COMPARING THE MICROBIAL DIVERSITY OF *SENNA ITALICA* LEAVES AND ROOTS WITH THE RHIZOSPHERE

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(Received 1st Feb 2024; accepted 8th Jul 2024)

Abstract. This study used metagenomic sequencing via Illumina high-throughput sequencing of V3-V4 16S rRNA to investigate and analyze the endophytic and rhizosphere bacterial communities associated with the soil, roots, and leaves of the desert medicinal plant *Senna italica*. Among the ten samples analyzed, those from the roots (1) had the highest read count (101,486), while those from the leaves (3) had the lowest read count (69,799). Operational taxonomic unit (OTU) analysis yielded 1351 units, with rhizosphere soil samples showing significantly greater OTU counts than endophytes. Taxonomic analysis at the phylum and order levels revealed distinct bacterial communities between the rhizosphere and endophytic environments, with Actinobacteria (ranging from 27.55% to 10.97%) and Proteobacteria (33.16% to 28.59%) dominating across samples. Actinomycetales, Rhizobiales, and Bacillales notable orders and mainly enriched in rhizosphere soil (>1% abundance). Microbial composition analysis via UPGMA tree and PCoA highlighted microbial diversity variations, revealing similarities between specific leaf samples and distinctive clustering among root samples. This study underscores the diverse microbial communities within *S. italica*, emphasizing the differential composition and structure of the rhizosphere (which is more prevalent in specific taxa such as Actinobacteria and Proteobacteria) and endophytic habitats. Overall, these findings shed light on the intricate relationships between *S. italica* and its associated microbial communities, offering insights into the ecological dynamics within its ecosystem and potential implications for plant health and growth.
Keywords: *Senna italica, bacterial communities, metagenomic sequencing, endophytic bacteria, drought stress*

Introduction

Senna italica, a member of the Fabaceae family (subfamily: Caesalpinaceae), has noteworthy medicinal properties and has played a significant role in human healthcare for millennia (Tshikalange et al., 2005). *Senna italica* has gained popularity for its ability to enhance health, prevent illnesses, and treat various ailments (Adjou et al., 2021). For instance, different Senna species are effective at treating sexually transmitted infections (STIs), and some of these species exhibit notable antibacterial effects (Tshikalange et al., 2005), underscoring the pharmacological significance of *S. italica* and justifying its use in treating diverse diseases (Dabai et al., 2012).

Deserts represent some of the most challenging terrestrial ecosystems due to intense solar radiation, limited rainfall, and extreme temperatures (Eida et al., 2018). Bacterial communities in soil, on leaves, and within roots play crucial roles in shaping plant growth and well-being. Despite the harsh conditions for plant life, desert vegetation can actively shape the microbial environment, favoring beneficial bacteria that support plant growth in such demanding surroundings (Alsaedi et al., 2022).

By 2030, the world's population is projected to reach 8 billion and is expected to increase further, exceeding 9 billion by 2050. This rapid population growth will exert

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_65136525

tremendous pressure on the global agri-food system, leading to unprecedented challenges in terms of food production, distribution, and sustainability (Serraj et al., 2019).

all parts of a plant contain a community of microbes. This section will specifically focus on the rhizosphere and endosphere (internal tissues). The rhizosphere surrounds the space surrounding plant roots and plays a crucial role in nurturing mutually beneficial chemical interactions between plants and soil microorganisms. This partnership positively enhances plant productivity and fortifies plant capacity to withstand different stresses encountered during various growth stages (Gull et al., 2019; Barra Caracciolo and Terenzi, 2021).

The endophytic bacteria found in *Senna italica* have been identified as crucial agents for improving plant growth, enabling nutrient accessibility, enhancing tolerance to various stresses, such as abiotic stress (Alswat et al., 2023; Madouh and Quoreshi, 2023), and offering biological protection against pathogens. These significant contributions substantially augment the overall well-being and vigor of the plant (Prasad et al., 2019; Alsaedi et al., 2022; Fadiji et al., 2022; Alswat et al., 2023).

In recent studies, metagenomics has been utilized to gain deeper insights into the microbial communities found in diverse ecosystems) (Ullah et al., 2019; Baeshen et al., 2020).

The characterization of microbial community composition and the identification of both cultivable and nonculturable bacteria have been facilitated through metagenomics. Phytobiomes, categorized according to plant tissue components, encompass microorganisms residing in the rhizosphere and phyllosphere (Quiza, St-Arnaud and Yergeau, 2015).

This study used metagenomic techniques to examine and analyze the endophytic and rhizosphere bacterial communities associated with the soil, roots, and leaves of the desert medicinal plant *Senna italica* and their involvement in the ability of plants to withstand abiotic stress.

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Materials and methods

Study area and sample collection

Ten samples of *Senna italica*, including 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 with a depth of 18-25 cm below the first layer, and the fourth sample, a free-soil sample, was used as a control same depth as the samples, which was accumulated from a close area of non-plant growth., were collected in triplicate from the challenging environment of the Asfan region in Saudi Arabia in April 2021. The geographical coordinates of the collection site were recorded as follows: latitude 21.53'13.3" N, longitude 39.15'06.6" E, and altitude 2.8 meters. The Asfan region has a challenging environment characterized by extreme conditions, including scorching temperatures reaching 52°C, arid sandy terrain, and minimal rainfall. Notably, control soil samples devoid of plant material were also collected for comparison. To maintain their integrity, the samples were carefully placed in separate sterile plastic bags in a 4 °C insulation box during transportation. Upon arrival at the laboratory, DNA extraction was conducted to analyze the plant's endophytic and rhizosphere bacterial communities. This process ensured the preservation of the samples for further molecular analyses.

