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Microbial Occurrence and Distribution at Different Rhizosphere Depths of *Musa sapientum var parasidiaca* and *Senna occidentalis*

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Authors' contributions

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

Research Article

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ABSTRACT

In this study, we focussed on the isolation, enumeration, distribution and occurrence of rhizomicroflora of *Musa sapientum* var parasidiaca and *Senna occidentalis*. The population, occurrence and distribution of culturable bacteria, fungi and actinomycetes in 5, 10, 15, and 20 cm depths rhizosphere samples of *Musa sapientum* var parasidiaca and *Senna occidentalis* growing in the botanical garden of the University of Lagos, Akoka, Nigeria, were investigated using standard plate count and biochemical techniques. Bacteria were the most predominant in the rhizosphere of both plants, followed by fungi, then actinomycetes. The culturable microbial population was at its maximum for depths 10 and 15 cm in *M. sapientum* var parasidiaca. In *S. occidentalis*, bacterial population was highest at 5cm, fungi at 10cm and actinomycetes at 15cm depth of the rhizosphere. *Bacillus cereus* had 100% distribution in the rhizosphere of both plants and *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* each had 75% distribution in both

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rhizospheres. Rhizosphere depth of 10 cm had 100% distribution of bacteria, and the least bacterial distribution was found at 20cm. Fungi were most distributed at 15cm rhizosphere of *M. sapientum* var parasidiaca and at 10 and 15 cm rhizosphere of *S. occidentalis*. *Rhizopus stolonifer* had 100% distribution and the highest % occurrence in the rhizosphere of both plants with *Aspergillus niger* having 100 and 75% distribution in the rhizosphere of *M. sapientum* and *S. occidentalis* respectively. Actinomycetes were most distributed at 10 cm (60 and 80% in rhizosphere of *M. sapientum* and *S. occidentalis* respectively). *Streptomyces* sp had the highest distribution in the rhizosphere of both plants and 58.33 and 55.17% occurrences in rhizosphere of *M. sapientum* and *S. occidentalis* respectively. *Streptomyces alanosinicus* and *S. gancidicus* were absent among the rhizosphere isolates of *M. sapientum*. Similarly, *S. globosus* and *S. sampsonii* were not found in the rhizosphere of *S. occidentalis*. The abundance of the microorganisms in these rhizospheres is typical of an environment with high species richness and functional diversity.

Keywords: Musa sapientum var parasidiaca; Senna occidentalis; rhizosphere; microbial occurrence; microbial distribution.

1. INTRODUCTION

Although rhizomicroflora of many plants and their roles have been described in numerous reports, little has been reported on rhizomicroflora of tropical plants in West Africa. Also, few reports on distribution and occurrence relative to rhizospheric depths are available. It is known that the diversity and composition of microbial taxa in the rhizosphere can be affected by several factors including plant species, soil type [1], soil management practices [2], microbial interactions [3], soil organic matter (4) and other environmental variables, among which rhizosphere depth may be significant. Rhizosphere microorganisms are indispensable to plant health and development and soil microorganisms, in general, are significant determinants of soil agricultural quality [4]. Observations have shown that the concentration of microorganisms found in rhizosphere of plants is generally much greater than in the bulk soil and that the rhizosphere supports higher microbial growth rates and activities as compared to the bulk soil [5,6]. One of the main reasons for this higher growth rate is the increased availability of soluble organic compounds that results from plant root exudation. Plant roots exude substantial quantities of low molecular weight organic compounds, typically carbohydrate monomers, amino acids, sugars, and organic acids, but the composition and quantity of root exudates varies depending on plant species [7] and abiotic conditions such as water content and temperature [6]. In turn, rhizosphere microorganisms increase root exudation through production of plant hormones or more directly by physically damaging the roots [8]. The composition of microbial community in the rhizosphere is important for the performances of the plant, as microbes can have beneficial, neutral or harmful relationships with the roots [9,10,11,12].

