COMPARISON OF ENDOPHYTIC MICROBIOME COMMUNITY AND RHIZOSPHERE IN THE DESERT PLANT *SENNA ITALICA*

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Abstract. *Senna italica* is indigenous to the desert and has important ecological and economic value in Kingdom of Saudi Arabia (KSA). Plant microbial endophytes and rhizosphere contribute significantly to plant growth, development, health, and ecological function. The variety of endophytic bacteria and rhizosphere associated with *S. italica* is still unknown. In this work, the Illumina MiSeq sequencing of bacterial 16S rDNA was used to examine the structure of the bacterial communities associated with various tissues, including roots, leaves, and rhizosphere soils. A total of 840.242 sequences and 1142 operational taxonomic units (OTUs) were obtained. *Cyanobacteria* were the most abundant bacterial phylum in endophyte samples, followed by *Actinobacteria*; and *Actinobacteria* and *Proteobacteria* were the most abundant phyla in rhizosphere soil samples. According to predictive metagenome research, endophytic bacteria and rhizosphere serve vital functional roles in *S. italica's* ability to tolerate abiotic stress. This conclusion could facilitate the study of the ecological functions of endophytic bacteria and rhizosphere and their interactions with *S. italica* to identify novel organisms that may have a role in abiotic stress resistance in the plant. Where there is still much about the endophytes and rhizosphere microbiome that may be discovered with more study.
Keywords: *metagenomics, Illumina amplicon, medicinal plant, abiotic stress, drought*

Introduction

Bacterial communities inhabit plant leaves, roots, and soil, and they play a crucial role in plant health and growth. Although deserts are one of the most hostile settings for plant development, desert plants have the potential to affect their surrounding microbial community and choose beneficial bacteria that promote their growth in these harsh conditions (Alsaedi et al., 2022). According to the United Nations, the current world population of 7.6 billion people is expected to approach 9.8 billion by 2050 (United Nations, 2019). Abiotic stressors, such as drought, account for more than half of major agricultural output losses. As a result, securing food supply for a growing population has become an essential, important, and intensive study area (Alsaedi et al., 2022).

A microbial population may be found in almost every tissue of a plant. We will concentrate on the rhizosphere and endosphere (internal tissues) in this section. The

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rhizosphere is a region of rich, mostly soil-derived microbial diversity that is influenced by plant mucilage and root exudate deposition (Kent and Triplett, 2002). Epiphytes are microbial occupants of the rhizosphere, whereas endophytes (the endophyte) are germs that live within plant tissues, either in leaves, roots, or stems. Microbes in these niches can form helpful, neutral, or harmful interactions with their host plants of varied closeness (Turner et al., 2013). Specific interactions between microorganisms and model plants, such as in Rhizobium-legume symbioses (Oldroyd et al., 2011), are well known, but the bulk of the plant microbiome, and its contribution to the extended phenotype of the host, is not yet well defined. Therefore, due to the scarcity of knowledge on the *Senna italica* microbiome, we compared rhizosphere and endophyte microbial populations in *Senna italica*. Plant roots are the principal locations where plants acquire nutrients from the soil and discharge organic molecules into the soil, facilitating plant-soil interactions (Lynch, 1995). Plant health, nutrient acquisition and absorption, biomass output, and stress tolerance are all influenced by root-associated bacteria (Castrillo et al., 2017; Puri et al., 2019).

Senna italica belongs to the Fabaceae family, (subfamily Caesalpinaceae) (Yagi et al., 2013; Adjou et al., 2021). The Fabaceae or Leguminosae Family, often known as the legumes, is the third largest plant family, with over 730 genera and over 19,000 species. Senna is an important genus of flowering plants, with over 350 species (Rahman and Parvin, 2014; Khalaf et al., 2019).

The metagenomic technique has enabled the discovery of both culturable and unculturable microorganisms, as well as the definition of microbial community structure. Phytobiomes are classified as rhizospheric and phyllospheric microorganisms microorganisms based on the host plant tissue components (Quiza et al., 2015).

This study aimed to investigate and compare the endophytic and rhizosphere bacterial communities associated with the leaves and roots and soil of the desert medicinal plant *Senna italica* by applying metagenomics techniques and their role in abiotic stress resistance in plants.

Materials and Methods

Study location

The study region was situated in Asfan, Saudi Arabia, northeast of Jeddah (latitude: 21.53’13.3” N, longitude: 39.15’06.6” E, and altitude: 2.8 m) above sea level. In the Asfan area, the climate is described as hot, dry, and sandy with decrease a in precipitation level of rainfall percentage (of 10%), the average April temperature in 2021 was between (29 and 33°C). Despite all these traits, the *Senna italica* plant communities are expanding considerably in this area.

Sample collection

A total of ten samples were collected from a single desert plant, the *Senna italica* plant was collected. six samples were collected, three samples were taken from roots, and the other three were collected from leaves. while the other samples were collected rhizosphere area, three samples were taken from the rhizosphere (same depth), and the fourth sample, a free-soil sample, was used as a control, which was accumulated from a close area of non-plant growth. The control sample was used to compare microbes in the

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rhizosphere. Each root and leaf were cut into small pieces before being frozen in liquid nitrogen (-196°C) and stored at -20°C until further examination.

