DIVERSITY OF RHIZOSPHERIC AND ENDOSPHERIC BACTERIA ASSOCIATED WITH *Zea mays* L. CULTIVATED IN THE SAHARAN OASES OF EL MINEA (ALGERIA)

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Abstract. The study of bacterial diversity in *Zea mays* L. cultivated in the Saharan oases of El Minea, Algeria, revealed that microbial diversity was higher in bulk soil compared to the rhizosphere and endosphere, although the number of bacterial phyla was similar among the three compartments. The main bacterial phyla identified were Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria, present in all three studied compartments. The abundance percentages of these phyla were higher in the rhizosphere and endosphere compared to the bulk soil, indicating a preference of microorganisms for root environments. The study also revealed the presence of unclassified bacterial genera, suggesting the existence of bacterial groups hitherto uncultured. Variations in the abundance of bacterial genera among bulk soil, rhizosphere, and endosphere compartments highlighted the complex interactions between host plants and soil microorganisms. Some bacterial genera, such as *Acinetobacter* and *Pseudomonas*, were found to be abundant in the rhizosphere, positioning them as potential candidates for agricultural biotechnology due to their ability to confer resistance to maize plants and improve yield. This information can be utilized to optimize agricultural practices and develop soil management strategies aimed at enhancing productivity and sustainability of maize crops in Saharan environments.

Key words: Algeria (El Minea); endospheric bacteria; rhizospheric bacteria; Saharan oases; *Zea mays*.

INTRODUCTION

The rhizosphere, referring to the soil region surrounding roots, harbors a beneficial bacterial microbiota for plants [9, 10, 32, 42]. Bacteria are also present in the endosphere, where they colonize the internal tissues of roots and symbiotically live with plants. They thereby contribute to their nutrition and enhance their tolerance to biotic and abiotic stresses [12, 18, 23, 28, 43, 47].

The growing study of plant-associated bacterial microbiome highlights its importance [5, 7]. However, the understanding of microbial community structure and influencing factors remains incomplete [42]. Nonetheless, some influences on the diversity and abundance of plant-associated bacteria have been elucidated, such as soil conditions, chemical signals from bacteria [32, 42, 48], genotype [29, 34], as well as soil phosphorus level [23]. Interactions between plants and soil, which can evolve depending on site history and previous cultivation practices, also influence the diversity and abundance of associated bacterial microbiota [6, 47, 49].

High-performing bacteria from the microbiome can be isolated and formulated into new biological products for inoculation to enhance crop yield [26, 27, 32, 42]. This utilization offers a promising alternative to improve soil fertility, unlike the use of chemical products [40]. Recent results from the inoculation of these bacteria highlight their importance for maize (*Zea mays* L.) cultivation. Indeed, inoculation with cyanobacteria presents significant advantages, such as reducing nitrogen use, improving soil fertility, and enhancing maize crop health [40]. Inoculation of plant growth-promoting rhizobacteria (PGPR) has also conferred maize tolerance to abiotic stresses, notably soil salinity [7, 39, 51, 52].

The bacterial diversity associated with maize is of great importance [35]. Maize has been cultivated in the Saharan oases of Algeria for many centuries [8]. Although it exhibits high genetic variability enabling adaptation to temperate conditions, climate change exacerbates the aridification of the Algerian Sahara, posing a threat to its production [3, 4, 8, 17]. However, studies have revealed a rich and extremely resilient bacterial diversity associated with plants in these regions, endowing them with resistance to thrive [48]. Indeed, despite extreme environmental conditions, maize continues to thrive in these oases, raising questions about the interactions between this plant and the bacterial microbiome present in its rhizosphere and endosphere [28]. Studies on bacteria associated with maize in the Algerian Sahara oases are scarce. In this study, we characterized the diversity of rhizospheric and endophytic bacteria of maize roots cultivated in El Minea, aiming to evaluate their biotechnological potential for sustainably improving agricultural practices in Saharan regions.

MATERIALS AND METHODS

Research experimental field station and maize cultivation

The experimental plot is located at the Experimental Research Station (30.6264°N and 2.88130°E) of the University of Science and Technology Houari Boumediene (USTHB), situated in El Minea, Ghardaia Province, Algeria. This region is characterized by a hot and dry Saharan climate, with an average annual temperature of 25°C and low rainfall of less than 50 mm per year. Precipitation is concentrated between the months of November and March. The soil of the experimental plot is sandy-loamy, with high alkalinity, moderate salinity, low organic matter

content, and the presence of essential nutrients (Table 1). In the past, various crops have been cultivated on this experimental plot, including wheat, barley, and maize.