Illumina amplicon sequencing

The soil, leaf, and root samples were processed at Macrogen, Inc., in Seoul, South Korea. After genomic DNA extraction, PCR amplification of the bacterial 3-V4 16S rRNA gene segments was performed, and subsequent sequencing was performed using Illumina SBS (Sequencing by Synthesis) technology. DNA purity and quantity were

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quantified utilizing the PicoGreen fluorescence-based quantification method (Invitrogen, cat. # P7589).

PCR amplification utilized universal primers (Bakt-341F: 5'-CCTACGGGNGGCWGCAG-3' and Bakt-805R: 5'-GACTACHVGGGTATCTAATCC-3') targeting region V3-V4 of the bacterial 16S rRNA gene (Risse et al., 2001), involving an initial denaturation step at 95°C for five minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 1 minute and 30 seconds, with a final elongation at 72°C for 10 minutes (Lorenz, 2012), resulting in amplicons containing a unique sequence as required by Illumina Technology (Illumina, Inc., CA, USA).

The sequencing libraries were prepared using Illumina SBS technology, selectively targeting 300 bp paired-end reads corresponding to the V3 and V4 regions following Illumina's recommended criteria (Quast et al., 2013). Illumina's prescribed library preparation strategy for MiSeq sequencing has notably highlighted the significance of the V3 and V4 regions in microbiota studies (Liu et al., 2020).

Statistical analysis

The raw sequencing data, stored in each sample's FASTA file of each sample along with quality files, were initially processed using Quantitative Insights Into Microbial Ecology (QIIME) software (Caporaso et al., 2010) (http://qiime.org/). QIIME facilitated quality preprocessing, taxonomic categorization, phylogenetic reconstruction, diversity analysis, operational taxonomic unit (OTU) selection, and graphical display.

The CD-HIT-OTU tool (http://weizhongli-lab.org/cd-hit-otu/) was used to create OTU clusters from Illumina platform ribosomal RNA (rRNA) tags. This multistep process involved filtering and trimming V3-V4 16S rRNA sequence data. Additionally, the CD-HIT-OTU-MiSeq method was utilized to cluster the spliced paired-end reference database and samples together to derive OTUs.

To address low-quality sequences, next-generation paired-end sequencing reads were merged using the FLASH program (http://ccb.jhu.edu/software/FLASH/), aiming for sequences within specific length and quality parameters (length: 350–450 bp; quality threshold: 20). OTUs generated with a 97% identity cutoff were employed for sequence set linkage and classification. The Ribosomal Database Project (RDP) Classifier determined the taxonomic composition.

Results

Bacterial community structure

The microbial community composition and diversity of ten *S. italica*-associated samples were investigated through metagenomic analysis utilizing Illumina SBS and the 16S rRNA gene. *Figure 1* shows the distribution of reads among the ten samples. Within the rhizosphere soil, the sample with the highest read count was observed in soil1 (92,112), whereas the control sample had the lowest count (76,899). Among the endophyte samples, Roots (1) had the highest read count (101,486), while the lowest read count was detected for Leaves 3 (69,799) (*Figure 2*).

At a similarity threshold of 97%, a total of 1351 operational taxonomic units (OTUs) were formed after filtering the sequences for quality. These OTUs, which ranged in number from 26 to 279 across the clustered samples, demonstrate the breadth of our

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research. When comparing the rhizosphere samples to the endophyte samples [soil (3); 297, soil (1); 275, soil (2); 270, and control; 243], a statistically significant increase in the OTU count was observed. specifically, roots1 and leaves2 exhibited higher OTU counts, with 117 and 30 OTUs, respectively. Conversely, the roots (3), roots (2), leaves (2), leaves (1), and leaves (3) had lower OTU counts compered to control sample, with 38, 29, 30 and 26 OTUs, respectively (*Figure 3*).

Figure 1. *Pictures showing ths location of the sampling site (A) types of habitats in its surroundings, (B) Senna italica plant (leaves and stems), (C) the soil and rhizosphere area*

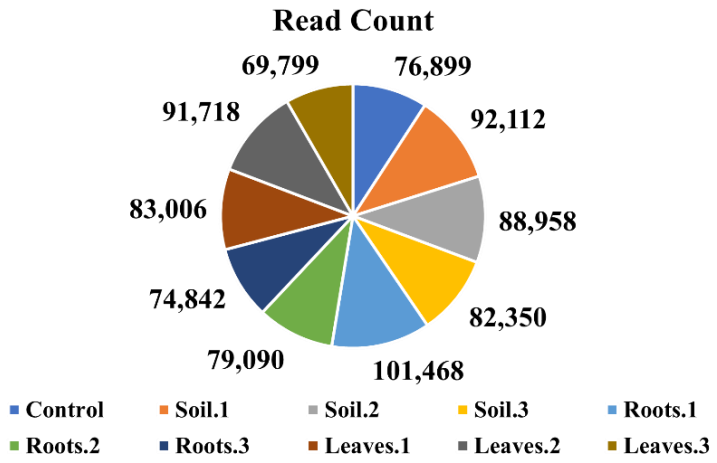

Figure 2. *Read count analysis for soil, root, and leaf samples from S. italica plants*

Microbial taxonomic analysis was conducted at multiple levels, including phylum, order, and family levels, utilizing QIIME to gather tag numbers for each OTU or taxonomic rank across various samples. A comparison between endophytic and rhizosphere soils highlighted distinct bacterial communities at the phylum level, revealing differences among 22 bacterial phyla observed in this study.

