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Soil Rhizosphere Microbial Properties of Selected Farmlands in Rumuokparali Community

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Authors' contributions

This work was carried out in collaboration between all authors. Author HOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JA and CJU managed the analyses of the study. Author JA managed the literature searches. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

This study was conducted to determine the rhizosphere soil microorganisms associated with the cultivation of *Manihot esculentum*, *Comelina bengalensis*, *Talinum triangulare* and *Telfairia occidentalis*. The plants were obtained from newly cultivated, ready for harvest and fallowed farmlands. The rhizosphere microorganisms were enumerated and compared with bulk soil microorganisms. The heterotrophic bacterial count for newly cultivated farmland ranged from 2.9 $\times 10^7$ -6.3 $\times 10^8$ cfug⁻¹ and total fungal ranged from 5.6 $\times 10^4$ -7.0 $\times 10^6$ sfug⁻¹, while the bulk soil total heterotrophic bacterial count for ready for harvest farmland ranged from 1.82 $\times 10^8$ -1.80 $\times 10^9$ cfug⁻¹ and total fungal ranged from 2.3 $\times 10^4$ -3.57 $\times 10^6$ sfug⁻¹, while the bulk soil total heterotrophic bacterial count for ready for harvest farmland ranged from 1.82 $\times 10^8$ -1.80 $\times 10^9$ cfug⁻¹ and total fungal ranged from 2.3 $\times 10^4$ -3.57 $\times 10^6$ sfug⁻¹, while the bulk soil total heterotrophic bacterial count for fallowed farmland ranged from 5.65 $\times 10^8$ -1.50 $\times 10^9$ cfug⁻¹ and total fungal ranged from 1.33 $\times 10^6$ -3.05 $\times 10^6$ sfug⁻¹, while bulk soil total heterotrophic bacterial count for fallowed farmland ranged from 5.65 $\times 10^8$ -1.50 $\times 10^9$ cfug⁻¹ and total fungal ranged from 1.33 $\times 10^6$ -3.05 $\times 10^6$ sfug⁻¹. The rhizosphere soil bacterial count was 1.74.0 $\times 10^9$ cfug⁻¹ and total fungal count was 1.07 $\times 10^6$ sfug⁻¹. The rhizosphere soil bacterial isolates belong to the genera: *Staphylococcus*, *Hafnia*, *Acinetobacter*, *Bacillus*, *Bacteroides*, *Klebsiella*, *Tatumella*, *Enterobacter*, *Corynebacterium* and *Pseudomonas* while fungal isolates belong to the

genera: Aspergillus, Epicocum, Chrysosporium, Trichosporon, Cryptococcus, Fusarium, Penicillium and Chaetomium. The bulk soil bacterial isolates belong to genera: Staphylococcus, Hafnia, Acinetobacter, Bacillus, Klebsiella, Tatumella, Corynebacterium and Pseudomonas while the fungal isolates belong to genera: Aspergillus, Epicocum, Chrysosporium, Trichosporon, Cryptococcus and Chaetomium. Microbial diversity of rhizosphere soil was more compared to bulk soil.

Keywords: Rhizosphere; bulk soil; microorganisms; farmlands.

1. INTRODUCTION

The rhizosphere is a narrow region of soil that is at direct proximity to plants roots and is influenced by root secretions and actions of associated soil microorganisms [1]. The actual extent of rhizosphere is dependent on the influence zone of the plant's roots and associated microorganisms. Soil which is not part of rhizosphere is known as bulk soil. The rhizosphere is generally considered to be the most biodiverse and dynamic habitat on earth and also, a metabolically busier and more competitive environment than surrounding bulk soil [2]. Unlike the soil associated with rhizosphere, the bulk soil is not penetrated by plant roots, and generally has lower microbial communities [3].

In rhizosphere, symbolic relationships exist between plant roots and microorganisms. Different water soluble nutritive compounds containing sugars and amino acids are exuded by plant roots which sustain and provide for microbial existence. Plants roots have strong impacts on physical and chemical environment in rhizosphere, such that a shift in this environment, which is influenced by manipulations of plant's roots, significantly impacts on the availability and forms of substances used for microbial metabolism, which in turn strongly affect the prevalence and diversity of different microbial species and functions, as well as their total biomass within the rhizosphere [1]. The microbial population in rhizosphere carries out processes, such as the transformation of carbon, nitrogen and other minerals, to plant benefit [4]. In addition to this, soil bacterial species produce biofilms which act as an aggregating mechanism for mineral particles and organic matter [5]. Also, mycorrhizal fungi have been shown to increase aggregation through the physical construction of fungal hyphae, as well through the secretion of their exopolymer deposit.