Table 1. Soil Analysis of the Experimental Station at El Menia.

Parameters	Analysis	Values
Texture	Clays	6.60%
	Fine silts	9.80%
	Coarse silts	11.40%
	Fine sands	24.80%
	Coarse sands	47.40%
Limestone	Total $CaCO_3$	6.75%
	Active $CaCO_3$	-
Acidity / Alkalinity	HCO_3	40.50 meq/100g
	pH (water/soil - 1/5)	8.62
Salinity	EC (saturated paste extract)	5.60 mmhos/cm
Organic matter		0.34%
Carbon		0.20%
Mineral elements	Nitrogen	7‰
	K^+	0.32 meq/100g
	Na^+	2.72 meq/100g
	Ca^{2+}	0.32 meq/100g
	Mg^{2+}	0.03 meq/100g

The sampling sites were randomly selected from the maize fields of the experimental station at El Minea. maize seeds of the TWC-352 variety were sown at a density of 50 cm × 50 cm in a plot with an area of 100 m² (10 m × 10 m). The plants were irrigated with water throughout the trial period.

Soil and plant material sampling

Soil bulk, rhizospheric soil, and root samples were collected following the method by Schneijderberg *et al.* (2020) [44], which is based on Lundberg *et al.* (2012) [36] approach. Soil, rhizosphere, and endosphere samples were stored separately at -4°C until further processing. They were kept in sterile plastic bags and transported on ice to the laboratory for subsequent treatment. Three replicates were performed for each analysis, grouping five plants into a single sample.

Rhizospheric soil was obtained by washing roots with a sterile phosphate buffer composed of 6.33 g of $NaH_2PO_4{\cdot}H_2O$, 10.96 g of $Na_2HPO_4{\cdot}2H_2O$, and 200 µL of Silwet L-77 per liter. Roots were thoroughly cleaned through multiple washes with the phosphate buffer until the wash solution remained clean. Then, washed roots were placed in an ultrasonic bath, alternating periods of sonication for 30 seconds followed by 30 seconds of rest, for a total of 10 minutes. Subsequently, the roots were dried on filter paper in a flow hood. Soil and root samples were frozen in liquid nitrogen and stored at -80°C until DNA isolation.

The roots of each plant were sterilized using a method involving a 5-minute soak in a 5.25% calcium hypochlorite solution ($CaCl_2O_2$), followed by a 1-minute soak in 70% ethanol, and then three rinses in sterile distilled water [14]. Sterilization verification was conducted by spreading 100 µL of the final rinse water onto tryptic soy agar (TSA) medium (GranuCult™, Merck KGaA Germany). Next, sterilized roots (1g) were macerated with 9 mL of 0.9% physiological saline solution, followed by serial dilutions up to 10-6. The obtained dilutions were plated in triplicate on TSA plates containing a sterile solution of cycloheximide at 0.45 µm (50 mg/L) (AppliChem GmbH, Darmstadt, Germany). The plates were incubated at 30±2°C for one week, and the colony-forming units (CFU) per gram of root weight were counted. Bacterial colonies were manually selected based on morphological characteristics such as shape, elevation, margin, surface, opacity, and pigmentation. These bacterial colonies were then purified through several streaking steps. In total, twenty-six endophytic bacterial colonies from plant roots were randomly selected, cultured on TSA medium, and stored at -20°C in 2 mL cryotubes containing tryptic soy broth (TSB) (GranuCult™, Merck KGaA Germany) and 30% glycerol.

DNA extraction and analysis of 16S ribosomal DNA sequences

DNA was extracted from bulk soil, rhizosphere, and endosphere samples using the Mo Bio PowerSoil kit (Qiagen), and from roots (EC) using the Fast DNA Spin for Soil kit (MP Biomedicals). DNA quality and quantity were assessed using the Thermo Scientific NanoDrop and 1% agarose gel electrophoresis. Approximately 300 ng of DNA per sample were sent to a genomics institute in Beijing for analysis.

To study microbial diversity, the V4 region of the 16S ribosomal RNA gene was amplified and sequenced using the 515F and 806R primers with an Illumina HiSeq 2500 sequencer. Raw sequence data were processed using the DADA2 microbiome pipeline [13]. Each sequence was examined and filtered using the "filterAndTrim" function of DADA2 with a maxEE = 2 threshold to remove unwanted raw reads. Additionally, reads corresponding to the phiX genome were excluded from the analysis. Error rates were assessed by randomly selecting 1e8 bases from all pooled samples. These error profiles were then used to infer Amplicon Sequence Variants (ASVs) by applying the "dada" function with default parameters on non-replicated sequences. Sequence tables from each sample were merged into a single table using the "mergeSequenceTables" function. To ensure the reliability of our results, we identified and removed chimeras, which are artificial sequences resulting from non-specific reactions, using the "removeBimeraDenovo" function of DADA2 with the "consensus" method.

