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## Diversity of Endophytic and Rhizospheric Bacteria Associated with Sugarcane and Soil Nutritional Characteristics in Assiut governorate, Egypt

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### ABSTRACT

Bacterial communities can vary dramatically across different locations. The diversity of rhizospheric and endophytic microorganisms associated with sugarcane (*Saccharum* sp. cv. C 9) are significantly influenced by various environmental factors, which are crucial in shaping plant health and resilience. The present study aimed to investigate the soil nutrients impact on the diversity and abundance of the rhizospheric and endophytic bacteria associated with sugarcane. Moreover, to study the impact of some specific environmental stressors, such as nutrient deficiency in alteration the endophytic bacterial community structures using indicator species and this alternation from richness, dominance and degree of significance of bacterial groups in each plant part for the studied locations. Total of 63 bacterial isolates were isolated from 2 different sites in Assiut. 79.4% of bacteria were isolated from the first location (Faculty of Agriculture farm) and 20.6% from the second location (Faculty of Science, Botany dep. farm). Endophytic bacterial isolates were 42 isolates while rhizospheric bacterial isolates were 21. The most essential ions for plant growth and bacterial diversity and richness were significantly higher in site 1 especially nitrate, phosphate, sodium and potassium ions. The nutrient deficiency was noticeable in the bacterial diversity, a complete absence of and showed the significance contribution of bacilli, filamentous bacteria in soil of site 1 as well as rods in sugarcane roots of site 2 soil. The scanning electron microscopy imaging was used for further verification of the bacterial shape and confirmation of the shape of the bacteria. SEM photos showed actinomycetes and short rod bacteria attached to the sugarcane roots.

By deep understanding of how environmental factors influence the bacterial diversity associated with sugarcane, we can develop the strategies of improving crop resilience and productivity in diverse ecological contexts.

## INTRODUCTION

Sugarcane (*Saccharum* spp.) is a perennial grass (family *Poaceae*, subfamily *Panicoide*), cultivated in tropical and warm-temperate regions with the best temperature between 20 and 35 °C. Most commercial sugarcane varieties are hybrids with *Saccharum officinarum*. It is considered the primary source of sugar production and is used in bioethanol, animal feed and energy (1). It is considered a vital crop in Egypt with a yield reach of fifteen million tons which ranks Egypt as one of the top 20 countries in the world in its cultivation (the 15<sup>th</sup>) and the highest crop yield in Egypt (2).

Plants deal with a wide variety of microorganisms including bacteria, fungi and algae could generally be classified depending on the site of their nest related to plant part to epiphytic, endophytic and rhizospheric. The microbes that inhabit the surface part of plant even phylloplane or rhizoplane or both known as epiphyte, while endophytic microorganisms nest in the internal plant tissues for a part or all stages of their life cycle without causing any obvious damage or harm including symbiotic and commensalism relations (3). This relation suggests that the plant acts as the host providing the niche and nutrients for microbes on the other hand microbes can supply the plant with tools used for the improvement of nutrition quality and protect the plant's immunity against pathogens (4). The rhizosphere is a habitat for numerous organisms and is considered as a complex ecosystem (5). Because of their high diversity and direct involvement in plant growth and diseases, rhizospheric bacteria, especially plant growth-promoting rhizobacteria, were extensively studied to investigate functions. Plant growth promoting rhizobacteria can promote plant growth via phytohormone production, nutrient solubilization, and nitrogen fixation and metabolism (6). Other effects of plant growth promoting rhizobacteria could be indirectly by the suppression of soil-borne pathogenic and deleterious microorganisms (7). Many studies have shown that the diversity of the rhizospheric bacteria is highly correlated to plant genotype and the soil type (7,8). Endophytes are defined as microorganisms that colonize the internal plant tissues without causing disease including mutualistic, commensalism and unknown-function microbes (9). The form of endophyte colonization in plants varies from being inside the plant cells called "intra-cellular" or limited in the intercellular space between cells called "inter-cellular". Many bacterial endophytes common in wide range of plants and showed a positive impact on their host plant health as

*Azoarcus*, *Burkholderia*, *Gluconobacter*, *Klebsiella*, *Pantoea*, *Herbaspirillum*, *Rahnella*, and *Pseudomonas* (10) and common recorded before in sugarcane were *Pseudomonas*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Pantoea*, *Brevibacillus*, *Staphylococcus* (11), *Gluconacetobacter diazotrophicus* (12), and *Acinetobacter* (13). It investigated the significant role of endophytes on plant growth either direct way by producing growth stimulator compounds such as phytohormones as described in (14) investigated the ability of *Enterobacter hormaechei* to produce Indole-3-Acetic Acid, reducing the sensitivity to heavy metals by minimal the plant abilities to accumulate them as shown in (15) or through indirect way as a part of plant immunity as mentioned in (16) that refer to the vital role of *Bacillus thuringiensis* in antagonist wheat flag smut agent (*Urocystis tritici*).