The rhizosphere is an environment that allows a diverse range of soil microorganisms to thrive.

This study aimed to determine both quantitatively and qualitatively, the rhizosphere soil microorganisms associated with certain plants from different farmlands.

2. MATERIALS AND METHODS

2.1 Sample Collection

Rhizosphere soil samples were collected from farmlands at Rumuokparali community in Obio Akpor Local Government Area of Rivers State. Nigeria. The rhizosphere soils were obtained from the plant roots, ranging from 0-10 cm depth. The control for each farmland was obtained from surface soil between 0-1 cm thickness. Using the composite sampling technique, rhizosphere soil samples were aseptically obtained with a sterile spatula from Manihot esculentum, Talinum triangulare. Telfairia occidentalis and Comelina bengalensis from newly cultivated and ready for harvest farmland. Manihot esculentum, Talinum triangulare and Comelina bengalensis rhizosphere soil samples were aseptically obtained from fallowed farmland. The bulk soils from farmlands, which served as controls, were collected using a composite sampling technique, with aid of a quadrate.

2.2 Microbiological Analysis

Soil samples were serially diluted to 10⁻⁵ dilutions. These dilutions were used to carry out soil bacteria and fungi isolation and enumeration from the soil.

2.3 Enumeration of Total Heterotrophic Bacteria Count (THBC)

This test was done to screen for total viable aerobic, mesophilic and heterotrophic bacteria present in each soil sample. The rhizosphere soil samples were serially diluted to 10^{-5} dilution using physiological saline. Aliquots 0.1ml from 10^{-3} , 10^{-4} and 10^{-5} dilutions were spread aseptically with a sterile glass rod on freshly

prepared dry nutrient agar plates. The plates were incubated at 37°C for 24 to 48 hours. After incubation, the THBC for each sample was recorded and expressed in colony forming unit per gramme (cfug⁻¹) after colony count.

2.4 Enumeration of Total Fungal Count (TFC)

This test was carried out to enumerate total heterotrophic fungal species contained in effluent samples. Aliquots of 0.1 ml from 10^{-1} , 10^{-2} and 10^{-3} dilutions were spread aseptically with a sterile glass rod on freshly prepared dry potato dextrose agar plates and incubated at 28° C for 3-5 days. After incubation, the TFC for each sample was recorded and expressed as spore-forming unit per gram (sfug⁻¹) after colony count.

2.5 Purification and Characterisation

After incubation, distinct colonies observed on both nutrient agar plates and potato dextrose agar plates were isolated and purified by repeated sub-culturing. The bacterial isolates were characterised using their macroscopic, microscopic and biochemical attributes. The biochemical tests performed on bacterial isolates were: catalase, oxidase, motility, hydrogen sulphide production (H_2S), indole, Voges-Proskauer (VP), Methyl red (MR), citrate, and sugar fermentation. The fungal isolates were characterised on basis of their macroscopic and microscopic appearance with reference to the standard manual.

2.6 Physicochemical Analysis

The physicochemical parameters monitored for soil samples were temperature, pH, moisture content, total organic carbon and total nitrogen. The physicochemical parameters were assessed according to methods as described by American Public Health Association [6].

3. RESULTS

The results for the total heterotrophic bacterial count (THBC) and total fungal count (TFC) are shown in Table 1. The rhizophere THBC ranged from 2.9×10^7 - 1.80×10^9 cfug⁻¹ while rhizophere TFC ranged from 2.3 $\times 10^4$ - 7.0×10^6 sfug⁻¹. The bulk soil THBC ranged from 1.74- $4.96.0 \times 10^9$ cfug⁻¹, while bulk soil TFC ranged from 1.07 $\times 10^6$ - $5.87.0 \times 10^6$ sfug⁻¹.

Table 2 shows bacterial isolates from rhizophere soil. The bacterial isolates belong to genera: Staphylococcus, Hafnia, Acinetobacter, Bacillus, Bacteroides. Klebsiella. Tatumella, Corynebacterium, Pseudomonas and were Enterobacter. The fungal isolates characterized to belong to genera: Aspergillus, Epicocum, Chrysosporium, Trichosporon, Cryptococcus, Chaetomium, Microsporium, Penicillium and Fusarium (Table 3).