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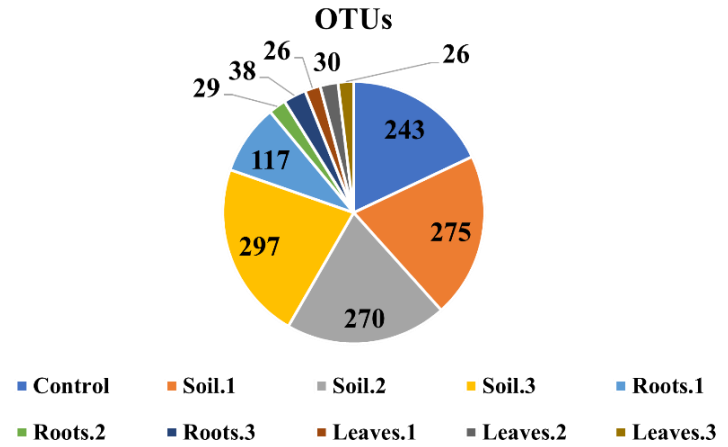

Figure 3. *The quantity of OTUs generated in each sample. The soil (3) sample yielded the highest number of OTUs, totaling 297, while the leaf (1) sample had the lowest number of OTUs, with 26 OTUs*

Actinobacteria, with their noticeable presence ranging from 27.55% to 10.97%, and Proteobacteria, both of which varied significantly across different tissues, are key findings of our research. Rhizosphere soil contained significantly more abundant bacterial communities than endophytes, with Actinobacteria and Proteobacteria constituting 34.41% to 28.06% and 33.16% to 28.59%, respectively, of the total bacterial sequences (*Figure 4B&C*). Additionally, Firmicutes (7.61% to 4.40%) and unclassified phyla (2.42% to 1.59%) were among the next most prevalent phyla observed (*Figure 4A&D*).

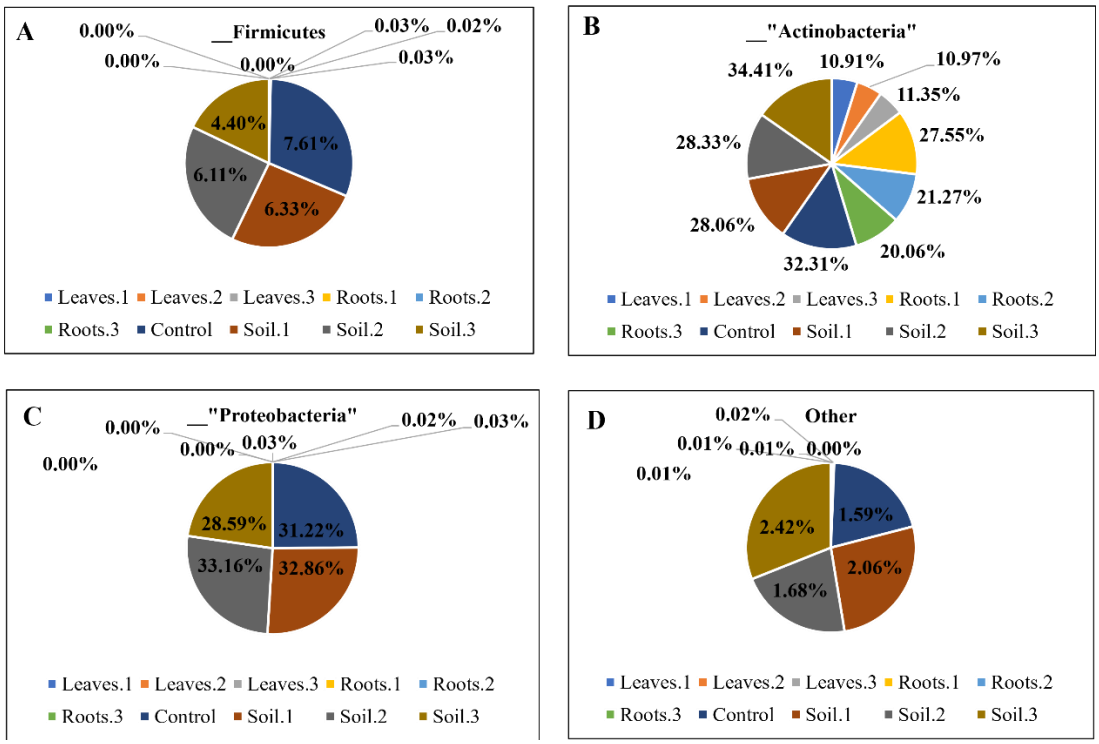

Figure 4. *Pie charts representing the taxonomic classification of bacterial communities at the phylum level within various samples associated with Senna italica plants*

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At the order level, Actinomycetales predominated significantly, ranging from 27.55% to 10.91%. Conversely, Rhizobiales, Bacillales, and other unclassified orders were present in in proportions less than 1% in the other tissue samples (*Figure 5A*). Rhizobiales and Bacillales were identified in the sequence reads of all the endophytic samples but in very minimal amounts, whereas in the rhizosphere soil, Actinomycetales accounted for 31.84% to 26.73%, Rhizobiales accounted for 21.11% to 18.28%, and Bacilli accounted for 7.58% to 4.40% (*Figure 5B*).