Soil is the preeminent source of plant endophytes from the beginning of seed growth and the signals associated with the germination process considering one of the main factors

that shape the endophytes community in the plant along with other factors which may be biotic and abiotic ones including the plant genotype, plant developmental stage, soil properties and nutrients, climatic conditions, and agricultural practices which affect and modulate the composition of the endophytic microbiota (17). As a reservoir, soil plays a critical role in the nutrition of organisms' habitat and acts as a shelter, elements like nitrogen and phosphorus act as main contributors for genetic material, enzyme generation, maintenance and formation of chlorophyll that relies on plant health and growth process. In addition, potassium is important for vascular system functions and nutrient transport. Thus, the macronutrients are considered as limiting agents (18). Along with macronutrients, other micro-nutrients in soil have also an important role in the improvement of plant metabolism and photosynthesis rate working as co-factors for enhancing plant productivity (19). For decades, studying endophytic and rhizospheric bacteria has taken a great interest in many aspects especially their relation to the host plant and the nature of interactions that occur between themselves without considering what they rely on their biodiversity and if this biodiversity can act as a sign for plant health state or if it was considered as a condition for plant state or how does soil nutrients including the concentration of some essential ions and elements determine their diversity within the host plant (20–22). Rhizosphere nutrient availability is clearly a key factor in mediating plant-microorganism interactions, especially soil nitrogen availability. Nitrogen can restrict the plant growth and microbial communities across diverse ecosystems. The three forms of nitrogen including organic nitrogen, nitrate nitrogen, and ammonium nitrogen play a crucial role in plant-microorganism interactions through nitrogen allocation among species (23,24). The limited understanding of the ecological role of endophytic bacteria makes the full use of them in agriculture especially in other fields generally a critical challenge. One of the ways to facilitate that is by trying to connect between environment and endophytic bacteria and get out of limited view through the internal state of the plant only (25). The aims of the current study are: 1) To isolate the endophytic and rhizospheric bacteria associated to sugarcane, 2) to investigate the soil nutrients effect on the diversity and abundance of the isolated bacteria and 2) to study the impact of some specific environmental stressors, such as nutrient deficiency in the alteration of the endophytic and rhizospheric bacterial community structures using indicator species and this alternation from richness, dominance, and degree of significance of bacterial groups in each plant part for the studied locations.

## MATERIALS AND METHODS

### *a. Study locations and isolation of bacteria (endophytic and rhizospheric)*

Sugarcane plant (*Saccharum sp.*, sugarcane cv. C 9) samples were collected from two sites at Assiut University. The first site is Faculty of Agriculture farm. The second site is the Faculty of Science, Botany dep. farm. Fresh plant parts and soil of rhizosphere were collected in sterile plastic bags and brought directly to the lab. The sample collection occurred in winter weather which met with the sugarcane growing season.

Plant parts were washed several times with tap water followed by rinsing with distilled water and then weighed to record the fresh weight. The surface disinfection protocol involved using NaOCl

(2%) on the roots for three minutes and leaves for 1 min, followed by treatment with ethanol (70%) for 2 min, and subsequently rinsing several times with sterilized distilled water (26). Afterward, the plant parts were homogenized in a sterile 5% sucrose solution using a sterile pestle and mortar, then 100  $\mu$ L of that aliquot was plated onto various media using the pour plate method (except for semi-solid LG media). For the stem, the outer surface was disinfected by swabbing it into ethanol (70%), wait until dry and then a section of the internal stem was cut with a sterilized knife (27) and homogenized as described before. Soil samples were diluted in 9 mL of 5% sucrose and then prepare serial dilutions. The mixtures were cultured on different agar media as shown in figure. 1.