Taxonomic assignment of sequence reads and diversity indexes

The Green Genes database was utilized to assign taxonomic classification to each ASV using the "assignTaxonomy" function, providing us with information on the genus they belong to. In cases where precise classification was challenging, we indicated "unclassified" to represent a higher taxonomic level.

For α and β diversity analyses, we generated a subset of the measurable ASV table based on the research question. To characterize bacterial community

diversity, we employed several indices, among which three are particularly significant: the Good's coverage index [24], the Shannon index [45], and the Simpson index [46].

Statistical analysis

The obtained data were analyzed using Analysis of Variance (ANOVA) and subsequently grouped into homogeneous groups using the Newman and Keuls method. In the case of data expressed in percentages or proportions, which are limited between 0% and 100%, power transformations are not suitable as they differently alter each end of the distribution [20]. A commonly used approach is to apply an angular transformation, particularly the arcsine transformation, which is well-suited for percentages [41].

RESULTS

Diversity index and number of detected taxa

The index values generally indicate that the bulk soil is richer than the rhizosphere and endosphere (Table 2). The Shannon index is highest in the bulk soil (1.77), followed by the endosphere (1.49) and the rhizosphere (1.24), indicating a decrease in microbial diversity from bulk soil to the inner root tissues. Similarly, the Simpson index is highest in the bulk soil (0.76), showing a more balanced community compared to the rhizosphere (0.57) and the endosphere (0.67).

Taxa are classified into phylum, class, order, family, and genus (Table 3). This information allows for the assessment of the taxonomic richness of microbial communities associated with maize. The total number of phyla is relatively stable across compartments, with 24 phyla detected overall. However, the number of taxa decreases progressively from bulk soil to endosphere at higher taxonomic levels, with 67 classes, 108 orders, 229 families, and 473 genus identified in total. The bulk soil hosts the highest taxonomic diversity, while the endosphere contains fewer taxa, reflecting a selective microbial colonization inside plant tissues.

Phylum Diversity

The most abundant phyla were Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria in the bulk soil. In the rhizosphere, the most abundant phyla were Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria. Finally, in the endosphere, the most abundant phyla were Proteobacteria, Actinobacteria, Planctomycetes, and Firmicutes (Fig. 1).

Genus Diversity

The *Acidobacteria* Gp4, *Acidobacteria* Gp6, *Pseudomonas,* unclassified *Oxalobacteraceae*, and unclassified *Bacteriovoracaceae* showed relatively high abundance in the bulk soil. In the rhizosphere, the most abundant genera include *Acinetobacter*, *Pseudomonas*, *Chryseobacterium*, and *Acidobacteria* Gp4. Regarding the endosphere, the most abundant genera were *Pseudomonas*, unclassified *Rhizobiales*, unclassified *Planctomycetaceae*, and *Arthrobacter* (Fig. 2).

Table 2. Good's, Shannon's, and Simpson's index values in bulk soil, rhizosphere, and endosphere of maize cultivated in El Minea.

Diversity index	Bulk soil	Rhizosphere	Endosphere
Good's coverage	0.96	0.96	0.88
Shannon	1.77	1.24	1.49
Simpson	0.76	0.57	0.67

Table 3. Number of taxa detected in bulk soil, rhizosphere, and endosphere of maize cultivated in El Minea.

Taxa	Total	Number of Taxa (% unclassified)		
		Bulk soil	Rhizosphere	Endosphere
Phylum	24	23 (0%)	23 (0%)	21 (0 %)
Class	67	38 (29%)	50 (30%)	25 (20%)
Order	108	65 (43%)	74 (37 %)	51 (33%)
Family	229	156 (27%)	153 (33 %)	114 (26%)
Genus	473	326 (24%)	323 (25%)	232 (26%)