S/N	Soil sample	Bacterial count (cfug ⁻¹)	Fungal count (sfug ⁻¹)
1.	NME	3.1 x10 ⁸	1.91 ×10 ⁶
2.	NCB	1.55 x10 ⁸	7.0 x10 ⁶
3.	NTT	6.3 x10 ⁸	5.6 x10⁴
4.	NTO	2.9 x10 ⁷	1.6 x10 ⁶
5. 6.	RME	18.2 x10 ⁸	3.57 ×10 ⁶
6.	RCB	180 x10 ⁹	2.0 x10 ⁶
7.	RTT	9.0 ×10 ⁸	2.3 x10 ⁴
8.	RTO	6.2 x10 ⁸	3.32 x10 ⁶
8. 9.	FME	1.5 x10 ⁹	1.83 x10 ⁶
10.	FCB	6.55 x10 ⁸	3.05 ×10 ⁶
11.	FTT	5.65 x10 ⁸	1.33 x10 ⁶
12.	NC	4.96 x10 ⁹	5.87 x10 ⁶
13.	RC	1.9 x10 ⁹	2.30 x10 ⁶
14.	FC	1.74 x10 ⁹	1.07 x10 ⁶

 Keys: NME - Newly Cultivated Manihot esculentum; NCB - Newly Cultivated Comelina bengalensis; NTT - Newly Cultivated Talinum triangulare; NTO - Newly Cultivated Telfairia occidentalis;
RME - Ready for Harvest Manihot esculentum; RCB - Ready for Harvest Comelina bengalensis;
RTT - Ready for Harvest Talinum triangulare; RTO - Ready for Harvest Telfairia occidentalis;
FME - Fallowed Manihot esculentum; FCB - Fallowed Comelina bengalensis; FTT - Fallowed Talinum triangulare; NC - Nearly Cultivated Control; RC - Ready for Harvest Control; FC - Fallowed Control

S/N	Soil sample	Bacterial isolates			
1.	NME	Staphylococcus sp, Hafnia sp, Acinetobacter sp, Bacillus sp,			
		Bacteroides sp			
2.	NCB	Staphylococcus sp, Hafnia sp, Corynebacterium sp, Klebsiella sp,			
		Bacillus sp			
3.	NTT	Klebsiella sp, Bacillus sp, Corynebacterium sp			
4.	NTO	Bacillus sp, Enterobacter sp, Tatumella sp			
5.	RME	Klebsiella sp, Hafnia sp, Acinetobacter sp, Tatumella Sp, Bacteroides			
		sp, Bacillus sp			
6.	RCB	Klebsiella sp, Tatumella Sp, Acinetobacter sp, Bacillus sp			
7.	RTT	Bacillus sp, Hafnia sp			
8.	RTO	Bacillus sp, Enterobacter sp, Tatumella sp, Klebsiella sp, Hafnia sp,			
		Acinetobacter sp			
9.	FME	Staphylococcus sp Bacillus sp, Corynebacterium sp, Hafnia sp,			
		<i>Tatumella</i> sp, <i>Klebsiella</i> sp			
10.	FCB	Pseudomonas sp, Klebsiella sp, Hafnia sp, Acinetobacter sp, Tatumell			
		Sp, <i>Bacillus</i> sp			
11.	FTT	<i>Bacillus</i> sp, <i>Hafnia</i> sp, <i>Tatumella</i> sp, <i>Klebsiella</i> sp			
12.	NC	Bacillus sp, Acinetobacter sp, Staphylococcus sp Tatumella Sp			
13.	RC	Bacillus sp, Acinetobacter sp, Staphylococcus sp Tatumella Sp,			
		<i>Klebsiella</i> sp			
14.	FC	Bacillus sp, Acinetobacter sp, Staphylococcus sp Klebsiella sp,			
		Pseudomonas sp, Corynebacterium sp, Tatumella sp			
		Keys: Idem			

Table 2. Bacterial isolates obtained from different soil samples

Keys: Idem

Table 3. Fungal isolates obtained from different soil samples

S/N	Soil sample	Fungal isolates
1.	NME	Aspergillus sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp
2.	NCB	Trichosporon sp, Cryptococcus sp, Aspergillus sp
3.	NTT	Aspergillus sp, Epicocum sp, Trichosporon sp, Chaetomium sp
4.	NTO	Aspergillus sp, Chrysosporium sp Chaetomium sp, Trichosporon sp, Cryptococcus sp, Epicocum sp
5.	RME	Aspergillus sp, Trichosporon sp, Epicocum sp, Chrysosporium sp, Cryptococcus sp, Chaetomium sp
6.	RCB	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp
7.	RTT	Aspergillus sp, Epicocum sp, Trichosporon sp, Chaetomium sp
8.	RTO	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp
9.	FME	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp
10.	FCB	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp Penicillium sp
11.	FTT	Aspergillus sp, Epicocum sp, Chaetomium sp, Trichosporon sp, Cryptococcus sp
12.	NC	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp
13.	RC	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp
14.	FC	Aspergillus sp, Microsporium sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus sp, Chaetomium sp