***b. Media used in isolation, purification, and preservation of bacteria:***

Different agar media were used in the current study including **i.** Nutrient agar (NA) media: the standard media used from HiMedia company, India. In addition, NA medium was used for purification and preservation of culture. **ii.** Modified LGIP media: as described by (28) contained (g/L):  $K_2HPO_4$ , 0.2;  $KH_2PO_4$  0.6;  $MgSO_4 \cdot 7H_2O$ , 0.2;  $CaCl_2 \cdot 2H_2O$ , 0.02;  $NaMoO_4 \cdot 2H_2O$  0.002;  $FeCl_3 \cdot 6H_2O$ , 0.01; 0.5% Bromothymol blue in 0.2 M KOH, 5; agar, 2.0; and carbon substrate, 100. The pH was adjusted to 5.5 with acetic acid. **iii.** Ashby media: Mannitol 5 g, Dipotassium Phosphate 0.2 g,  $MgSO_4 \cdot 7H_2O$  0.2 g, NaCl 0.2 g,  $CaSO_4$  0.1 g,  $CaCO_3$  0.1 g,  $H_2O$  1000 ml (29). **iv.** Starch casein agar (SCA): soluble starch: 10 g,  $K_2HPO_4$ : 2 g,  $KNO_3$ : 2 g, casein: 0.3 g,  $MgSO_4 \cdot 7H_2O$ : 0.05 g,  $CaCO_3$ : 0.02 g,  $FeSO_4 \cdot 7H_2O$ : 0.01 g, agar: 15 g, filtered sea water: 1000 ml and pH:  $7.0 \pm 0.1$  (30) v. PG11. **vi.** Modified PDA: used as described (31) which supplemented by 10% sucrose instead of glucose. This media used for subculturing the bacterial growth from LGIP semisolid media.

Then different media were incubated at  $35 \pm 2^\circ C$  for 1 day for Nutrient agar, 2 days for Ashby, 4 to 6 days for starch casein agar and at  $30^\circ C$  for 7 days for LGIP and for 14 days in PG11. The growth in LG media was cultured in solid LG media then purified in modified PDA media which supplemented by crystallized sugar 10% instead of glucose.

***c. Detection of indole acetic acid (IAA) production and phosphate solubilization potential of the isolated bacteria***

To differentiate between the same bacterial shapes isolated on different media, their potential to produce indole acetic acid (IAA) (36) and to solubilize phosphate. IAA detection were done using tryptone broth medium as described in (37). Bacteria were incubated for 3 days at  $32^\circ C$  under shaking at 100 rpm then, the bacterial culture centrifugated at 4000 rpm for 5 minutes for harvesting. The concentration of IAA production determined by using Salkowski as in (38). The reagent was added to the bacterial supernatant (1:2) to determine IAA producing capability and measured at 530 nm wavelength using spectrophotometer.

For detecting phosphate solubilization capability, the isolates were cultivated on Pikovskaya agar medium (PVK) media as mentioned in (39) and by formation of halo zone around and under the colony as an indication for phosphate solubilization.

***d. Scanning electron microscope examination of sugarcane root tissues.***

Three samples of 0.5 to 1 cm were taken from the sugarcane root sample and fixed in  $5^\circ C$  cold buffered glutaraldehyde for two days. The samples were then washed by cacodylate buffer three times for 13 minutes each and post fixed in 1 % osmium tetroxide for two hours. Samples were then washed in cacodylate buffer three times for thirteen minutes each and then dehydrated by using an ascending series of ethanol 30,50,70,90 for two hours, 100% for two days, and then to amyl acetate for two days. Drying was applied to the samples by using liquid carbon dioxide. Each

sample was stuck on metallic blocks using silver paint. By using gold sputter coating apparatus, samples were evenly gold coated in a thickness of 15nm. Samples were examined by using JEOL JSM 5400 LV scanning electron microscope 15- 25. kv and photographed (32).

#### *e. Quantification of soil nutrients*

Measuring magnesium and calcium ions were determined by the volumetric titration method using 10 mN EDTA(33), chloride content quantified as silver chloride and titrated volumetry by 0.005 N AgNO<sub>3</sub> (34) and bicarbonate ions quantified by volumetric titration with 10 mN HCl. Sodium and potassium ions are determined by a Carl-Zeiss DR LANGE M7D flame photometer, sulphate ions quantified by colorimetric method using acidic NaCl and Gum acacia then waiting for 30 minute and measure the turbidity at 420 nm. Phosphate ions determined using molybdate sulfuric acid and ascorbic acid 10% as reagents that result in appearance of blue color which was measured at 720 nm-(34). Nitrate ions determined by using hydrazine sulphate (0.0188 M ) , salphanilic acid , copper sulphate 0.33 mM and alpha-naphthyl amine which gave pink color and measured at 540 nm (35).