Illumina amplicon sequencing, 16S rRNA gene sequencing, and PCR

Soil, leaf, and root samples were sent to Macrogen Inc. in Seoul, South Korea, where genomic DNA was isolated from each sample. A Picogreen fluorescence-based quantification method (Invitrogen, cat. # P7589) was used to assess DNA purity and quantity.

By employing the universal primers (Bakt 341F: CCTACGGNGGCWGCAG) and (Bakt 805R: GACTACHVGGGTATCTAATCC), PCR was used to amplify bacterial V3-V4 16S rRNA gene segments. Amplification was performed after five minutes of denaturation at 95 °C. After 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 40 s, and extension at 72 °C for 1.30 s, a final elongation at 72 °C for 10 minutes is performed (Lorenz, 2012).

Illumina SBS technology was used to deep sequence the purified amplicons and create libraries. 300 bp pair-end reads of the V3 and V4 sections were then isolated and chosen from the Illumina-recommended library (Quast et al., 2013). The V3 and V4 regions have emerged as the most popular amplicon targets in microbiota studies since Illumina revealed a recommended library preparation approach for sequencing on the MiSeq technology (Wu et al., 2020).

Processing and statistical analysis of the 16S dataset

The raw sequencing data was transferred as FASTA files for each sample, as well as sequencing quality files. The files were accessed using the bioinformatics tool Quantitative Insights Into Microbial Ecology (QIIME), where they were processed and analyzed in accordance with the general protocols indicated recommended by (Caporaso et al., 2010). An open-source bioinformatics tool called QIIME can analyze the microbiome using raw DNA sequencing data provided by Illumina or other sequencing programs. Further features offered by QIIME include raw read quality pretreatment, taxonomic assignment, operational taxonomic unit (OTU) selection, phylogenetic reconstruction, diversity analysis, and graphical presentations. The QIIME tool was used for all statistical analyses (Macrogen, 2017).

OTU analysis

The CD-HIT-OTU tool is a multi-step workflow that generates OTU clusters for ribosomal RNA (rRNA) tags from Illumina platforms after trimming and filtering the V3-V4 16S rRNA sequencing data. Furthermore, the CD-HIT-OTU-MiSeq can cluster the samples and the spliced Paired-End reference database, allowing the OTUs to be identified.

To weed out low-quality sequences, paired-end reads from next-generation sequencing investigations were combined using the FLASH tool. Sequences were filtered by length and quality, de-replicated, and assigned to particular samples (length: 350–450 bp; quality threshold: 20). With UCLUST (a cutting-edge clustering technique used for clustering sequences within a similarity threshold to a reference sequence that would cluster to an OTU) and 97% detected clustering, the resulting sequences were assigned and sorted into OTUs. From each OTU, the sequence with the highest abundance was chosen. Chimera

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Slayer was used to eliminate the chimeric sequences. Using the Ribosomal Database Project Classification, bacterial taxonomy was determined based on the OTUs results.

Results

Analysis of sequencing data and bacterial community diversity

840.242 high-quality tag sequences were obtained with an average length of 250 bp across endophyte and rhizosphere after sequence denoising and quality filtering. The obtained sequences ranged from 69.799 to 101.468, with an average of 84.0242±9.54 (mean±SD) sequences across all ten samples.

All quality-filtered sequences were clustered into 1142 OTUs at a 97% similarity. The number of clustered OTUs varied from 13.0 to 283. All rarefaction curves gradually saturated with increasing sequencing quantity in all 10 samples that covered the entire group. The results of rarefaction curves showed that OTU abundance was diverse in different endophyte and rhizosphere samples (*Figure 1*). The numbers of OTUs were significantly higher in the rhizosphere samples than in the endophyte samples (soil.3; 283, soil.1; 265, soil.2; 255, then control; 235). The root and leaves samples revealed a higher number of OTUs (24 and 18 respectively), while Leaves.1, Leaves.2, and Roots.2 and Roots.3 showed lower richness, with 13, 17, and 15 OTUs, respectively (*Figure 2*).