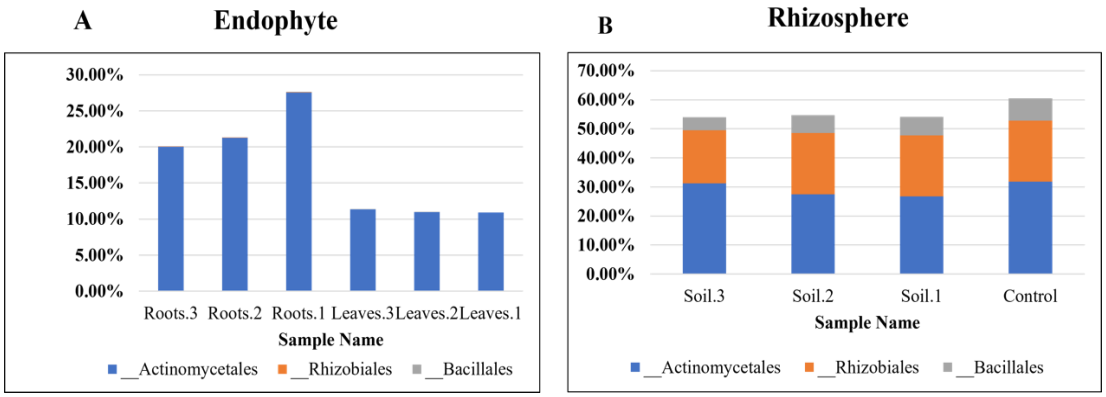

Figure 5. *The distribution of bacterial communities at the order level in the (A) Endophytic and (B) Rhizospheric samples among the various samples associated with the Senna italica plant*

Table 1 illustrates the orders with abundances surpassing 1% in individual tissues. The results revealed a considerably greater richness of bacterial communities in the rhizosphere soil than in the plant tissues.

Table 1. *The relative abundances of the bacterial orders exceeded 1% across all the samples*

The relative abundance (>1%)	Control	Soil (1)	Soil (2)	Soil (3)	Leaves (1)	Leaves (2)	Leaves (3)	Roots (1)	Roots (2)	Roots (3)
Actinomycetales	31.84	26.73	27.5	31.27	10.91	10.97	11.35	27.55	21.27	20.06
Rhizobiales	21.01	21.06	21.11	18.28	-	-	-	-	-	-
Bacillales	7.58	6.31	6.10	4.40	-	-	-	-	-	-

At the family level, within the endophytic samples, there was a notably greater abundance of Microbacteriaceae, ranging from 27.48% to 10.96%, whereas this family constituted less than 1% of the rhizosphere soil (*Figure 6A*). Conversely, the abundance of Micrococcaceae was significantly greater in the rhizosphere soil, varying from 17% to 37%, in contrast to being less than 1% in the endophytes (*Figure 6B*).

Microbial composition analysis

The UPGMA tree, based on the weighted UniFrac distance, illustrates the similarities and differences in the bacterial compositions of the examined leaf and root samples. There was a close resemblance between Leave 1 and 2, a slightly greater dissimilarity in Leave 3, and significant differences among the root samples, providing insights into microbial

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diversity (*Figure 7A*). Additionally, the proximity observed between Soil 1 and Soil 2 indicated considerable similarity in their bacterial composition or shared microbial taxa (*Figure 7B*).

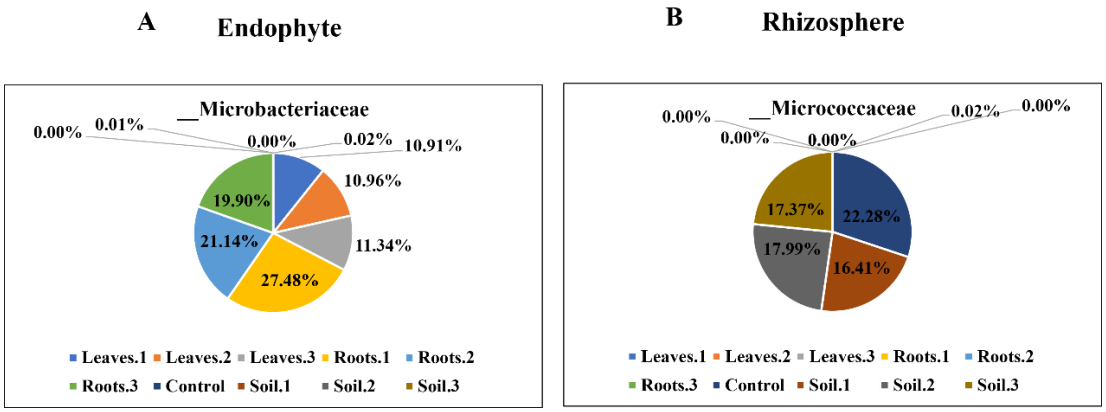

Figure 6. *The common families present in both the endophyte and rhizosphere were associated with the Senna italica plant*