#### *f. Data analysis:*

At the beginning of analysis, bacterial isolates grouped according to their shapes to eight main groups monobacilli, diplobacilli, streptobacilli, monococci, staphylococci, rods, short rods and filamentous for each location. Multivariate calculation for indicator species that shows dominance, significance of bacterial groups in each plant part in each location and Canonical Correspondence Analysis (CCA) for connecting between these bacterial groups and environmental factors measured using PAST v2.

## RESULTS

Through the isolation from two locations, total of 63 bacterial isolates were isolated. Fifty bacterial isolates were isolated from the first site, Faculty of Agriculture farm (31 isolates were endophytic and 19 from rhizospheric) which represent 79.4% of total no of isolates and 13 bacterial isolates were isolated from the second site, Faculty of Science, Botany dep. farm (11 endophytic isolates and 2 rhizospheric isolates) with 20.6% (Table 1). Some of the isolates were able to produce IAA and solubilize phosphate as shown in table 1. As shown in table 2, the most essential ions for plant and bacterial endophytes growth and richness were greatly high in site 1 especially nitrate, phosphate, sodium and potassium ions.

Table 1: The table describes the source of bacterial isolates, the shape and ability to form spores, potential to produce indol acetic acid (IAA), to solubilize phosphate and CFUs. Samples for isolation were collected from the farms of Faculty of agriculture and Faculty of Science, Botany dep. in Assiut University farm and, Assiut Governorate, during October 2021 on different culture media at 37±2 °C (NA refers to nutrient agar media and SCA to starch casein agar media).

Location 1	Sample No.	Source of sample	Media of isolation	Shape	Spore formation	Phosphate solubilization	IAA Production	CFU
	1	Roots	NA	Short rods	Absent	Positive	Positive	$307 \times 10^5$
	2	Roots	NA	Strptobacilli	Present	Positive	Positive	$54 \times 10^5$
	3	Stems	NA	Staphylococci	Absent	Positive	Negative	$273 \times 10^5$
	4	Stems	NA	Staphylococci	Absent	Negative	Negative	$249 \times 10^5$
	5	Stems	NA	Short rods	Absent	Positive	Negative	$10^3$
	6	Leaves	NA	Staphylococci	Absent	Negative	Positive	$10^3$
	7	Leaves	NA	Diplobacilli	Present	Positive	Negative	$10^3$
	8	Leaves	NA	Monococci	Absent	Negative	Negative	$21 \times 10^3$
	9	Leaves	NA	Staphylococci	Absent	Negative	Negative	$4 \times 10^3$
	10	Leaves	NA	Short rods	Absent	Negative	Negative	$9 \times 10^3$
	11	Rhizosphere	NA	Strptobacilli	Present	Positive	Negative	$10^8$
	12	Rhizosphere	NA	Strptobacilli	Present	Positive	Positive	$3 \times 10^7$
	13	Rhizosphere	NA	Strptobacilli (white)	Present	Negative	Negative	$6 \times 10^7$
	14	Rhizosphere	NA	Strptobacilli (yellow)	Present	Negative	Negative	$3 \times 10^8$
	15	Rhizosphere	NA	Short rods	Absent	Negative	Negative	$2 \times 10^7$
	16	Rhizosphere	NA	Diplobacilli	Present	Positive	Negative	$2 \times 10^7$
	17	Rhizosphere	NA	Diplobacilli	Present	Negative	Negative	$3 \times 10^7$
	18	Rhizosphere	NA	Monobacilli	present	Positive	Negative	$2 \times 10^8$
	19	Rhizosphere	NA	Monococci	absent	Positive	Positive	$10^7$
	20	Rhizosphere	NA	Staphylococci	absent	Negative	Negative	$2 \times 10^7$

