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AN INVESTIGATION OF BACTERIA BIODIVERSITY IN THE RHIZOSPHERE OF SELECTED PLANTS SPECIES

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Abstract
The aim of this study is to investigate the biodiversity of bacteria in the rhizophere and non-rhizosphere of cassava, fluted pumpkin, pawpaw and scent leaf plants the objectives are to isolate, enumerate and identify bacteria from the soil samples**.** A total of 40 rhizosphere and non-rhizosphere soil samples each were collected in duplicates from cassava, fluted pumpkin, pawpaw and scent leaf plants. Standard microbiological techniques were used to analysed for bacterial counts in the rhizosphere and non-rhizosphere soil samples and their corresponding rhizosphere effects estimated. The highest mean rhizosphere count, non-rhizosphere count and rhizosphere effect are as follows; cassava, 4.60 x 10^7 cfu/g, 1.78 x 10^8 cfu/g, 2.584; fluted pumpkin, 3.35 x 10^7 cfu/g, 7.35 x 10^7 cfu/g, 0.931; pawpaw, 9.8 x 10^7 cfu/g, 9.00 x 10^7 cfu/g, 7.368 and scent leaf, 5.15 x 10^6 cfu/g, 4.00 x 10^6 cfu/g, 11.182. Bacteria isolated include *Pseudomonas* sp., *Leuconostoc* sp., *Bacillus* sp., *Acinetobacter* sp., *Enterobacter* sp., *Providencia* sp., *Citrobacter* sp., *Klebsiella* sp. and *Escherichia coli*. The study had revealed that there are diverse groups of bacteria in the rhizosphere of the plants which could have contributed to their healthy growth, hence serve as biofertiliser and phyto-stimulator for improved crop yield.

Keywords: Bacteria, Biodiversity, Plant species, Rhizosphere, Soil

Introduction

Cassava (*Manihot esculenta)* is a member of the *Euphorbiaceae*, a perennial herb plant, native to South America (Frediansyah, 2018), and widely cultivated in both tropical and subtropical regions. On a global scale in 2017, cassava cultivation has been grown up to 201 million hectares with Africa accounting for more than 60% of the total. Frediansyah *et al*. (2012) had reported that Nigeria was the largest producer of cassava, followed by Thailand and Indonesia.

Fluted pumpkin (*Telfairia occidentalis*) which is an important staple vegetable grown in Nigeria, is a plant which produces luxuriant edible green leaves that are rich in iron and vitamins. Harvesting of fluted pumpkin takes place 120-150 days after sowing (Oboh *et al.,*2006; Kayode *et al.,*2009). *Telfairia occidentalis* leaf is rich in minerals (such as iron, potassium, sodium, phosphorus, calcium and magnesium), antioxidants, vitamins (such as thiamine, riboflavin, nicotinamide and ascorbic acid, phyto-chemicals such as phenols. The amino acid profile of *T. occidentalis* had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cystine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine (Fasuyi, 2006).
Pawpaw (*Carica papaya*) is a semi-woody, usually single-stemmed plant, widely distributed in tropical and subtropicals (Jimenez *et al*., 2024). The very flesh is deep yellow or orange to salmon. The unripe fruit contains a milky juice in which is present a protein-digesting enzyme known as papain. This juice is

used in the preparation of various remedies for indigestion and in the manufacture of meat tenderizers (The Editors of Encyclopaedia Britannica, 2024). Scent leaf (*Ocimum gratissimum*) is a traditional herb locally known as "Ahuji" (Igbos) or "Daidoya" (Hausas) according to Effraim *et al*. (2004) and is also grown across the tropic of the world. Earlier reports showed that scent leaf possesses a wide range of phytochemicals such as flavonoids, polyphenols and volatile compounds like eugenol, thymol, and geraniol. Its medicinal uses include vasorelaxation, anti-inflammatory, anti-mycotoxigenic and antioxidant activities (Bhavani *et al*., 2019).

Plants do encourage the growth of protective bacteria by producing certain types of root exudates. These materials produced certainly create a distinctive environment called the rhizosphere for soil microorganisms (Willey *et al.,* 2008). According to Ambreen *et al*. (2012), rhizosphere can be defined as the region of soil surrounding plant roots. This is the region of soil surrounding a plant where the biology and chemistry of the soil are influenced by the roots (Nasrin and Rahman, 2007). The rhizosphere is an important 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 promote plant growth and protect plants from pathogen attack by a range of mechanisms such as altering plant hormones, secretion of organic compounds, increasing abiotic stress tolerance in plants, rhizoremediation and enhancing nutrient availability and uptake and disease control (Raaijmakers, 2009; Mendes *et al*., 2013).