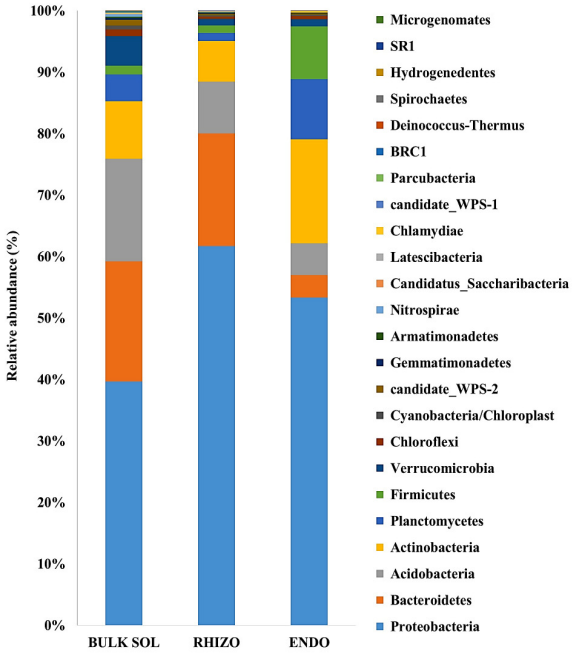

Figure 1. Relative abundance of bacterial phyla detected in the bulk soil, rhizosphere, and endosphere of maize cultivated in the Algerian Sahara oases (El Minea).

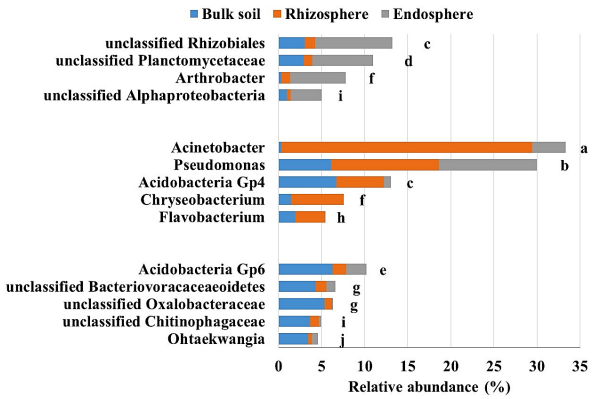

Figure 2. Relative abundance of major bacterial genera detected in the bulk soil, rhizosphere, and endosphere of maize cultivated in the oasis agroecosystems of the Algerian Sahara (El Minea). No statistically significant difference was observed between bars bearing the same letter of the alphabet, based on the results of the Newman-Keuls test.

DISCUSSION

Diversity index and number of detected taxa

The results are consistent with previous studies that have shown lower microbial diversity in the maize rhizosphere compared to bulk soils [22, 38]. This reduction in diversity in the rhizosphere could be explained by the selection of bacteria by maize plants, thereby promoting their growth and development by contributing to nitrogen fixation, nutrient provision, and metabolite supply for resistance. Indeed, plants influence the soil microbiome through various root exudates, enabling them to nourish and form symbioses with bacteria of interest [25].

According to Table 3, the evaluation of the composition and abundance of rhizospheric and endospheric bacterial communities associated with maize cultivated in El Minea reveals a total number of genera (473) higher than that obtained in other studies [15, 19, 22, 33]. Percentages of 24.46% and 26.29% of the sequences found in the rhizosphere and endosphere, respectively, corresponded to unclassified bacteria, indicating the presence of bacterial groups not previously cultured. A high percentage of unclassified sequences has also been reported in the rhizosphere of maize plants [19]. These results suggest that microbial communities in the endosphere may be slightly less diversified than those present in the bulk soil and rhizosphere.

Phylum Diversity

According to previous studies, the main bacterial phyla present in maize rhizosphere include Proteobacteria, Firmicutes, Actinobacteria, Acidobacteria, and Bacteroidetes [15, 38]. In a study conducted by Correa-Galeote *et al.* (2016) [15] on maize cultivated in sandy-loam soil with a pH of 6.3, the major members of the bacterial phylum detected in the rhizosphere were Proteobacteria (29.10%), Bacteroidetes (17.03%), Acidobacteria (16.62%), Actinobacteria (6.40%), Verrucomicrobia (4.48%), and Firmicutes (3.55%). In our study, conducted in sandy-loam soil with an alkaline pH of 8.62, the ranking of phyla remains generally similar, but these variations could be attributed to the influence of the soil's alkaline pH. Specifically, we found that the abundance percentage of Proteobacteria was twice as high (61.70%). Bacteroidetes (18.32%) and Actinobacteria (6.57%) do not seem to be affected by the alkaline pH. However, the percentage of Acidobacteria is reduced by approximately half (8.46%). The percentages of Firmicutes (1.08%) and Verrucomicrobia (0.32%) appear to be significantly reduced.