21	Rhizosphere	NA	Rods	absent	Negative	Positive	500
22	Rhizosphere	NA	Filamentous	present	Negative	Positive	$10^7$
23	Roots	SCA	Short rods (green pigmentation)	Absent	Negative	Positive	$21 \times 10^5$
24	Roots	SCA	Short rods	Absent	Negative	Positive	104
25	Roots	SCA	Short rods	Absent	Positive	Negative	$13 \times 10^4$
26	Roots	SCA	Monobacilli	Present	Negative	Positive	$6 \times 10^5$
27	Roots	SCA	Streptobacilli	Present	Negative	Positive	$15 \times 10^4$
28	Roots	SCA	Monobacilli	Present	Positive	Positive	$7 \times 10^4$
29	Roots	SCA	Filamentous	Present	Positive	Positive	103
30	Roots	SCA	Rods	Absent	Negative	Positive	$5 \times 10^4$
31	Roots	SCA	Rods	Present	Positive	Positive	$14 \times 10^4$
32	Stem	SCA	Diplobacilli	Present	Negative	Negative	$2 \times 10^4$
33	Stem	SCA	Diplobacilli	Present	Positive	Positive	$21 \times 10^3$
34	Stem	SCA	Streptobacilli	Present	Positive	Negative	$2 \times 10^6$
35	Stem	SCA	Monobacilli (white )	Present	Positive	Negative	$2 \times 10^7$
36	Stem	SCA	Monobacilli (opaque)	Present	Positive	Negative	$2 \times 10^4$
37	Stem	SCA	Staphylococci	Absent	Negative	Negative	$2.13 \times 10^7$
38	Leaves	SCA	Staphylococci (white)	Absent	Negative	Negative	$43 \times 10^2$
39	Leaves	SCA	Monococci	Absent	Negative	Negative	$5 \times 10^2$
40	Leaves	SCA	Staphylococci (yellow)	Absent	Negative	Negative	$5 \times 10^2$
41	Rhizosphere	SCA	Short rods	Absent	Negative	Negative	$10^7$
42	Rhizosphere	SCA	Diplobacilli	Present	Positive	Negative	$10^7$

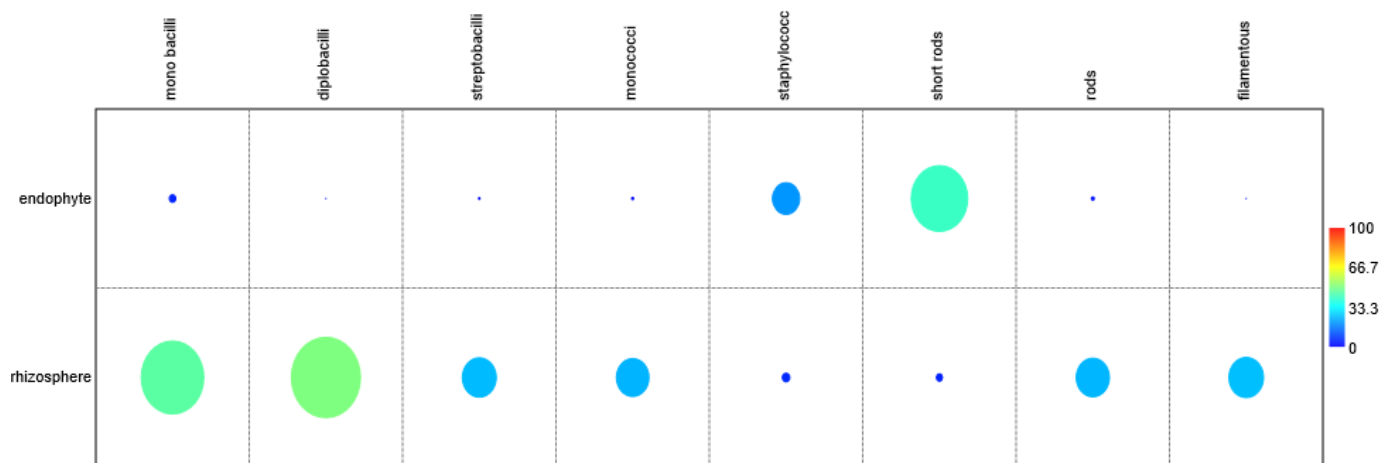
43	Rhizosphere	SCA	Diplobacilli	Present	Negative	Negative	$3 \times 10^7$
44	Rhizosphere	SCA	Rods (white)	Absent	Positive	Negative	$2 \times 10^8$
45	Rhizosphere	SCA	Rods (yellowish white)	Absent	Positive	Negative	$10^7$
46	Rhizosphere	SCA	Streptobacilli	Present	Positive	Positive	$10^7$
47	Rhizosphere	SCA	Streptobacilli	Present	Positive	Negative	$3 \times 10^7$
48	Stem	LGIP	Rods	Absent	Positive	Negative	$8 \times 10^5$
49	Stem	LGIP	Monococci	Absent	Positive	Positive	$6 \times 10^5$
50	Roots	Ashby	Rods sp.	Absent	Negative	Positive	150

Table 2: The measured parameters of soil of location of study in ( $\mu\text{g} / \text{gm}$ ) a1, a2 refer to Agriculture farm location, s1, s2 for Botany and microbiology farm, Faculty of science.