Hasan *et al*. (2024) stated that the production of auxin and siderophores, solubilization of phosphate and nitrogen fixation are the most common desirable traits always looked for in plant growth promoting rhizobacteria (PGPR). Seed germination, seedling vigour, plant growth and development as well as nutrition, diseases and productivity of the host plant are some of the profound effects by many rhizosphere - associated microorganisms as shown by an overwhelming number of studies (Mendes *et al*., 2013). Consequent upon the above importance of rhizosphere, the present investigation is carried out to investigate the bacteria biodiversity in the rhizosphere of selected plants species (cassava, fluted pumpkin, pawpaw and scent leaf) collected from Captain Elechi Amadi Polytechnic, Rumuola, Obio-Akpor Local Government Area, Rivers State.

Materials and Methods

The study was carried out in Captain Elechi Amadi Polytechnic, Rumuola, Obio-AkporLocal Government Area, Rivers State. Five rhizosphere soil samples of cassava (*Manihot esculenta*) fluted pumpkin (*Telfairia occidentalis*), pawpaw (*Carica papaya* L.) and scent leaf (*Ocimum gratissimum*) plants were collected by gently uprooting the plants using sterile shovel. The plants were shaken to remove unwanted soil particles. The soil particles adhered to the roots was transferred to already labelled sterile polyethylene bags for each sample. Also, five soil samples adjacent few centimeters (5cm) away from each cassava, fluted pumpkin, pawpaw and scent leaf plant roots was also collected, transferred to already labelled sterile polyethylene bags for each plant and was considered as non-rhizosphere soil, serving as controls. The samples were carried aseptically to the Biology/Microbiology Laboratory 1, Science Laboratory Technology Department Polytechnic, Rumuola and processed within 1 hour of collection.

One gramme (1g) from each soil sample (rhizosphere and non-rhizosphere) was weighed into a test tube containing 9ml of sterile distilled water (10^{-1}), from which a ten-fold serial dilution was carried out by pipetting 1ml into several test tubes containing 9ml of sterile distilled water up to 10^{-5}. From 10^{-5} dilution, an aliquot of 0.1ml was plated onto a freshly prepared Nutrient Agar plates. The plates were inoculated in duplicates employing spread plate method using sterile gas rod. The inoculated plates were incubated at 37^{o}C for 24 hours. Discrete colonies that developed (30-300) were counted and recorded as bacteria and estimated using the formula:

$$\text{cfu/ml} = \frac{\text{Average number of colonies x dilution factor}}{\text{Volume plated}}$$

The quantitative rhizosphere effect of the plants was calculated using the formula according to Subabarao (2000):

$$\text{R/S} = \frac{\text{Number of microorganism per gramme of rhizosphere soil}}{\text{Number of microorganism in a gramme of non-rhizosphere soil}}$$

Discrete colonies were then picked and subcultured onto Nutrient Agar plates using streak plate method. Stock cultures were prepared on sterile Nutrient Agar in Bijou bottles, coded for ease of identification and stored in the refrigerator (4°C) until needed for further tests such as biochemical test. The isolates were identified on the basis of their cultural, morphological and physiological characteristics in accordance with schemes and methods described by Ogbulie *et al*. (2001) and Cheesborough (2006). Microscopic examination of isolates was carried out using oil immersion objectives (x100). The following tests were carried out: Gram staining and Biochemical tests which were catalase, coagulase, citrate utilisation, indole, methyl red, Voges Proskauer and Hydrogen Sulphide.

Results

The results of the bacterial counts obtained from cassava rhizosphere soil, non-rhizosphere soil and rhizosphere effects are presented in Table 1. The rhizosphere bacterial counts are in the following order: 4.6 x 10^7 cfu/g (Sample D) > 3.0 x 10^7cfulg (Sample A) > 1.75 x 10^7cfu/g (Sample C) > 1.15x10^7cfu/g (Sample B) > 1.6 x 10^7cfu/g (Sample E). The non-rhizosphere bacterial count is in the following order: 7.0 x10^6 cfu/g (Sample B) > 6.3 x 10^7cfu/g (Sample C) > 3.65 x 10^7 cfu/g (Sample A) > 1.78 x 10^8 cfu/g (Sample D) > 1.40 x 10^8 cfu/g (Sample E). The rhizosphere effects values are in the following order: 2.584 (Sample D) >1.142 (Sample E) > 0.822 (Sample A)> 0.278 (Sample C) >0.164 (Sample B).