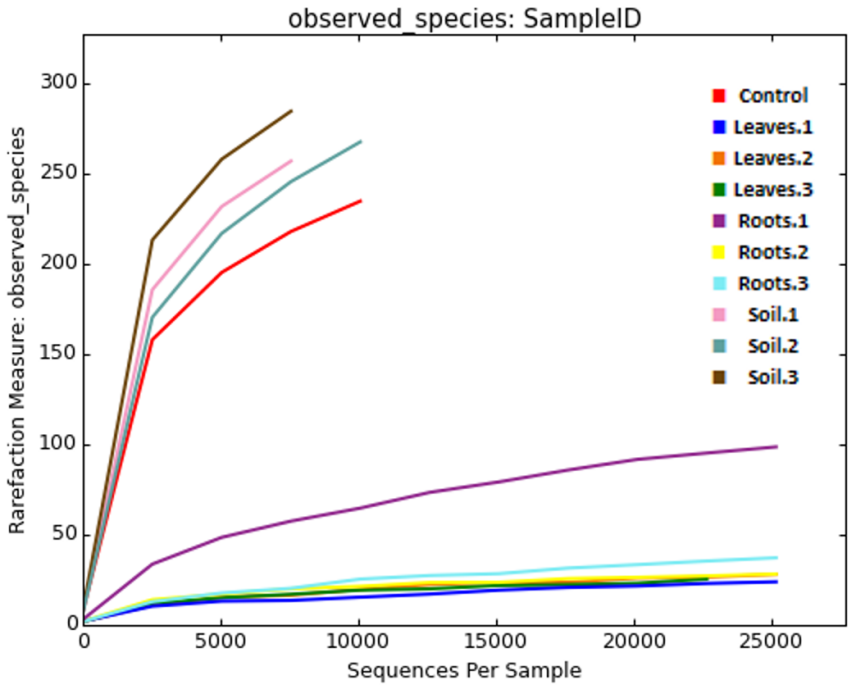

Figure 1. *Alfa rarefaction curve observed based on observed species (OTUs) value. The curve has shown flatter to the right, which indicates the comparatively high species richness that is associated with Senna italica samples*

Among the types of samples, the highest richness and diversity of the bacterial community were found in the rhizosphere soil samples, the richness of the bacterial community in the soil.3 was higher than that in the soil.1, and the lowest richness and diversity were observed in the control (*Table 1*).

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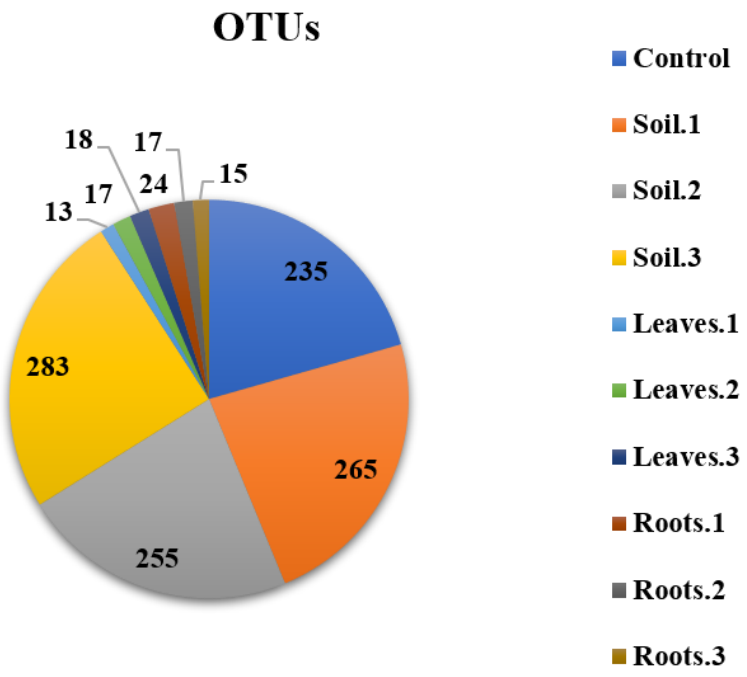

Figure 2. *The number of OTUs generated for each sample. The Soil.3 sample had the most OTUs of 283, while the Leave.1 sample had the fewest of 13*

Table 1. *Number of OUTs and Alpha diversity of endophytic and rhizosphere bacteria in Senna italica*

Sample Name	Number of sequences	OTUs	Alpha diversity			
			Chao1	Shannon	Inverse Simpson	Good's Coverage
Control	76.899	235	313.12	5.06690179	0.92266147	0.99
Soil.1	92.112	265	322.6	5.41719838	0.941282189	0.99
Soil.2	88.958	255	328.7	5.29433893	0.936824728	0.99
Soil.3	82.350	283	303.8	5.73210289	0.950355062	0.99
Leaves.1	83.006	13.0	13.5	0.61571822	0.198657743	0.99
Leaves.2	91.718	17.0	23.0	0.63584698	0.200129437	0.99
Leaves.3	69.799	18.0	28.0	0.66064383	0.206815614	0.99
Roots.1	101.468	24.0	25.0	1.20627592	0.431553623	0.99
Roots.2	79.090	17.0	17.0	0.944092778	0.347332416	1.0
Roots.3	74.842	15.0	15.0	0.902923313	0.3315832831	1.0

In the endophyte samples, Leaves.3 and Roots.1 had a relatively close diversity, whereas the richness of the bacterial community in the Leaves.3 was higher than that in Roots.1, and the lowest richness and diversity were observed in Leaves.1, Roots.3, Roots.2 (*Table 1*).

Taxonomic examination of microbial at the phylum and class levels

With the aid of QIIME, the tag numbers for each OTU or taxonomic rank, from phylum to species, in various samples were compiled. The grouping of genomes also

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demonstrated phylum-level variations in bacterial communities between endophytic and rhizosphere soils. The sequences in this study were grouped into 22 different bacterial phyla.

comparable to the phyla, was different than rhizosphere soil samples of the endophyte samples, it was obvious that *Cyanobacteria* was the most dominant phylum, accounting for 72.40–89.08% of all bacterial sequences in the different tissues, followed by *Actinobacteria* (27.55–10.91%). The abundance of each phylum varied in the various tissues, whereas the plenty of these *Firmicute*, *proteobacteria*, and other phyla was less than 1% in other tissues (*Figure 3-A*).