Location 2	Sample No.	Source of sample	Media of isolation	Shape	Spore formation	Phosphate solubilization	IAA Production	CFU
	1	Stem	NA	Diplobacilli	Present	Negative	Negative	$2 \times 10^4$
	2	Stem	NA	Diplobacilli	Present	Positive	Positive	$21 \times 10^3$
	3	Stem	NA	Streptobacilli	Present	Positive	Negative	$2 \times 10^6$
	4	Stem	NA	Monobacilli	Present	Positive	Negative	$2 \times 10^4$
	5	Stem	NA	Rods	Absent	Negative	Positive	200
	6	Stem	NA	Short rods	Absent	Negative	Negative	20
	7	Leaves	NA	short rods	absent	Negative	Negative	40
	8	Leaves	NA	monobacilli	present	Negative	Negative	$4 \times 10^3$
	9	Leaves	NA	streptobacilli	present	Negative	Negative	$1.5 \times 10^3$
	10	Roots	NA	Rods	Absent	Positive	Positive	$8.16 \times 10^6$
	11	Roots	NA	Short rods	Absent	Negative	Positive	$21 \times 10^5$
	12	Rhizosphere	NA	diplobacilli	present	Positive	negative	$2 \times 10^4$
	13	Rhizosphere	NA	monobacilli	present	Positive	negative	$3.5 \times 10^4$

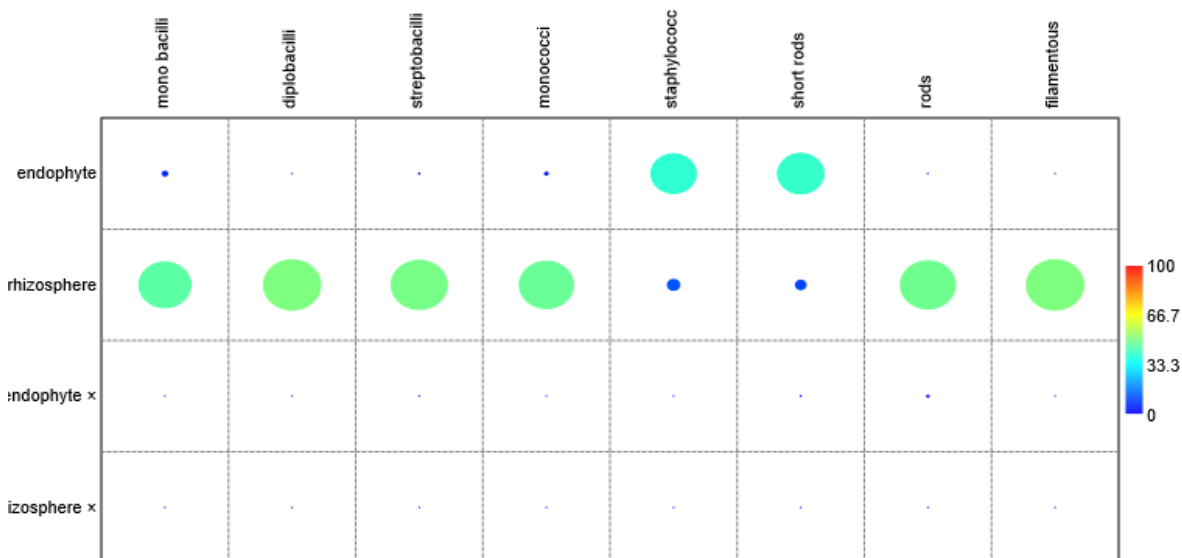
	pH	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>+2</sup>	Mg <sup>+2</sup>	Cl <sup>-</sup>	HCO <sub>3</sub>	Phosphate	Sulphate	Nitrate
<b>a1</b>	7.15	569	160	0.38	0.0915	0.623	7.1167	127.9105	679.9978	781.0609
<b>a2</b>	7.23	323	145.6	0.25	0.0305	0.534	12.2	141.5469	838.3311	470.5847
<b>s1</b>	7.1	575	86.4	0.175	0.061	0.534	6.1	19.72869	1129.998	444.3942
<b>s2</b>	7.21	294	107	0.15	0.0915	0.445	3.05	19.72869	834.9978	404.8704

Using the number of bacterial individuals in both sites (endophytic and rhizospheric) in two locations, the diversity indices for both locations were calculated to compare the dominances and the significance of the effect of each bacterial group on the diversity of each community. Figure 2 presents the biodiversity of the studied areas in both locations. The current results showed that short rods bacteria represent the highest group of endophytic bacteria followed by staphylococci while they were nearly absence in rhizosphere in contrast the other groups that show notable appearance.