Table 1 Bacterial Counts of Cassava Rhizosphere Soil, Non-Rhizosphere Soil and Rhizosphere Effect

Sample	Bacterial Counts (cfu/g)		Rhizosphere Effects
	Rhizosphere	Non- Rhizosphere	
A	3.0 x10^7	3.65 x 10^7	0.822
B.	1.15 x10^7	7.0 x 10^6	0.164
C	1.75 x 10^7	6.3 x 10^7	0.278
D	4.6 x 10^7	1.78 x 10^8	2.584
E	1.6 x 10^7	1.40 x 10^8	1.142

Key: cfu/g Colony forming unit per gramme

The bacterial counts of fluted pumpkin rhizosphere soil, non-rhizosphere soil and rhizosphere effect are represented in Table 2. The bacterial counts of fluted pumpkin rhizosphere are in the following order: 3.35 x 10^7 cfu/g (Sample D) > 2.3 x 10^7 cfu/g (Sample A) > 2.0 x 10^7 cfu/g (Sample E) > 1.2 x 10^7 cfu/g (Sample B) > 1.0 x 10^7 cfu/g (Sample C). The bacterial counts of non-rhizospere are in the following order: 7.35 x 10^7 cfu/g (Sample A) > 4.9 x 10^7 cfu/g (Sample C) > 3.7 x 10^7 cfu/g (Sample D) > 3.6 x 10^7 cfu/g (Sample B) > 2.2 x 10^7 cfu/g (Sample E). The rhizospere effect values are in the following order: 9.318 (Sample E) > 9.054 (Sample D) > 3.333 (Sample B) > 3.129 (Sample A) > 2.041 (Sample C).

Table 2 Bacterial Counts of Fluted Pumpkin Rhizosphere Soil, Non-Rhizosphere Soil and Rhizosphere Effect

Sample	Bacterial Counts (cfu/g)		Rhizosphere Effect
	Rhizosphere	Non- Rhizosphere	
A	2.3×10^7	7.35×10^6	3.129
B.	1.2×10^7	3.6×10^7	3.333
C	1.0×10^7	4.95×10^7	2.041
D	3.35×10^6	3.7×10^7	9.054
E	2.05×10^6	2.2×10^7	9.318

Key: cfu/g Colony forming unit per gramme

The results of the bacterial counts obtained from pawpaw rhizosphere soil, non-rhizosphere soil and rhizosphere effects are presented in Table 3. The rhizosphere bacterial count is in the following order: 5×10^6 cfu/g (Sample E) < 1.5×10^6 cfu/g (Sample D) < 4.5×10^7cfu/g (Sample A) < 5.5×10^6 cfu/g (Sample B) < 9.8×10^7 cfu/g (Sample C). The non-rhizosphere bacterial count is in the following order: 5×10^6 cfu/g (Sample D) < 8×10^6 cfu/g (Sample E) < 9×10^7 (Sample A) < 1.11×10^7 cfu/g (Sample B) < 1.33×10^7 cfu/g (Sample C). The rhizosphere effects are in the following order: 0.3 (Sample D) < 0.48 (Sample A) < 0.58 (Sample B) < 6.25 (Sample E) < 7.35 (Sample C).

Table 3 Bacterial counts obtained from Pawpaw Rhizosphere soil, Non-Rhizosphere Soil and Rhizosphere Effects

Sample	Bacterial Counts (cfu/g)		Rhizosphere Effects
	Rhizosphere	Non- Rhizosphere	
A	4.3×10^7	9×10^7	0.48
B.	5.5×10^6	1.11×10^7	0.58
C	9.8×10^7	1.33×10^7	7.35
D	1.5×10^6	5×10^6	0.3
E	5×10^6	8×10^5	6.25

Key: cfu/g Colony forming unit per gramme

The bacterial counts obtained from scent leaf (*Ocimum gratissimum*) rhizosphere soil, non-rhizosphere soil and rhizosphere effect are represented in Table 4. The bacterial counts of scent leaf (*Ocimum gratissimum)* rhizosphere is in the following order: 1.95×10^6 cfu/g (Sample B) < 2.25×10^6 cfu/g (Sample E) < 2.5×10^6 cfu/g (Sample A) < 2.6×10^6 cfu/g (Sample D) < 5.15×10^6 cfu/g (Sample C). The bacterial counts of non-rhizosphere soil is in the following order: 1.65×10^5 cfu/g (Sample B) < 3.05×10^5 cfu/g (Sample E) < 3.55×10^5 cfu/g (Sample D)< 4.0×10^6 cfu/g (Sample C) < 9.5x105 cfu/g (Sample A). The rhizospere effect values are in the following order: 0.732 (Sample D) < 1.182 (Sample B) < 1.288 (Sample C) < 1.8 (Sample E) < 2.632 (Sample A).