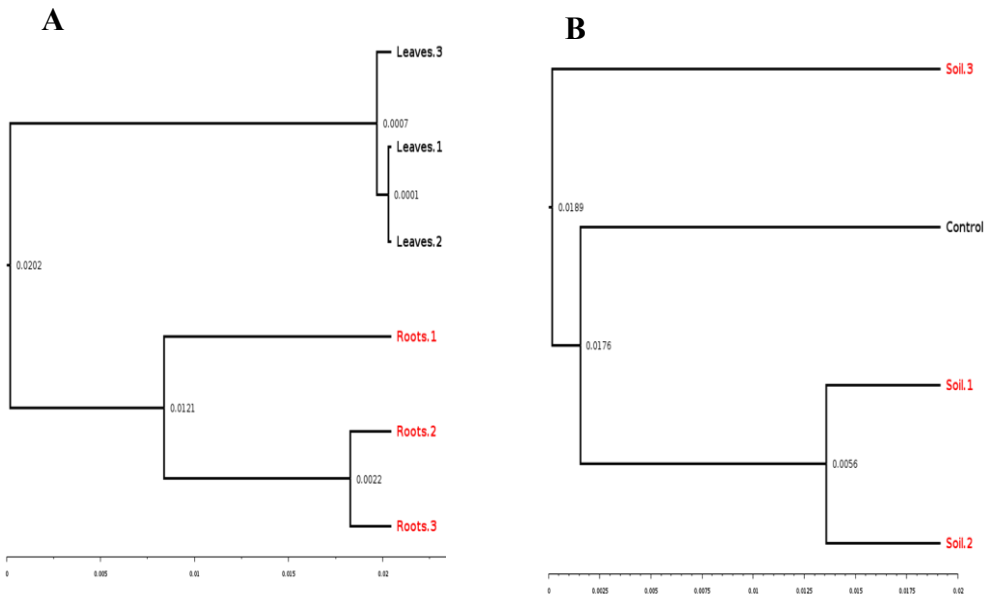

Figure 7. *UPGMA tree analysis revealed microbial similarities and relationships among Senna italica soil, root, and leaf samples*

The similarity between endophytic and rhizospheric bacterial populations was assessed via principal coordinate analysis (PCoA) at the OTU level to depict variations in OTU composition across samples. Among the endophytes, *Figure 8A* (PC1 vs. PC2) *and 8B* (PC2 vs. PC3) accounted for 60.83% and 5.08% of the total variance in the bacterial communities. Conversely, in rhizosphere soil, the PCoA and plot analysis, specifically for PC1 vs PC2, explained 53.09% and 44.21% of the total variance in the bacterial communities, as depicted in *Figure 8C*.

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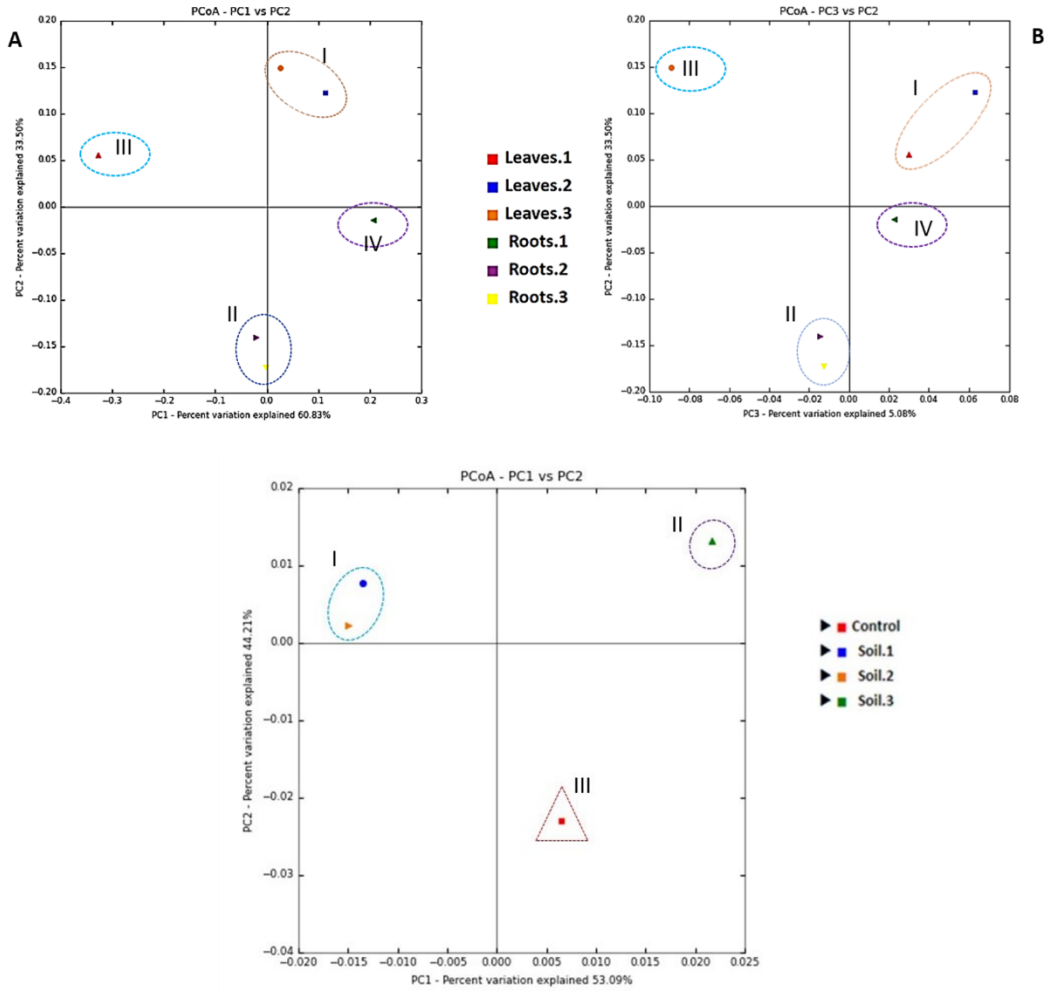