At the experimental station in El Minea, where maize is cultivated, Proteobacteria dominate in all three studied compartments (bulk soil, rhizosphere, and endosphere). Their abundance increases remarkably in the rhizosphere and endosphere, suggesting their preferential adaptation to the vicinity of maize roots. This observation is consistent with previous studies that have identified Proteobacteria as being associated with beneficial interactions between plants and soil microorganisms [15, 38]. Proteobacteria are involved in many important microbial functions, such as nitrogen fixation, organic matter degradation, nutrient solubilization, and plant growth promotion [16].

Bacteroidetes and Acidobacteria are known for their role in organic matter degradation. Additionally, Bacteroidetes are involved in nitrogen fixation processes, while Acidobacteria are adapted to environmental conditions [21]. At the experimental station, although these two phyla are initially present in the bulk soil, their abundance decreases in the rhizosphere and endosphere. This may indicate stricter selection exerted by maize on these phyla or competition with other more adapted phyla. In comparison to other phyla, only Acidobacteria abundance is higher in the bulk soil than in the rhizosphere and endosphere. The results of this study suggest that Acidobacteria may be less well adapted to maize root exudates and tissues cultivated at the El Minea experimental station, which could explain their relatively low abundance in the rhizosphere and endosphere.

Actinobacteria are known to produce beneficial secondary metabolites for plants, Planctomycetes play a role in the nitrogen cycle, and Firmicutes may contribute to nutrient release [30, 50]. These roles could explain the increased presence of these three phyla in the endosphere, suggesting their involvement in specific interactions with maize tissues. They could potentially provide additional nutrients or contribute to the regulation of microbial balance within maize.

Genus Diversity

Variations observed in the abundance of bacterial genera among bulk soil, rhizosphere, and endosphere compartments indicate complex interactions between host plants and soil microorganisms (Fig. 2). Compared to the abundance percentage in the soil of the El Minea experimental station, maize significantly increased the abundance of the *Acinetobacter*, *Pseudomonas*, *Chryseobacterium*, *Acidobacteria* Gp4, and *Flavobacterium* genera in its rhizosphere, as well as the unclassified *Rhizobiales*, unclassified *Planctomycetaceae*, and *Arthrobacter* genera in its endosphere. These results suggest that these bacterial genera are favored in the maize root zone, which may be related to beneficial symbiotic interactions and specific adaptation mechanisms to the endospheric environment, allowing them to benefit from the resources and conditions provided by plant tissues. Correa-Galeote *et al.* (2016) [15] reported that maize increased the relative abundance of genera Gp4, Gp6, *Flavobacterium*, *Subdivision3 genera incertae sedis* of the phylum Verrucomicrobia, *Gemmatimonas*, *Dechloromonas*, *Ohtaekwangia*, *Rhodoferax*, *Gaiella*, *Opitutus*, Gp7, *Spartobacteria genera incertae sedis*, Terrimonas, Gp5, Steroidobacter, and Parcubacteria genera incertae sedis in its rhizosphere. The difference in the genera increased by maize reported by Correa-Galeote *et al.* (2016) [15] and our results could be

attributed, on the one hand, to the variety in question, i.e., genotype. Indeed, the maize genotype plays a role in microbiome modification [11, 38]. On the other hand, the soil characteristics of El Minea (high alkalinity, moderate salinity, low organic matter content) can influence soil biological fertility and therefore require adapted management approaches, such as improving organic matter, adjusting pH, managing irrigation, and monitoring salinity. However, in these Saharan conditions, two genera, *Acinetobacter* and *Pseudomonas*, stand out for their abundance in the rhizosphere, making them excellent candidates potentially valued by biotechnology due to their ability to confer resistance to maize plants and improve yield. These two genera have been the subject of recent intensive research [1, 2, 31, 37]. Some strains of *Acinetobacter* have been studied for their ability to promote plant growth and stimulate their immune system. They may also be involved in the degradation of soil chemical contaminants [1, 2]. *Pseudomonas* species are often used in agricultural biotechnology due to their functional diversity. Some strains of *Pseudomonas* can help plants resist pathogens by stimulating their immune system or producing antimicrobial metabolites [31, 37].

The findings of this study can be used to optimize agricultural practices in the Saharan oases of El Minea. By better understanding the bacterial diversity associated with maize, it is possible to develop soil management strategies aimed at improving crop productivity and sustainability. For example, selecting or applying beneficial bacteria belonging to genera such as Acinetobacter and Pseudomonas, which could promote maize plant growth and reduce dependence on chemical fertilizers and pesticides. Further studies are needed to deepen our understanding of these complex interactions and to develop specific management approaches.

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