Keys: Idem

Table 4 shows the physicochemical parameters of farmlands. The rhizosphere soils from Manihot esculentum for the three farmlands had their temperature as 27°C, while pH ranged from 5.265-6.190; moisture content, 10.8-12.9%; total carbon content, 0.927-1.614 mgL⁻¹ and total nitrogen content, 1.40-1.68 mgL^{-1} . The rhizosphere soils from Comelina bengalensis for the three farmlands had their temperature values as 27°C, while their pH values ranged from 5.901-6.682; moisture content, 41.0-43.6%, total carbon content, 12.232-17.001 mgL $^{-1}$; nitrogen content, 4.321-4.968 mgL $^{-1}$. total The rhizosphere soils from Talinum triangulare for the three farmlands had their temperature values as 27°C, while their pH values ranged from 4.987-6.230; moisture content, 16.29-33.20%, total carbon content, 8.002-12.280 mgL⁻¹; nitrogen content, 3.200-5.212 mgL⁻¹; rhizosphere soils from T from T total The rhizosphere soils from Telfairia occidentalis for the three farmlands had their temperature values as 27°C, while their pH values ranged from 4.890-6.009; moisture content, 28.2-32.0%, total carbon content, 3.210-7.980 mgL⁻¹; total nitrogen content, 5.101-5.210 mgL⁻¹. The bulk soil samples from the three farmlands had their temperature values as 27°C, while their pH values ranged from 6.628-6.983; moisture content, 6.69-7.21%, total carbon content, 10.01-11.29 mgL⁻¹; total nitrogen content, 11.68-12.90 mgL^{-1} .

4. DISCUSSION

This study was conducted to determine, both quantitatively and qualitatively, the diversity of rhizosphere soil microorganisms associated with

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Manihot esculentum, Comelina bengalensis, Talinum triangulare and Telfairia occidentalis obtained from newly cultivated, ready for harvest and fallowed farmlands. The heterotrophic bacterial count obtained from different rhizosphere soils for different plant species in ready for harvest farmland ranged from 1.82 $x10^8$ -1.80 $x10^9$ cfug⁻¹ and total fungal count ranged from 2.3 $x10^4$ -3.57 $x10^6$ sfug⁻¹, while bulk soil heterotrophic bacterial count was 1.9 x10⁹ and fungal count was 2.3x10⁶ sfug⁻¹. The ready to harvest soil had more bacterial load than the bulk soil which had a more fungal load. For fallowed farmland, heterotrophic bacteria count ranged from 5.65x10⁸-1.50x10⁹ cfug⁻¹ and total fungi ranged from 1.33-3.05x10⁶ sfug-1, while heterotrophic bacteria count for bulk soil sample was 1.74x10⁹cfug⁻¹ and total fungi count was 1.07x10⁶ sfug⁻¹. Just like in ready to harvest soil, followed farmland had more bacteria load than the bulk soil which had the more fungal load.

The microbial counts obtained from different rhizosphere soils of the different plant species at newly cultivated farmland ranged from 2.9×10^{7} - 6.3×10^{8} cfug⁻¹ for heterotrophic bacteria and 5.6 $\times 10^{4}$ -7.0 $\times 10^{6}$ sfug⁻¹ for the total fungi, while bulk soil had a heterotrophic bacterial count of 4.96 $\times 10^{9}$ cfug⁻¹ and total fungal count 5.87 $\times 10^{6}$ sfug⁻¹. Soil-root system affects soil microbial population [1]. The heterotrophic bacterial count range was lower compared to other farmlands. The lower heterotrophic bacterial content of rhizosphere soil could be as a result of regulatory mechanisms of the crop root system to maximise environmental properties to enhance their well being.