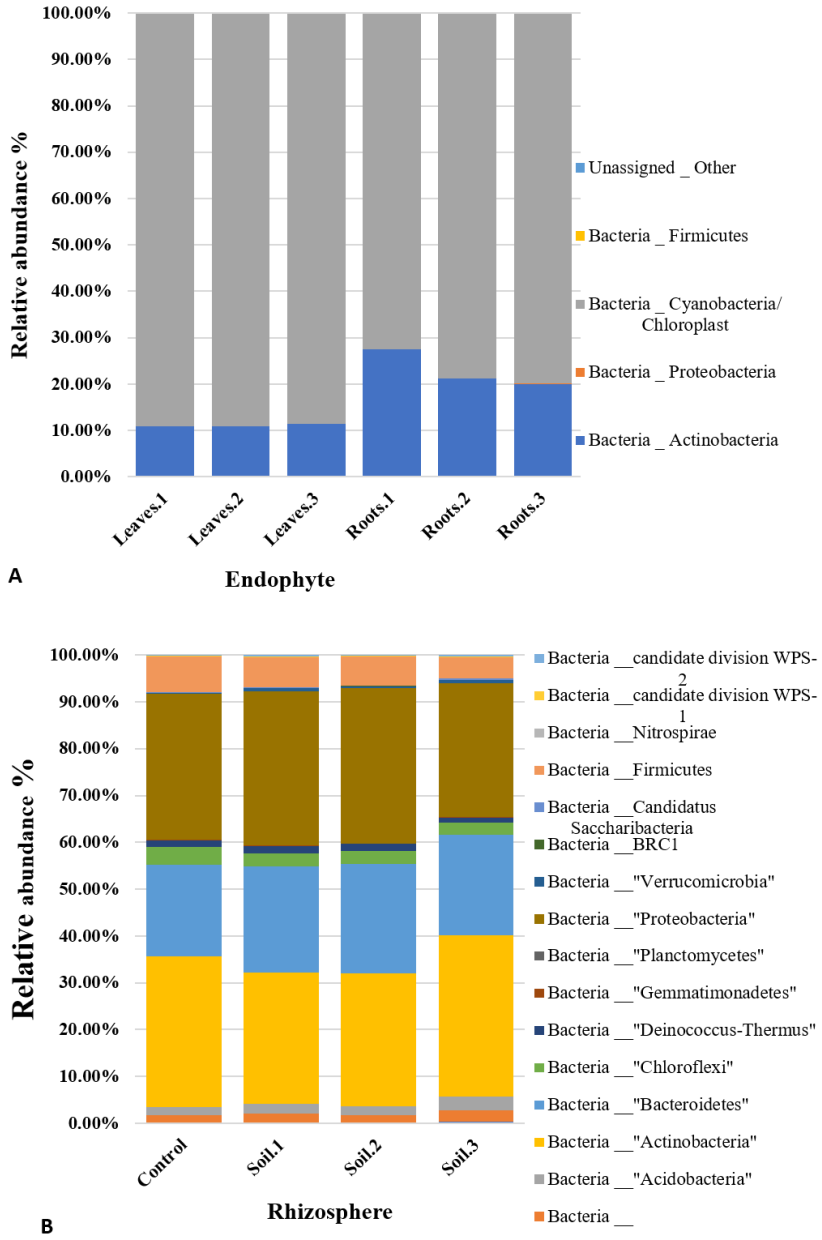

Figure 3. *The relative abundances of bacterial communities at the phylum level; (A) Endophyte and (B) Rhizosphere in the different samples that are associated with Senna italica plant*

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The abundance of bacterial communities was significantly higher in the rhizosphere soil than in the endophytes. *Actinobacteria* and *Proteobacteria* were the dominant phyla, accounting for 34.41–28.06% and 33.16–28.59% of all bacterial sequence respectively, followed by *Bacteroidetes* (23.48) -(19.45%) *Firmicutes* (7.61–4.4%), unclassified phyla (2.42-1.59%) (*Figure 3-B*).

At the class level, species in *Chloroplast* and *Actinobacteria* showed virtually absolute dominance, representing 89.08% -72.40% and (27.55%-10.91%). However, *Proteobacteria*, *Firmicutes* and Other whereas their abundance was less than 1% in other tissues (*Figure 4-A*). According to Macrogen (2017) analysis, Firmicutes and unidentified bacteria were identified and were found in the sequence of the read in all samples of endophytic but in very negligible proportions.