Musa sapientum var parasidiaca is a perennial monocotyledonous large shrub from the banana family; Musaceae. It is usually tree – like in appearance and widely distributed in the tropics. It is significant as a food and medicinal plant in most West African countries. *Senna occidentallis* (L) Link is a dicotyledonous perennial shrub of the family Fabaceae. At maturity, it grows to 5 – 8 m tall. It is cosmopolitan in distribution and is more common in the tropical areas. It is cultivated for ornamental purposes as well as medicine in the treatment of some diseases such as malaria in many West African countries. These two plants are widely distributed in West Africa. This present study, therefore, focused on the distribution and

occurrence of rhizomicroflora of *M. sapientum* var parasidiaca and *S. occidentalis* relative to rhizospheric depths as a first – step toward screening for antimicrobial activity

2. MATERIALS AND METHODS

Using the method of [13], 100g soil samples were collected, with the aid of sterile soil corers, at 5, 10, 15, 20 cm depths of rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* growing in the botanical garden of the University of Lagos. Rhizosphere soil samples were collected from three different plants each of *M. sapientum* var parasidiaca and *S. occidentalis.* Each sample was collected into sterile universal bottles and stored in the refrigerator at 4ºC for less than 48 h before use.

2.1 Isolation of Microorganisms

The soil samples were sun dried for 8 h after which 1 g of each soil sample was added to 100 ml distilled water, carefully shaken on a rotatory shaker (200 rev/min) for 30 min. This soil suspension was then serially diluted to the 10^{-5} dilution, from which 0.1 ml was separately added to the surface of nutrient agar (NA), Saboraud dextrose agar (SDA) (supplemented with 50 mg/L penicillin, streptomycin and tetracycline respectively to inhibit bacterial growth) and Starch Casein agar (SCA) plates and carefully spread on the agar surface using sterile bent glass rod. The plates were then incubated at 37ºC for 24 – 48 h (NA plates), 25ºC for 48 – 72 h (SDA plates) and 25ºC for 10 - 14 days (SCA plates). After the incubation periods, distinct colonies were counted and the microbial population determined. Distinct colonies were subcultured twice to obtain pure cultures. Morphologically distinct bacterial and actinomycete colonies were subjected to biochemical characterization according to [14]. The fungal isolates were identified using both cultural and microscopic (the lactophenol – in – cotton blue stain) techniques [15,16]. The distribution of each organism across the rhizosphere depths of each plant was determined as the percentage of depths at which the organism was present. Also, the distribution of each group of microorganisms at each rhizosphere depth of each plant was determined as the percentage of members of the group present at that depth.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Physical Characteristics of the rhizosphere samples

The rhizosphere samples were loamy. The results in Table 1 show that samples of rhizosphere of *M. sapientum* var parasidiaca were very slightly acidic, while those of *Senna occidentalis* were very close to neutral pH.

Table 1. Mean Temperature and pH of the rhizospheric soil samples

Rhizosphere sample	Soil type	pH	Temperature (ºC)
Musa sapientum var parasidiaca			
5	Loamy	6.0	25.0
10	Loamy	6.0	25.0
15	Loamy	6.3	26.0
20	Loamy	6.5	26.0
Senna occidentalis			
5	Loamy	7.2	26.0
10	Loamy	7.2	26.0
15	Loamy	7.5	26.0
20	Loamy	7.5	26.0

3.1.2 Microbial population

The results in Table 2 show that bacteria were the most predominant culturable organisms in the rhizosphere of both plants, followed by fungi, then actinomycetes. The culturable microbial population was at its maximum for depths 10cm and 15cm in *M. sapientum* var paraidiaca. In *S. occidentalis*, bacteria population was highest at 5cm, fungi at 10cm and actinomycetes at 15cm depth of the rhizosphere.

3.1.3 Bacterial occurrence and distribution

Results in Table 3 show that *Bacillus cereus* had 100% distribution in the rhizosphere of both plants. *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* each had 75% distribution, in both rhizosphere, with all absent at 20 cm. *Escherichia coli* and *Enterobacter aerogenes* each had 50% distribution in both rhizosphere, and were not found at 15 and 20 cm (*E. coli*), and 5 and 20 cm (*E. aerogenes*). Rhizosphere depth of 10 cm had 100% distribution of bacteria, followed by 5 cm (75 and 87% for rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively). The least bacterial distribution was found at 20cm.

A total of 35 and 38 bacterial isolates were obtained from the rhizosphere of *M. sapientum* and *S. occidentalis* respectively. *Bacillus cereus* had the highest % occurrence of 34.3 and 29.0 in the rhizosphere of *M. sapientum* and *S. occidentalis* respectively, followed by *Ps. aeruginosa* with 14.3 and 18.4% also respectively. The least occurring bacteria were *Acinetobacter* sp with 2.9% in rhizosphere of *M. sapientum* and *Micrococcus* sp with 5.3% in rhizosphere of *S. occidentalis*.