**Figure 2:** The dominance and the diversity contribution degree of different bacterial groups in two sites of the study between endophytes and rhizospheric bacteria. The colour for diversity contribution degree was referred in scale.

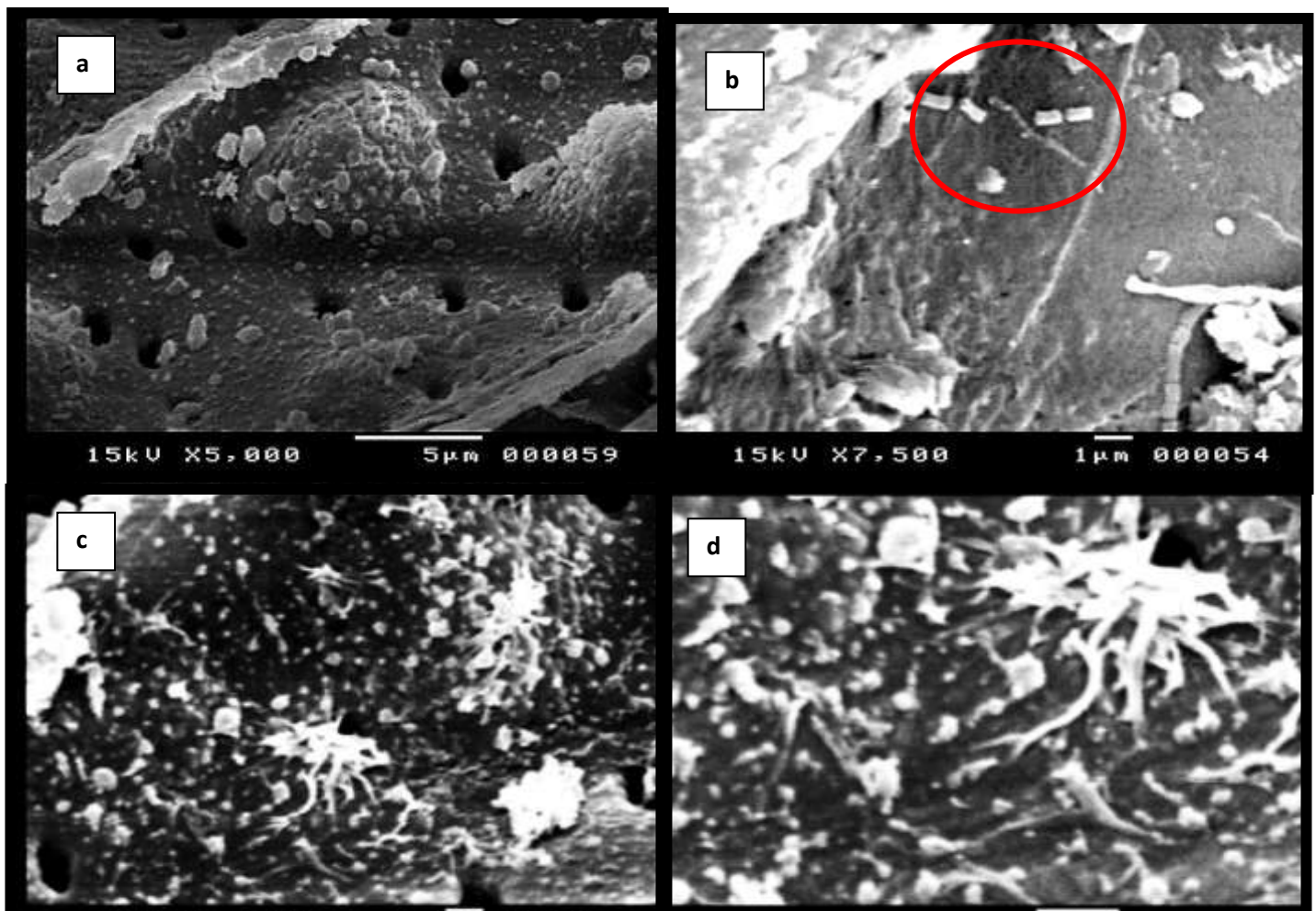
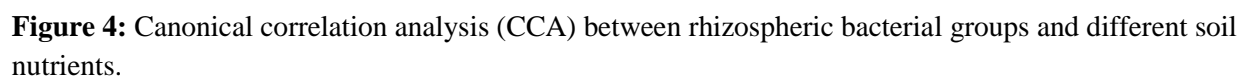
With a close view of the community structure of both locations using indicator species as a comparison factor, Figure 3 record the major contribution in diversity occurred by first location community especially rhizospheric one and most bacterial groups were present in rhizosphere in location 1 with comparable dominance that represented in circle width and a degree in biodiversity contribution in circle color. Location 1 was nourished with bacterial communities with high richness and abundance compared to location 2 which was severely poor community in biodiversity.