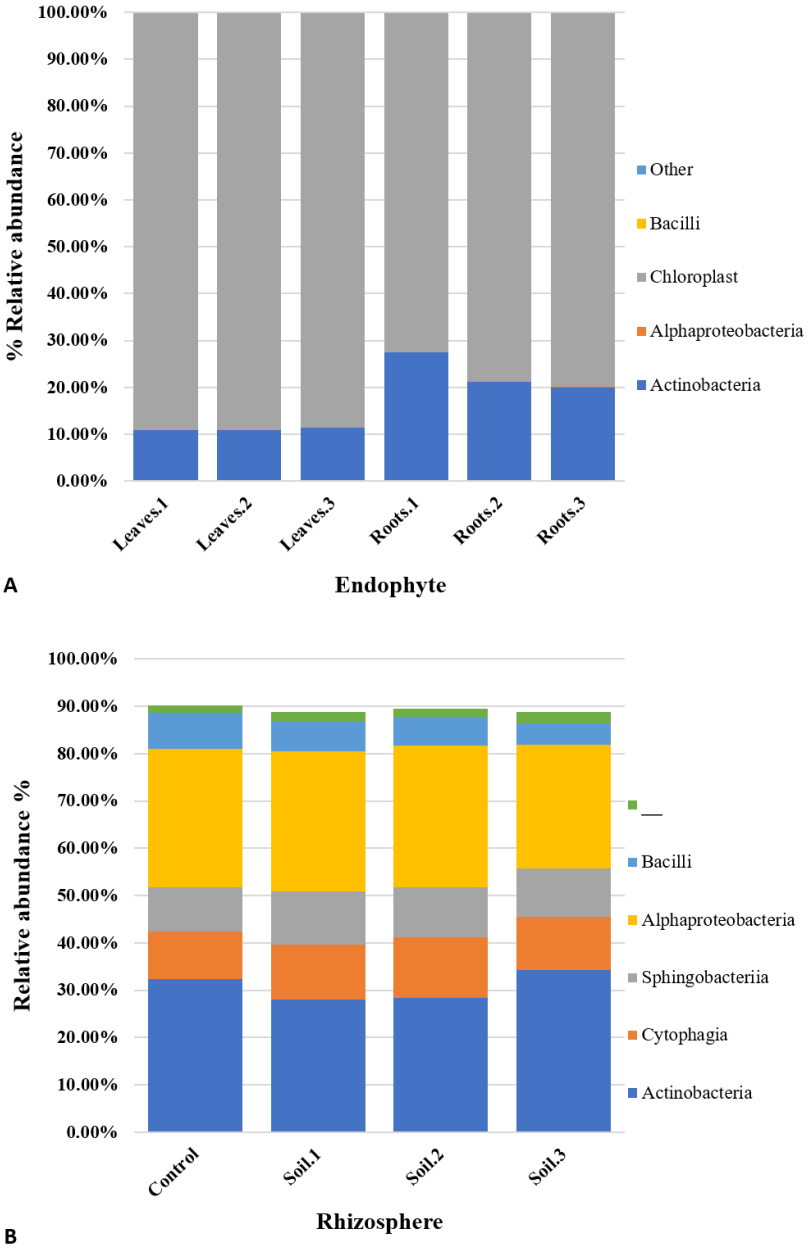

Figure 4. *The relative abundances of bacterial communities at the class level; (A) Endophyte and (B) Rhizosphere in the different samples that are associated with Senna italica plant*

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In the rhizosphere soil, *Actinobacteria* (34.35%-28.05%) followed by *Alphaproteobacterial* (29.90%-26.26%), cytophagua (12.86%-10.13%), *Sphingobacteria* (11.13%-9.31%), *Bacilli* (7.58%-4.40%) and others were also predominant (> 1% relative abundance) in soil samples (*Figure 4-B*).

In the endophyte, at the guns level, *Okibacterium* and *Streptophyta* genera were found in all endophyte samples, where it was found in the samples with percentages were (10.91% - 27.48%) and (72.40% - 89.08%) respectively (*Figure 5-A*).