Table 4 Bacterial counts obtained from Scent Leaf Rhizosphere soil, Non-Rhizosphere Soil and Rhizosphere Effect

Sample	Bacterial Counts (cfu/g)		Rhizosphere Effect
	Rhizosphere	Non- Rhizosphere	
A	2.5×10^6	9.5×10^5	2.632
B.	1.95×10^5	1.65×10^5	1.182
C	5.15×10^5	4.0×10^6	1.288
D	2.6×10^6	3.55×10^5	0.732
E	2.25×10^5	3.05×10^5	1.8

Key: cfu/g Colony forming unit per gramme

The characterisation and identification of bacteria isolated from the cassava, fluted pumpkin, pawpaw and scent leaf rhizosphere reveals *Pseudomonas* sp., *Leuconostoc* sp., *Bacillus* sp., *Acinetobacter* sp., *Enterobacter* sp., *Providencia* sp., *Citrobacter* sp., *Klebsiella* sp. and *Escherichia coli*.

Discussion

The present study investigated the biodiversity of bacteria in the rhizophere and non-rhizosphere of cassava, fluted pumpkin, pawpaw and scent leaf plants. The study shows that the non-rhizosphere soils have higher bacterial counts than the rhizosphere soils of cassava and fluted pumpkin. Arotupin and Akinyosoye (2008) had earlier reported lower bacterial counts for cassava rhizosphere soil and non-rhizosphere soil respectively. Lesser ranges of bacterial population were obtained in the rhizosphere soil comparably due to selective inhibition by the exudate in the rhizosphere.

Pawpaw and scent leaf recorded high bacteria counts in their rhizosphere soil, which may be due to the adequate nutritional status of the soils which are available to the microorganisms; in addition, are the favourable physical chemical properties of the soils (Arotupin and Akinyosoye, 2006). The nutritional versatility of bacteria may also be responsible for their ecological sources within the study plants rhizosphere. Generally, the high bacteria counts obtained from the study plants rhizosphere may be due to availability and accessibility of soil nutrients to the micro-organism (Arotupin and Akinyosoye, 2008). Green *et al*. (2006) also stated that bacteria are specially concentrated in the rhizosphere, a narrow region next to the root exudates which actually encourages the growth of protective bacteria.

The differences in bacteria counts in the rhizosphere and non-rhizosphere soils obtained in this study could have been due to difference in plant age in the fields and pH of the soils (Musyimi *et al*., 2018). Plant age increases microbial activity and which declines towards maturity probably due to the plant secreting exudates in reduced quality and quantity that may contain antimicrobial metabolites (Makut and Owolewa, 2011). Furthermore, according to Alexopoulos *et al*. (2002), the disparity in the rhizosphere and non-rhizosphere soil microbial population could be due to the study plants roots releasing exudates containing different organic and inorganic compounds that stimulated development of active microbial population.

Rhizosphere effect obtained from this study indicates that they were generally high between the soils and the study plants. Tamilarasi *et al*. (2008) reported a rhizospere effect of 1.22×10^4 for *Ocimum basilium*. The greater the rhizosphere effect the higher will be microorganism number. The rhizosphere effect greater decrease as we move away from the root (Curl and Love, 1986). According to Grayston and Campbell (1996), the varying types and quantities of rhizodeposits have been postulated to act as key factors influencing the density and diversity of the rhizosphere microorganisms.

The bacteria isolated and identified from the cassava, fluted pumpkin, pawpaw and scent leaf rhizosphere are *Pseudomonas* sp., *Leuconostoc* sp., *Bacillus* sp., *Acinetobacter* sp., *Enterobacter* sp., *Providencia* sp., *Citrobacter* sp., *Klebsiella* sp. and *Escherichia coli*. Gupta and Kaushal (2017) and Pandey *et al*. (2018)

have reported the isolation of *Pseudomonas* and *Kiebsiella* from the rhizosphere of solanaceous vegetable crops. Tamilarasi *et al.* (2008) and Obire and Abuba (2010) isolated *Pseudomonas*, *Enterobacter* and *Bacillus* from *Ocimum basilium*. *Pseudomonas* was occurring in all the rhizosphere while *Bacillus* occurred in the rhizosphere of scent leaf, cassava and fluted pumpkin. Differences in environmental variables such as pH and temperature might have influenced the variations in microbes (Musyimi *et al*., 2018). Also, different plant species host specific microbial communities (Alwathnani *et al*., 2012); the diversity and composition of the microbial taxa in the rhizosphere can be affected by plant species (Cappuccono and Sherma, 2008).

Conclusion and Recommendations

The study has confirmed that there is high population of bacteria in the rhizosphere soil samples of the study plants. The findings from the study also shows that bacteria were found in the rhizosphere of cassava, fluted pumpkin, pawpaw and scent leaf, confirming the plants' ability to host numerous and diverse microbes. The rhizosphere microbial community presence could be manipulated for improving the growth and health of the study plants.

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