3.1.4 Fungal occurrence and distribution

The results in table 4 show that *Rhizopus stolonifer* had 100% distribution in the rhizosphere of both plants. *Aspergillus niger* had 100 and 75% distribution in the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* (absent at 20 cm) respectively. The least distribution; 25% was obtained for each of *Microsporum canis, Microsporum audouinii, Trichophyton terrestre, Histoplasma capsulatum* in the rhizosphere of *M. sapientum* var parasidiaca and each of *M. audouinii, T. terrestre, Penicillium, Geotrichium* sp in the rhizosphere of *S. occidentalis*. Fungi were most distributed at 15 cm rhizosphere of *M. sapientum* var parasidiaca at 70% and 10 and 15 cm rhizosphere of *S. occidentalis* at 60%.

The least fungal distribution was at 20 cm (40 and 30% in the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively).

A total of 30 and 26 fungal isolates were obtained from the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively. *Aspergillus niger* had the highest % occurrence of 23.3 and 23.1 in the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively, followed by *Rhizopus stolonifer* with 20.0 and 19.2% respectively. The least occurring fungi in the rhizosphere of *M. sapientum* var parasidiaca were *M. canis, M. audouinii* and *Hist. capsulatum* each with 3.3% occurrence. In the rhizosphere of *S. occidentalis*, the least occurring fungi were *M. audouinii, T. terrestre, Penicillium* sp. and *Geotrichium* sp. each with 3.9% occurrence.

3.1.5 Actinomycete occurrence and distribution

Table 5 shows that *Streptomyces* sp had 100% distribution in the rhizosphere of *S. occidentalis* and 75% in the rhizosphere of *M. sapientum* var parasidiaca. *Micromonospora* and *Saccharomonospora* sp. each had 75% in the rhizosphere of both plants. At 25% each, *S. globosus* and *S. rochei* were the least distributed in the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively. Actinomycetes were most distributed at 10 cm (60 and 80% distribution in rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively). At 15 cm, the % distribution was 60 in rhizosphere of both plants. In the rhizosphere of *M. sapientum* var parasidiaca, actinomycetes were least distributed at 5 cm. In *S. occidentalis* rhizosphere, the least distribution was at 20cm.

A total of 24 and 29 actinomycete isolates were obtained from the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively. *Streptomyces* sp had 58.33 and 55.17% occurrence in rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis* respectively. *Streptomyces alanosinicus* and *S. gancidicus* were absent among the rhizosphere isolates of *M. sapientum* var parasidiaca. Similarly, *S. globosus* and *S. sampsonii* were not found in the rhizosphere of *S. occidentalis*.

Table 2. Population of culturable microorganisms in the rhizosphere of *Musa sapientum* var parasidiaca and *Senna occidentalis*

Group of microorganisms	Population (cfu g^{-1}) Rhizosphere depth (cm)				Group/Total microbes (%)
	5	10	15	20	
***Musa sapientum* var parasidiaca**					
Bacteria	5.5×10^4	5.7×10^4	5.8×10^4	3.5×10^4	80.9
Fungi	6.3×10^3	1.92×10^4	1.13×10^4	7.6×10^3	17.5
Actinomycetes	7.1×10^2	1.43×10^3	1.25×10^3	7.7×10^2	1.6
Senna occidentalis					
Bacteria	1.24×10^5	1.22×10^5	1.22×10^5	9.1×10^4	95.4
Fungi	4.0×10^3	6.3×10^3	4.1×10^3	3.2×10^3	3.7
Actinomycetes	7.3×10^2	1.19×10^3	1.4×10^3	1.01×10^3	0.9

*** Values are mean of three sets of data.*

Table 3. Occurrence and number distribution of bacteria in rhizosphere of *Musa sapientum* var parasidiaca (and *Senna occidentalis*)

Rhizosphere Depth (cm)	*S. aureus*	*B. Cerus*	*Ps. aeruginosa*	*E. coli*	*E Aerogenes*	*P. mirabilis*	*Micrococcus* sp.	*Acinetobacter* sp.	Overall % distribution by depth
5	1 (1)	2 (3)	2 (3)	2 (2)	0 (0)	2 (2)	2 (1)	0 (1)	75.0 (87.5)
10	1 (2)	5 (4)	2 (3)	1 (2)	1 (1)	1 (1)	2 (1)	1 (1)	100 (100)
15	1 (1)	3 (2)	1 (1)	0 (0)	1 (2)	1 (1)	1 (0)	0 (1)	75.0 (75.0)
20	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	12.5 (12.5)
% distribution	75 (75)	100 (100)	75 (75)	50 (50)	50 (50)	75 (75)	75 (50)	25 (75)	-
% occurrence {n = 35 (38)}	8.6 (10.5)	34.3 (29.0)	14.3 (18.4)	8.6 (10.5)	5.7 (7.9)	11.4 (10.5)	14.3 (5.3)	2.9 (7.9)	-