Figure 8. *β diversity analysis using an unweighted PCoA of UniFrac distances. This Principal Coordinate Analysis visually represents the distinctions among bacterial communities found in Senna italica soil, roots, and leaves*

Discussion

The results obtained by sequencing utilizing the MiSeq platform (IlluminaTM) demonstrated a high percentage of valid sequences (97%) and a high degree of coverage in the taxonomic classification process (0.99). Thus, the detailed information on the composition of the bacterial community found in this study is precise and allows comparative inference among *Senna italica* soil, roots, and leaves.

Understanding the microbial communities within soil and endophytes is vital for comprehending plant development, as suggested in prior research (Berendsen et al., 2012). This study employs modern taxonomic methods, focusing on the genetic materials of microorganisms for classification based on DNA similarities. Unlike traditional approaches relying on microbial cultures, this method aligns with contemporary metagenomics, revealing microbial abundance and diversity disparities. Moreover, this research addresses a gap in the literature exploring the taxonomy, structure, and functional genomes of prokaryotes in arid environments, helping to elucidate their ecological significance.

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This study aimed to explore the bacterial diversity within the endophytes and rhizosphere of *Senna italica* using Illumina amplicon sequencing. Previous research using this sequencing method has emphasized the pivotal role of endophytic bacteria in plant growth (Badri et al., 2009; Weyens et al., 2009; Köberl et al., 2011; Lu et al., 2022).

Compared to endophytes, the rhizosphere contained more significant proportions of Actinobacteria, Proteobacteria, Firmicutes, and unclassified phyla. As noted in several studies, Actinobacteria and Proteobacteria are commonly found in various plants and soils (Manter et al., 2010; Lin et al., 2019) .Actinobacteria and Proteobacteria constituted a significant portion of the bacterial sequences in the rhizosphere, comprising 34.41-28.06% and 33.16–28.59%, respectively. This dominance of Actinobacteria in the rhizosphere aligns with the findings of previous studies by Yadav et al. (2018). These phyla have been detected in various crop plants, such as rice, barley, maize, and grapevine (Bulgarelli et al., 2013; Hernández et al., 2015; Zarraonaindia et al., 2015; Alsaedi et al., 2023).

Earlier studies by Jeon et al. (2003); Kragelund et al. (2007); and Lin et al. (2019) highlighted the significant role of Proteobacteria in various ecological processes, including wastewater treatment, enhancing tolerance to pollutants and improving soil quality. Additionally, Proteobacteria actively participate in global sulfur, nitrogen, and carbon cycles, as Zhao et al. (2018) indicated. Actinobacteria were notably more abundant in all the samples from *Senna italica*. Moreover, Actinomycetales and Rhizobiales, which belong to Actinobacteria and Proteobacteria, respectively, emerged as the dominant orders in the rhizosphere soil, highlighting their prevalence and importance in this environment.

Our investigation across multiple phyla revealed the ability of these bacteria to withstand drought stress. Numerous studies conducted at the order level have highlighted the advantageous roles of bacteria in various sectors, including industry, pharmaceuticals, agriculture, and the environment. Within the Actinobacteria group present in rhizosphere soils, Actinomycetes was the most prevalent order. While Actinomycetes were present in all the rhizosphere samples, their abundance was notably more significant in the rhizosphere samples than in the endophytes. Despite this, there are currently no documented reports on this particular endophyte. Actinomycetes, as prokaryotes, possess diverse metabolic capabilities and produce essential compounds such as enzymes and antibiotics that contribute significantly to health, including immune modulation (Dzhembekova et al., 2018).

Micrococcaceae emerged as the predominant and distinct bacterial family in the rhizosphere soil, while its presence in endophytes remained below 1%. Our findings align with those of another study investigating the impact of drought on bacterial communities in rice, which confirmed the specific proliferation of Micrococcaceae in the drought-affected rhizosphere (Munoz-Ucros et al., 2022).

In contrast, the abundance of the bacteria in the rhizosphere was notably more significant in the endophytes than in the control group (below 1%). Despite this distinction, there needs to be more knowledge regarding the interactions of Microbacteriaceae endophytes with plants, and there have been no reports documenting the behavior or characteristics of these endophytes thus far.

These findings indicate an association between endophytic bacteria and soil microorganisms, suggesting that *Senna italica* might have acclimated effectively to its surrounding environment, fostering mutualistic interactions with microbes.