S/N	Soil sample	Temperature (℃)	рН	Moisture content (%)	Total carbon content (mgkg ⁻¹)	Total nitrogen content (%)		
1.	NME	27	5.265	12.9	0.927	1.40		
2.	RME	27	6.190	12.6	1.080	1.68		
3.	FME	27	5.695	10.8	1.614	1.555		
4.	NCB	27	6.682	43.6	12.232	4.968		
5.	RCB	27	6.002	43.2	14.621	4.712		
6.	FCB	27	5.908	41.0	17.001	4.321		
7.	NTT	27	4.987	33.20	12.280	5.212		
8.	RTT	27	6.230	16.29	8.002	3.200		
9.	FTT	27	5.760	21.30	10.621	5.111		
10.	NTO	27	4.890	32.0	3.210	5.210		
11.	RTO	27	6.009	28.2	7.980	5.101		
12.	NC	27	6.983	7.21	10.82	12.92		
13.	RC	27	6.801	7.20	10.01	11.68		
14.	FC	27	6.628	6.69	11.29	11.98		
	Keys: Idem							

Table 4. Physicochemical properties of farmland soil samples

The newly cultivated bulk soil had the highest bacterial load compared to rhizosphere soils. This might be due to the organic load present in the soil before cultivation, rather than to microorganisms-plant roots interaction. Plantderived compounds provide nutrients to a large variety of soil microorganisms [1]. The soil around Manihot esculentum at ready to harvest farmland had the highest bacterial load (1.8x10⁹ cfug⁻¹) among the rhizosphere soils. Manihot esculentum is a root crop with several branches, having a long duration for maturity before harvest. It follows that root exudates would have provided material for the proliferation of associated bacteria. The soil around Comelina bengalensis in newly cultivated farmland had the highest fungal load (7.0x10⁶ sfug⁻¹). The high fungal load from Comelina bengalensis a flowering plant and a common farmland weed in the area of study may be due to the plant material nature under decomposition within the soil vicinity.

Bacteria were the most dominant in soil given their population and diversity. The rhizosphere bacteria differ with the plant species. The bacteria isolated from the different rhizosphere soils belong to ten genera namely: Staphylococcus, Hafnia, Acinetobacter, Bacillus, Bacteroides. Klebsiella. Tatumella. Corynebacterium, Pseudomonas and Enterobacter. Seven bacterial isolates were obtained bulk which at soils were Staphylococcus sp, Acinetobacter sp, Bacillus sp, Klebsiella sp, Tatumella sp, Corynebacterium sp and Pseudomonas sp. The most common rhizosphere bacteria genera are Pseudomonas, Bacillus, Arthrobacter, Rhizobia, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas and Micrococcus [7]. Panaiyadiyan and Chellaia [8] reported Acinetobacter sp, Klebsiella sp, Staphylococcus sp, Bacillus sp and Pseudomonas sp among the 18 bacterial species isolated from rhizosphere soil. Bacillus sp and Pseudomonas sp are generally found in most rhizosphere soil, with their composition dependent on plant species [9]. In this study, Bacillus sp was dominant and present in both rhizosphere and bulk soil. Bacillus sp had been reported as most numerous rhizobacteria associated with rice and wheat [10].

The fungal rhizosphere isolates at bulk soil belong to genera *Aspergillus, Epicocum, Chrysosporium, Trichosporon, Cryptococcus, Chaetomium, Microsporium, Penicillium* and *Fusarium.* There were nine fungal isolates in total. Fungi are more tolerable to acidic soil. The rhizosphere soil had more fungal diversity, with all the fungal genera represented. The bulk soil had Microsporium sp, Aspergillus sp, Epicocum sp, Chrysosporium sp, Trichosporon sp, Cryptococcus Chaetomium sp, sp, with Penicillium and *Fusarium* not detected. Aspergillus sp was a dominant fungal isolate. Panaiyadiyan and Chellaia [8] similarly isolated Aspergillus sp from rhizosphere soil, which they described as one of the most important soil fungi because of their benefits to plants.

The results of physicochemical analyses of the samples showed variations. The pH values were generally mildly acidic. Soil pH affects soil and extends to chemistrv virtuallv all characteristic of soil that is affected by multiple interactions within the rhizosphere. Respiration by roots macro and microorganisms releases carbon dioxide which generates bicarbonate/carbonic acid which impact on soil acidity. The increased microbial activity also increases microbial transformations of chemical species to nitrate acid and sulphuric acid production [11]. The soil contributed to soil microbial population and diversity. According to Kent and Triplett [5], soil pH affects the nutrients availability and influence the abundance and diversity of associated microorganism. The soil temperature (27°C) supported the mesophilic microorganism present at the soil. The soil moisture content which ranged from 16.29-33.20% was adequate for both bacteria and fungi to thrive.

5. CONCLUSION

It has been established that rhizosphere soil microbes play various roles in the development of plants, and these roles confer additional benefits to plants. Several bacteria and fungi species are closely associated with plant roots, which together with soil environment influence microbial population and diversity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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