** Data for rhizosphere of S. occidentalis are in parentheses. ** Values are mean of three sets of data.*

Table 4. Occurrence and number distribution of fungi in rhizosphere of *Musa sapientum* var parasidiaca (and *Senna occidentalis*)

Rhizosphere Depth (cm)	*Mucor* sp.	*Fusarium Solani*	*Rhizopus stolonifer*	*Aspergillus sp*	*M. canis*	*M. audouinii*	*T. terrestre*	*Hist. capsulatum*	*Penicillium* sp.	*Geotrichium* sp.	Overall % distribution by depth
5	2 (2)	0 (0)	2 (2)	3 (3)	1 (1)	1(1)	0 (0)	0 (0)	0(0)	0(0)	50 (50)
10	2 (1)	1 (2)	2 (1)	2 (2)	0 (1)	0(0)	2 (1)	0 (0)	0(0)	0(0)	50 (60)
15	1 (1)	1 (1)	1 (1)	1 (1)	0 (0)	0(0)	0 (0)	1 (1)	1(1)	2(0)	70 (60)
20	0 (0)	0 (0)	1 (1)	1 (0)	0 (0)	0(0)	0 (0)	0 (1)	1(0)	1(1)	40 (30)
% distribution	75 (75)	50 (50)	100 (100)	100 (75)	25 (50)	25 (25)	25 (25)	25 (50)	50 (25)	50 (25)	-
% occurrence {n = 30 (26)}	16.7 (15.4)	6.7 (11.5)	20.0 (19.2)	23.3 (23.1)	3.3 (7.7)	3.3 (3.9)	6.7 (3.9)	3.3 (7.7)	6.7 (3.9)	10.0 (3.9)	-

**Data for rhizosphere of S. occidentalis are in parentheses. **Values are mean of three sets of data.*

Table 5. Occurrence and number distribution of actinomycetes in rhizosphere of *Musa sapientum* var parasidiaca (and *Senna occidentalis*)

Rhizosphere Depth (cm)	*Streptomyces* sp.	*S. Alanosinicus*	*S. globosus*	*S. gancidicus*	*S. rochei*	*Saccharomonospora* sp.	*Saccharopolyspora* sp.	*S. sampsonii*	*S. virginae*	*Micromonospora* sp.	Overall % distribution by depth
5	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	1 (1)	0(1)	10 (40)
10	1 (2)	0 (2)	0 (0)	0 (1)	1 (2)	2 (1)	2 (1)	2 (0)	0 (2)	2(2)	60 (80)
15	2 (2)	0 (1)	2 (0)	0 (1)	1 (0)	1 (1)	0 (2)	1 (0)	0 (0)	1(2)	60 (60)
20	3 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	1(0)	30 (20)
% distribution	75 (100)	0 (50)	25 (0)	0 (50)	50 (25)	75 (75)	25 (75)	50 (0)	25 (50)	75 (75)	-
% occurrence {n = 24 (29)}	25.0 (20.7)	0 (10.4)	8.3 (0)	0 (6.9)	8.3 (6.9)	16.7 (13.8)	8.3 (13.8)	12.5 (0)	4.2 (10.4)	16.7 (17.2)	-

** Data for rhizosphere of S. occidentalis are in parentheses. ** Values are mean of three sets of data.*

3.2 Discussion

This study revealed the predominance of bacteria in the rhizosphere of both *Musa sapientum* var parasidiaca and *Senna occidentalis*, constituting 80.9 and 95.4% respectively. These results justify the fact that prokaryotes are the most numerous soil inhabitants [17].