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Conclusion

This investigation explored the diversity of endophytic bacterial communities associated with *Senna italica*, encompassing soil, roots, and leaves. By utilizing the 16S rRNA gene as a marker, Illumina MiSeq technology was used to examine the bacterial diversity in these samples, shedding light on both endophytic and rhizospheric bacteria. Our study suggested that the presence of endophytes and rhizosphere bacteria could forecast plant growth and survival in harsh environments, a characteristic observed across the investigated phylum. Notably, Actinobacteria were found to be a prominent component of both endophytes and rhizosphere soil, emphasizing their significance in this complex ecosystem. Furthermore, our findings suggest the potential positive influence of these microbes on plant adaptation to drought stress. These insights contribute to our understanding of plant-microbe interactions, particularly under drought conditions, and have valuable implications for plant health.

REFERENCES

[1] Adjou, E. S., Koudoro, A. Y., Nonviho, G., Ahoussi, E. D., Sohounhloue, D. C. K. (2021): Phytochemical profile and potential pharmacological properties of leaves extract of *Senna italica* Mill. – American Journal of Pharmacological Sciences 9(1): 36-39.

[2] Alsaedi, Z. S., Ashy, R. A., Shami, A. Y., Majeed, M. A., Alswat, A. M., Baz, L., Baeshen, M. N., Jalal, R. S. (2022): Metagenomic study of the communities of bacterial endophytes in the desert plant *Senna italica* and their role in abiotic stress resistance in the plant. – Brazilian Journal of Biology 82: e267584.

[3] Alsaedi, Z. S., Alswat, A. M., Ashy, R. A., Shami, A. Y., Majeed, M. A., Baz, L., Baeshen, M. N., Jalal, R. S. (2023): Comparison of Endophytic Microbiome Community and Rhizosphere in the Desert Plant *Senna italica*. – Applied Ecology and Environmental Research 21(5): 4279-4294. Available at: https://doi.org/http://dx.doi.org/10.15666/aeer/2105_42794294.

[4] Alswat, A. M., Jalal, R. S., Shami, A. Y., Majeed, M. A., Alsaedi, Z. S., Lina, B. A. Z., Refai, M. Y., Baeshen, M. N., Bataweel, N. M., Ahmed, A.-H. (2023): Investigating the metagenomics of the bacterial communities in the rhizosphere of the desert plant *Senna italica* and their role as plant growth promoting factors. – Notulae Botanicae Horti Agrobotanici Cluj-Napoca 51(1): 13053.

[5] Badri, D. V., Weir, T. L., van der Lelie, D., Vivanco, J. M. (2009): Rhizosphere chemical dialogues: plant–microbe interactions. – Current Opinion in Biotechnology 20(6): 642-650.

[6] Baeshen, M. N., Moussa, T. A. A., Ahmed, F., Abulfaraj, A. A., Jalal, R. S., Majeed, M. A., Baeshen, N. A., Huelsenbeck, J. P. (2020): Diversity profiling of associated bacteria from the soils of stress tolerant plants from seacoast of Jeddah, Saudi Arabia. – Applied Ecology and Environmental Research 18(6): 8217-8231.

[7] Barra Caracciolo, A., Terenzi, V. (2021): Rhizosphere microbial communities and heavy metals. – Microorganisms 9(7): 1462.

[8] Berendsen, R. L., Pieterse, C. M. J., Bakker, P. A. H. M. (2012): The rhizosphere microbiome and plant health. – Trends in Plant Science 17(8): 478-486. Available at: https://doi.org/10.1016/j.tplants.2012.04.001.

[9] Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., Schulze-Lefert, P. (2013): Structure and functions of the bacterial microbiota of plants. – Annual Review of Plant Biology 64(1): 807-838.

[10] Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I. (2010): QIIME allows analysis of high-throughput community sequencing data. – Nature Methods 7(5): 335-336.