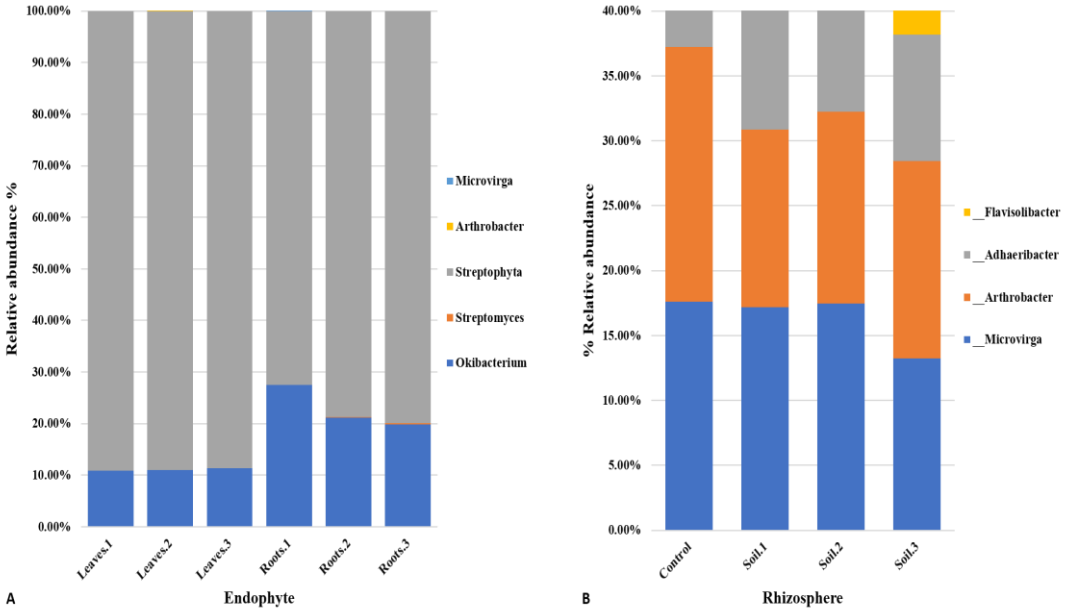

Figure 5. *The relative abundances of bacterial communities at the genus level; (A) Endophyte and (B) Rhizosphere in the different samples that are associated with Senna italica plant*

In the rhizosphere, at the genus level, species in *Flavislibacteria* and *Adhaeribacter* showed virtually absolute dominance in all rhizosphere soil samples, representing (10.45%) -(8.65%) and (10.34%) -(8.21%) (*Figure 5-B*).

At the genus level, species have common genera in endophytes and rhizosphere soils, *Arthropods* and *Microvirga* are found more in rhizosphere, while in endophytes >1% (*Figure 6*).

Table 2 displays the abundances of the classes over 1% in each tissue. These findings demonstrated that the relative richness of bacterial communities was substantially higher in the rhizosphere soil than in the plant tissues.

Comparison analysis of the bacterial populations in several samples

A principal coordinate analysis (PCoA) at the OTU level was used to assess the degree of similarity between the endophytic and rhizospheric bacterial populations, which helped to display the differences in OTU composition in the various samples (*Figure 5-A*). The PCA showed that the bacterial community structures were similar in the tissues of the endophytic and rhizosphere however, there were notable differences in the community structures between the tissues of the roots and soils, demonstrating that the roots and soils had their own distinct bacterial community structures (*Fig. 7-A*). Complete linkage clustering (CLC) tree analysis outcomes were comparable to PCA outcomes (*Fig. 7-B*).

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While the root and soil samples were grouped together, the roots and other tissues in the plant tissues were divided into two distinct groups (*Fig. 7-B*). Results showed that the microbiota in these rhizospheres was significantly different from those in other samples.

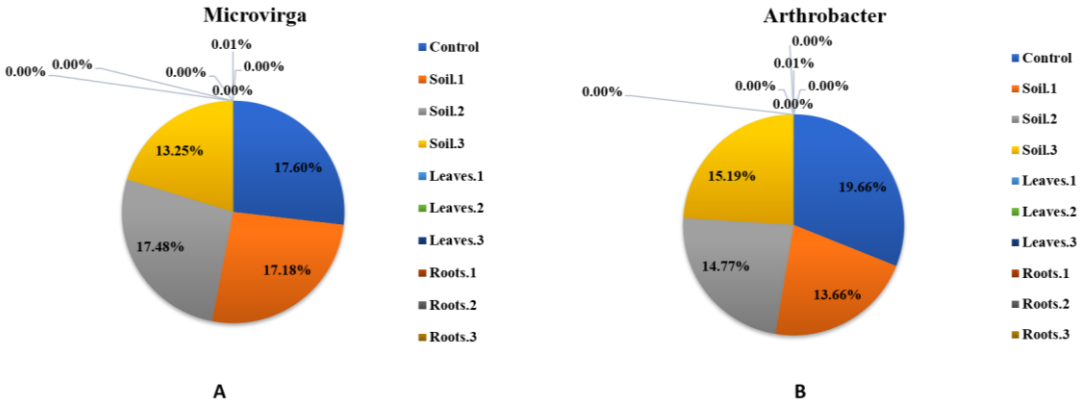

Figure 6. *The common genera in both the endophyte and rhizosphere are associated with the Senna italica plant*

Table 2. *The relative abundances of the classes exceeding 1% in all sample*

The relative abundance (>1%)	Control	Soil.1	Soil.2	Soil.3	Leaves.1	Leaves.2	Leaves.3	Roots.1	Roots.2	Roots.3
Cytophagia	10.13	11.66	12.86	11.10	/	/	/	/	/	/
Sphingobacteriia	9.31	11.13	10.62	10.20	/	/	/	/	/	/
Actinobacteria	32.31	28.05	28.33	34.35	10.91	10.97	11.35	27.55	21.27	20.06
Alphaproteobacteria	29.23	29.65	29.90	26.26	/	/	/	/	/	/
Bacilli	7.58	6.31	6.10	4.40	/	/	/	/	/	/
Other	1.59	2.06	1.68	2.42	/	/	/	/	/	/
Chloroplast	/	/	/	/	89.08	89.03	88.64	72.40	78.71	79.91

