogens around the seed and root of the crop.

Soil microorganisms play important role in almost every chemical transformation taking place in the soil. In particular, they can improve the fertility status of the soil and contribute to plant growth. ‘Bio-fertilizers’ which microorganisms that play these roles are receiving increased attention for use as microbial inoculants in agriculture. These microorganisms called ‘phytostimulators’ should be extensively studied for possible use as microbial inoculants to improve crop yield.

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