Population of culturable bacteria in the rhizosphere of *Senna occidentalis*, at all the studied depths, was greater than that in the rhizosphere of *Musa sapientum* var parasidiaca. The reverse of this trend was, however, observed for culturable fungal population. This trend is, probably, due to the fact that the rhizosphere samples of *M. sapientum* var parasidiaca tended a little acidic with average pH 6.2 while those of *S. occidentalis* were closer to neutrality with average pH 7.4. Acidity, generally, suppresses the growth of bacteria, thereby allowing fungi to grow with less competition for nutrient and space [17]. It may also be that, the rhizosphere of the two plants were naturally enriched with different organic molecules more suitable for the growth of one group of microbes than the other [18,19]. Actinomycetes were the least in population among the three groups of microbes isolated, with their populations in close proximity in the rhizosphere of both plants. The percentage of culturable actinomycetes to culturable bacteria in rhizosphere of *M. sapientum* var parasidiaca was 2.03 which were greater than 0.94 for the rhizosphere of *S. occidentalis*. These results fall within the range of actinomycetes/culturable bacteria (%) reported by [19] for rhizosphere of *Theobroma cacao.* Some other studies have, however, reported populations of actinomycetes more than 30% of the total culturable bacteria in rhizosphere of other plants [20]. The small population of actinomycetes obtained in this study may indicate that the rhizosphere environment of the studied plants were not so favourable to the growth of actinomycetes [19]. This indication will, however, require a greater number of samples studied over an extended period of time to confirm.

Bacillus cereus had 100% distribution in the rhizosphere depths of both studied plants and was the highest occurring bacterium in the rhizosphere samples of both plants. *Pseudomonas aeruginosa* coming next to *B. cereus* in occurrence in the rhizosphere of both plants justifies previous reports on these organisms as among leading soil bacteria and may be an indication of the important roles these bacteria play in protecting the roots of these plants from pathogens. Species of *Bacillus and Pseudomonas* secrete hydrolytic enzymes capable of degrading cell walls, iron – chelating siderophores, several cyclic lipodepsipeptides (LDPs), and a great variety of antibiotics and are, therefore, frequently used as biocontrol agents [21]. *Bacillus cereus* was the only bacterium isolated at 20 cm rhizosphere depth of both plants. This may be due to the adaptive capability to form endospores by *Bacillus sp*. which may be necessary for survival in a low oxygen environment as may exist at such depth [17].

In occurrence and distribution, *Aspergillus* sp, *Rhizopus stolonifer* and, to lesser extent, *Mucor* sp were the dominant fungi isolated in the rhizosphere samples of both plants. [22] reported *Aspergillus niger* and *Rhizopus stolonifer* as leading fungi isolated from soil environment in Keffi, Nassarawa state in Nigeria. These fungi, especially *Aspergillus* sp can be found in many soil types because of their saprophytic nature and their spores are easily dispersed in the air. Soil fungi are so crucial to the decomposition of dead plant materials, breaking down of complex macromolecules such as lignin, and cellulose [22]. The presence of *Microsporum canis, M audouinii* and *Trichophyton terrestre* among the fungi isolates from the rhizosphere samples may be an indication that these dermatophytic fungi are geotrophic in origin [23]. In this study, the culturable fungal population was highest at 10 and 15 cm rhizosphere depths. This can still be said to conform with the report that fungi, been mostly

aerobes, are primarily found in the 10 cm topsoil and are rarely found below 30 cm soil. The presence of high population of fungi in the 15 cm rhizosphere soil samples may be attributed to the higher nutrient content of rhizosphere soil as compared to bulk soil [5].

The actinomycetes isolated in this study were mostly distributed at the 10 and 15 cm rhizosphere depths of both plants. Very few actinomycetes were isolated in the 5 cm rhizosphere samples, especially those for *M. sapientum* var parasidiaca. This tend to confirm previous report that the deeper the soil depth the more the actinomycetes population [24]. Culturable actinomycetes distribution at the 20 cm rhizosphere depth was more than that at 5 cm but, was less than that at the 10 and 15 cm depth. This may be due to the reduced oxygen level at the 20 cm depth (actinomycetes being aerobes) [19]. *Streptomyces* sp was very dominant among the actinomycetes isolated in this study and distantly followed by *Micromonospora* sp, thereby, confirming several reports that *Streptomyces* sp are the dominant actinomycetes [25]. The predominance of *Streptomyces* sp., *Micromonospora* sp. and *Saccharomonospora* sp may be an indication of the important role these organisms play in the rhizosphere of the select plants. *Streptomyces* sp and other actinomycetes contribute greatly to biological buffering of soils [26], they provide many plant growth factors and produce great number of bioactive substances which are effective in protecting plants against phytopathogens [25].

4. CONCLUSION

The results of this study indicate that diverse types of bacteria, fungi and actinomycetes were distributed in the rhizosphere of *M. sapientum* var parasidiaca and *S. occidentalis*, growing on a Nigerian soil, with majority of the microbes at the 15 cm depth and above where about 80% of soil microbial activities take place. The abundance of the microorganisms in these rhizospheres typifies an environment rich in available, soluble organic nutrients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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