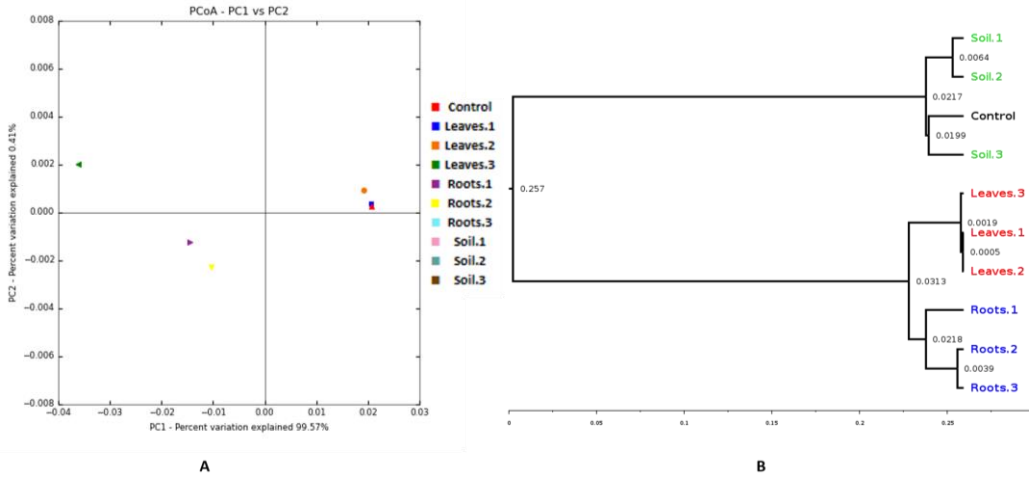

Figure 7. *Beta diversity analysis: (A) principal coordinate analysis (PCoA) based on the relative abundance of bacterial OTUs. (B) The complete linkage clustering (CLC) of the bacterial communities in different samples based on unweighted UniFrac*

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Discussion

The structure of the soil and endophytic microbial communities can reveal new information about microorganisms in the rhizospheric and endophytic microbiomes that may benefit plant development (Berendsen et al., 2012). Previous studies found that soil and endophytic bacteria play an important role in the maturity of tobacco and rice seeds (Okunishi et al., 2005; Xiaolin et al., 2015). The information presented here is intended to investigate the bacterial diversity of the medicinal plant *Senna italica's* rhizosphere and endophytes. Endophytic bacteria have been shown to be crucial for plant growth in research on plants using Illumina amplicon sequencing (Weyens et al., 2009; Badri et al., 2009; Müller et al., 2016; Lu et al., 2020). To our knowledge, this is the first report of Illumina amplicon sequencing being used to examine the makeup of the rhizosphere and endophytic bacterial community in the plant *Senna italica*. The sequence analysis revealed that endophytic bacteria were clustered into 5 phyla. In comparison, the rhizosphere was clustered into 17 phyla. Our results revealed that the proportions of *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and unclassified phyla in rhizospheres were higher compared with the endophytic bacterial phyla. Additionally, *Actinobacteria* and *Cyanobacteria* were the unique, dominant, endophytic bacterial phyla. Furthermore, the unique, dominant, endophytic bacterial genera *Okibacterium* and *Streptophyta* were the most abundant genera in root and leaf-associated endophyte communities, which fall under *Actinobacteria* and *Cyanobacteria*, respectively. In contrast, *Microvirga* and *Arthrobacter* were the unique, dominant, rhizospheres bacterial genera of the soil-associated rhizosphere communities. This was consistent with many previous studies we found in Huang (2012), Jin et al. (2014), Madhurama et al. (2014) and Zhang et al. (2019). Despite abiotic stress and nutritional shortage, desert plants were colonized by many endophytic and rhizospheric *Actinobacteria*, most notably *Streptomyces*, followed by other uncommon genera and novel species such as *Okibacterium*, *Streptophyta*, *Arthrobacter*, and *Flavisolibacter* (Singh and Dubey, 2018). However, investigations on desert plant endophytes and their rhizosphere remain unexplored.

In the endophytic communities, it is evident that *Cyanobacteria* was the most dominant phylum, accounting for 72.40–89.08% of all bacterial sequences in the different tissues, followed by *Actinobacteria* 27.55–10.91%. In desert-like settings, (high radiation, drought, salt stress, and so on) despite these circumstances, the *cyanobacteria* can maintain photosynthetic metabolism (Harel et al., 2004; Chen et al., 2013; Singh et al., 2013; Alsaedi et al., 2022). *Cyanobacteria* are well-known for their effective contribution to nitrogen fixation (Issa, 2014). For instance, as stated in a study by Mishra and Pabbi (2004) using *cyanobacteria* as a bio-fertilizer in rice fields might prove to be an affordable and ecologically friendly strategy to boost rice output. *Cyanobacteria* also work as plant growth promoters and dietary additives. In field bioremediation and biofertilizers, a variety of *cyanobacterial* species are utilized. Based on our findings, this is compatible with the study's objective; hence, the use of *cyanobacteria* as biofertilizers will reduce reliance on harmful fertilizers - that may reduce soil fertility - and may boost efforts for a more sustainable environment and ecosystem. *Cyanobacteria* are well-known bacteria with several pharmacologically active activities, including antibiotic, anti-inflammatory, and antioxidant properties (Sonani et al., 2014; Chaubey et al., 2019). *S. italica* has anti-inflammatory and antioxidative functions (Ondua et al., 2019), and *Cyanobacteria* is the dominant phylum in *S. italica's* endophytic bacterial community; thus, the medicinal value of *S. italica* may be associated with *Cyanobacteria*, and

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Cyanobacteria's pharmacological characteristics may contribute to *S. italica's* high-quality medicinal value.