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[11] Dabai, Y. U., Kawo, A. H., Aliyu, R. M. (2012): Phytochemical screening and antibacterial activity of the leaf and root extracts of *Senna italica*. – African Journal of Pharmacy and Pharmacology 6(12): 914-918.
[12] Dzhembekova, N., Moncheva, S., Ivanova, P., Slabakova, N., Nagai, S. (2018): Biodiversity of phytoplankton cyst assemblages in surface sediments of the Black Sea based on metabarcoding. – Biotechnology & Biotechnological Equipment 32(6): 1507-1513.
[13] Eida, A. A., Ziegler, M., Lafi, F. F., Michell, C. T., Voolstra, C. R., Hirt, H., Saad, M. M. (2018): Desert plant bacteria reveal host influence and beneficial plant growth properties. – PLoS ONE 13(12): e0208223. Available at: https://doi.org/10.1371/journal.pone.0208223.
[14] Fadiji, A. E., Babalola, O. O., Santoyo, G., Perazzolli, M. (2022): The potential role of microbial biostimulants in the amelioration of climate change-associated abiotic stresses on crops. – Frontiers in Microbiology 12: 829099.
[15] Gull, A., Lone, A. A., Wani, N. U. I. (2019): Biotic and abiotic stresses in plants. – Abiotic and Biotic Stress in Plants 7: 1-9.
[16] Hernández, M., Dumont, M. G., Yuan, Q., Conrad, R. (2015): Different bacterial populations associated with the roots and rhizosphere of rice incorporate plant-derived carbon. – Applied and Environmental Microbiology 81(6): 2244-2253.
[17] Jeon, C. O., Lee, D. S., Park, J. M. (2003): Microbial communities in activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor. – Water Research 37(9): 2195-2205.
[18] Köberl, M., Müller, H., Ramadan, E. M., Berg, G. (2011): Desert Farming Benefits from Microbial Potential in Arid Soils and Promotes Diversity and Plant Health. – PLoS ONE 6(9): e24452. Available at: https://doi.org/10.1371/journal.pone.0024452.
[19] Kragelund, C., Caterina, L., Borger, A., Thelen, K., Eikelboom, D., Tandoi, V., Kong, Y., Van Der Waarde, J., Krooneman, J., Rossetti, S. (2007): Identity, abundance and ecophysiology of filamentous Chloroflexi species present in activated sludge treatment plants. – FEMS Microbiology Ecology 59(3): 671-682.
[20] Lin, Y., Ye, Y., Hu, Y., Shi, H. (2019): The variation in microbial community structure under different heavy metal contamination levels in paddy soils. – Ecotoxicology and Environmental Safety 180: 557-564.
[21] Liu, P.-Y., Wu, W.-K., Chen, C.-C., Panyod, S., Sheen, L.-Y., Wu, M.-S. (2020): Evaluation of compatibility of 16S rRNA V3V4 and V4 amplicon libraries for clinical microbiome profiling. – BioRxiv.
[22] Lorenz, T. C. (2012): Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. – JoVE (Journal of Visualized Experiments) 63: e3998.
[23] Lu, L., Mok, B. W. Y., Chen, L. L., Chan, J. M. C., Tsang, O. T. Y., Lam, B. H. S., Chuang, V. W. M., Chu, A. W. H., Chan, W. M., Ip, J. D. (2022): Neutralization of severe acute respiratory syndrome coronavirus 2 omicron variant by sera from BNT162b2 or CoronaVac vaccine recipients. – Clinical Infectious Diseases 75(1): e822-e826.
[24] Madouh, T. A., Quoreshi, A. M. (2023): The function of arbuscular mycorrhizal fungi associated with drought stress resistance in native plants of arid desert ecosystems: A review. – Diversity 15(3): 391.
[25] Manter, D. K., Delgado, J. A., Holm, D. G., Stong, R. A. (2010): Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. – Microbial Ecology 60: 157-166.
[26] Munoz-Ucros, J., Wilhelm, R. C., Buckley, D. H., Bauerle, T. L. (2022): Drought legacy in rhizosphere bacterial communities alters subsequent plant performance. – Plant and Soil 471: 443-461.
[27] Prasad, M., Srinivasan, R., Chaudhary, M., Choudhary, M., Jat, L. K. (2019): Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. – PGPR Amelioration in Sustainable Agriculture, Chapter 7, pp. 129-157.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_65136525

[28] Quast, S., Berger, A., Eberle, J. (2013): ROS-dependent phosphorylation of Bax by wortmannin sensitizes melanoma cells for TRAIL-induced apoptosis. – Cell Death & Disease 4(10): e839-e839.
[29] Quiza, L., St-Arnaud, M., Yergeau, E. (2015): Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering. – Frontiers in Plant Science 6: 507.
[30] Risse, T., Cowles, M. G., Caporaso, J. (2001): Europeanization and Domestic Change: Introduction. – Transforming Europe: Europeanization and Domestic Change, Chapter 1, pp. 1-20.
[31] Serraj, R., Krishnan, L., Pingali, P. (2019): Agriculture and food systems to 2050: a synthesis. – Agriculture & Food Systems to 2050, Chapter 1, pp. 3-45.
[32] Tshikalange, T. E., Meyer, J. J. M., Hussein, A. A. (2005): Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. – Journal of Ethnopharmacology 96(3): 515-519.
Available at: https://doi.org/https://doi.org/10.1016/j.jep.2004.09.057.
[33] Ullah, A., Nisar, M., Ali, H., Hazrat, A., Hayat, K., Keerio, A. A., Ihsan, M., Laiq, M., Ullah, S., Fahad, S. (2019): Drought tolerance improvement in plants: an endophytic bacterial approach. – Applied Microbiology and Biotechnology 103: 7385-7397.
[34] Weyens, N., van der Lelie, D., Taghavi, S., Vangronsveld, J. (2009): Phytoremediation: plant–endophyte partnerships take the challenge. – Current Opinion in Biotechnology 20(2): 248-254.
[35] Yadav, A. N., Verma, P., Kumar, S., Kumar, V., Kumar, M., Sugitha, T. C. K., Singh, B. P., Saxena, A. K., Dhaliwal, H. S. (2018): Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. – In: New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, pp. 13-41.
[36] Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S. (2015): The soil microbiome influences grapevine-associated microbiota. – MBio 6(2): 10-1128.
[37] Zhao, Y., Song, C., Dong, H., Luo, Y., Wei, Y., Gao, J., Wu, Q., Huang, Y., An, L., Sheng, H. (2018): Community structure and distribution of culturable bacteria in soil along an altitudinal gradient of Tianshan Mountains, China. – Biotechnology & Biotechnological Equipment 32(2): 397-407.

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