**Figure 3:** Comparison between indicator species of the two locations of the current study. The color for species contribution degree was referred in the scale on the right side.

Location 2 shows severe poverty in these ions that reflected on the richness and abundance of studied bacteria, SEM photos for roots in location 1 figure 5. Photos showed the dominance of short rods in internal plant roots. Different nutrients correspond to form the rhizospheric bacterial communities by variable degrees and distinct ways as CCA in figure 4. The diagram shows the environmental factors efficient degree by closing the arrows that represent the factors, S refers to the richness and d to dominance (figure 4). The richness is greatly affected by phosphate and potassium ions, cocci bacteria (staphylo- and mono cocci ) share with filamentous bacteria their affected by pH . Short rods , rods and streptobacilli strongly related to calcium and nitrate ions and sulphate presence has an effect on monococci bacteria presence. It was noted the independence of diplobacilli bacteria and their presence far from any factor .So, nutrients such as nitrate, phosphate, calcium, and potassium lay in different quarter and affect on different bacterial groups that proof of the attribution of all these factors to form equilibrium diversity and their presence make them highly related to that diverse community and that strengthen the explanation of the presence of most bacterial group is related directly to variation of nutrients as in location 1.

Axis	Eigenvalue	% of constr. in.	% of total inertia
1	0.91602	99.99	91.58
2	5.24E-05	0.005725	0.005244
3	1.25E-16	1.37E-14	1.25E-14



## DISCUSSION

From the total cultivable samples isolated from two locations the agriculture farm (location 1) bacterial isolates represent around 79.4% of total isolates while the Faculty of Science, Botany dep. farm (location 2) with 20.6%, that great notable difference is attributed to complicated net conditions occurred that could be shown through the study of soil is considered the main source of endophytes in general (40).

Bacterial isolates able to produce IAA and solubilize phosphorous are interesting because phytohormones promote root cell proliferation and increase nutrient and water absorption through the overproduction of side cells and root hairs (46). In addition, phosphorus-solubilizing microorganisms are important in agricultural ecosystems and directly or indirectly influence physical, chemical, and biological soil properties (47-49).

The raising of essential ions in rhizosphere bacterial community in location 1 creates a quite balanced nest of studied bacteria for good biodiversity structure, getting it able to be suitable for the growth of a broad spectrum of bacteria excluding the staphylococci and short rods bacteria that show weak presence in rhizosphere while almost solitary members forms endophytic community. On the other hand, almost all groups of bacteria in location 2 were showed so weak appearance due to the poverty of this community with different nutrients that enabled their growth (41).

Bacterial stability is difficult, and could be caused by biofilm formation by some species such as *Acinetobacter* sp.(34,10). location 2 shows a relatively poor bacterial community, with the absence of bacteria in the soil. On the other hand, rods represent weak occurrence in endophyte the chance of rods and short rods to nourish endophytically is getting higher may that occur because of the ability of rod bacteria to adapt efficiently in plant internal tissues that known by limited spaces (38) as occurred in the internal plant tissues of location 2, that makes us suppose that rods bacteria have efficient mechanisms manage them to colonize well in the internal plant tissues and ability to self-provide the nutrients by ways as nitrogen fixation that enable them to survive in niches known as low nutrients availability (39). The community structure in this study was similar to that recorded by Velázquez *et al.* 2008 (40) as an endophytic community in healthy sugarcane, which indicates the good state of the studied plant in location 1 .

## CONCLUSION

The current study is considered one of few studies that highlight the significance of soil nutrients in endophytic bacterial diversity shaping. We conclude that soil nutrients had a significant impact on the presence and dominance of bacteria groups that were altered in different plant parts and sites. staphylococci and short rods bacteria showed terrific distribution in internal plant parts.

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