Actinobacteria and *Proteobacteria* are microbiome communities that are widespread in different plants and soils (Manter et al., 2010; Huang et al., 2019; Lin et al., 2019). In the rhizosphere soil communities, similarities were examined, *Actinobacteria* and *Proteobacteria* were the two phyla that made up the bulk of the rhizosphere soil, accounting for 34.41–28.06% and 33.16–28.59% of all bacterial sequences respectively. Our findings revealed a greater preponderance of *Actinobacteria* in rhizosphere soil communities than in endophyte communities, which was also seen in previous research such as a study by Yadav et al. (2018). Other research has also observed a variety of yield plants, including barley, maize, grapevine, and rice (Bulgarelli et al., 2013; Hernández et al., 2015; Zarraonaindia et al., 2015; Alsaedi et al., 2022). According to previous reports by Jeon et al. (2003), Kragelund et al. (2007) and Lin et al. (2019), *Proteobacteria* have been reported to play a significant part in natural processes and have the potential for use in wastewater treatment, increasing tolerance to contaminants, and enhancing the soil environment. In addition to its active participation in the global carbon, nitrogen, and sulfur cycles (Zhao et al., 2018). *Actinobacteria* were more ubiquitous in all samples of *Senna italica*, *Actinobacteria* are crucial in the production of antibiotics and have high UV and dehydration tolerance (Baeshen et al., 2020). They are adaptively resistant to drought and are most prevalent in desert soils, and it has been demonstrated that *Actinobacteria* can thrive in dry soil with low osmotic potential and a comparatively higher prevalence (Bouskill et al., 2013).

Our findings from several phyla demonstrated their capacity to withstand abiotic stress, such as salt and drought. Numerous studies at the genus level have proved the advantages of bacteria in environmental, medicinal, industrial, and agricultural applications. A comparison of endophyte communities reveals both universal and organ-specific groupings. *Okibacterium*, for example, was the most prevalent genus among root and leaf-associated communities, which are classified as *Actinobacteria*. Although the *Okibacterium* genus was discovered in all endophyte samples, it was found in roots samples at a greater incidence than in leaves samples. But until now no reports on this endophyte are known.

Between *Cyanobacteria*, the *streptophyte* was found significantly among all endophyte samples, with a higher proportion in the leaves than in the roots. *Streptophyta* is essential for primary production, nitrogen fixation, and nutrient cycling, and it can form a symbiotic connection with soil particles. They provide important ecological roles in primary production, water retention, and soil stability (Glaser et al., 2017). However, little is known about the interactions of *Streptophyta* endophytes with plants, and until now no reports on this endophyte are known.

Arthrobacter (Phylum: *Actinobacteria*) was detected in a soil sample and is frequently used in many applications. Its unique functions have been widely recognized by research institutions in fields, such as agriculture, medicine, industry, and the environment. *Arthrobacter* plays a crucial function in the nitrogen cycle in rhizospheric burnt soils, and plans for this type of ecological environment in the future (Fu et al., 2014).

The most prevalent genus in soil samples, *Microvirga*, is a member of the *Proteobacteria*, which can enhance soil nutrients, encourage plant growth, and prevent disease caused by soil-borne pathogens (Wang et al., 2017). However, there is currently little biochemical knowledge available regarding this genus (Li et al., 2020).

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In rhizosphere soil, two species of the genus *Adhaeribacter* and *Flavisolibacter* were found to be elevated, but we had no previous reports of their role in mitigation Abiotic factors deserve attention in future studies, which will help researchers better comprehend new candidates for biological agents employed to improve agricultural and industrial processes.

The findings showed a connection between soil microorganisms and endophytic bacteria. It is therefore possible that *Senna italica* has developed mutualistic relationships with microorganisms and has a great capacity for adaptation to local environmental conditions.

Conclusion

In this study, the variety of endophytic bacterial populations linked with the desert medicinal plant *Senna italic* (leaves, roots, and soils) is investigated. The bacterial diversity of *Senna italica* samples was examined using Illumina Miseq with the 16S rRNA gene as the biomarker to gain a better understanding of endophytic and rhizosphere bacteria. Our findings show that endophytes and rhizospheric bacteria may be utilized to predict plant growth rate and survival in harsh settings. This distinguishing characteristic was identified in all members of the phylum studied. In the endophytes area, two species of the genus *Okibacterium* and *streptophyte were found,* while in the rhizosphere, two species of the genus *Adhaeribacter* and *Flavisolibacter* were found in high proportions, but we had no previous reports of their role in the mitigation of abiotic factors. Finally, we recommend doing more bioinformatic and functional analyses to examine the influence of this microbiome on plant growth-promoting processes and to comprehend the symbiotic connections with host plants under drought-stress circumstances at the molecular level.

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