Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Metagenomic study of the communities of bacterial endophytes in the desert plant *Senna Italica* and their role in abiotic stress resistance in the plant

Estudo metagenômico das comunidades de endófitos bacterianos na planta do deserto *Senna Italica* e seu papel na resistência ao estresse abiótico na planta

Z. S. Alsaedi[a], R. A. Ashy[a], A. Y. Shami[b], M. A. Majeed[a], A. M. Alswat[a], L. Baz[c], M. N. Baeshen[a] and R. S. Jalal[a*]

[a]University of Jeddah, College of Science, Department of Biology, Jeddah, Saudi Arabia
[b]Princess Nourah bint Abdulrahman University, College of Sciences, Department of Biology, Riyadh, Saudi Arabia
[c]King Abdulaziz University, Faculty of Science, Department of Biochemistry, Jeddah, Saudi Arabia

Abstract

Plant leaves and roots are home to diverse communities of bacteria, which play a significant role in plant health and growth. Although one of the most unfriendly environments for plant growth is deserts, desert plants can influence their surrounding microbial population and choose favorable bacteria that encourage their growth under these severe circumstances. *Senna italica* is known for its excellent medicinal values as a traditional medical plant, but little is known about its associated endophytic bacterial community under extreme conditions. In the present study, metagenomic sequencing of 16S rRNA was used to report the diversity of endophytic bacterial communities associated with the leaves and roots of the desert medicinal plant *Senna italica* that was collected from the Asfan region in northeast Jeddah, Saudi Arabia. Analyses of the 16S rRNA sequences at the taxonomic phylum level revealed that bacterial communities in the roots and leaves samples belonged to five phyla, including *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and unclassified phyla. Results indicated that the most common phyla were *Cyanobacteria/Chloroplast* and *Actinobacteria*. Analysis of the 16S rRNA sequences at the taxonomic phylum level revealed that bacterial communities in the roots and leaves samples belonged to twelve genera at the taxonomic genus level. The most abundant ones were highlighted for further analysis, including *Okibacterium* and *Streptomyces* found in *Actinobacteria*, which were the dominant genus in roots samples. However, *Streptophyta* found in *Cyanobacteria/Chloroplast* was the dominant genus in leaf samples. Metagenomic analysis of medicinal plants leads to identifying novel organisms or genes that may have a role in abiotic stress resistance in the plant. The study of endophytic microbiome taxonomic, phylogenetic, and functional diversity will better know innovative candidates that may be selected as biological agents to enhance agricultural and industrial processes, especially for crop desert agricultural improvement.

Keywords: microbiome, endophytes, PGPEB, metagenomics, *Senna italica*.

Resumo

As folhas e raízes das plantas abrigam diversas comunidades de bactérias, que desempenham um papel significativo na saúde e no crescimento das plantas. Embora um dos ambientes mais hostis para o crescimento de plantas sejam os desertos, as plantas do deserto podem influenciar a população microbiana circundante e escolher bactérias favoráveis que encorajem seu crescimento sob essas circunstâncias severas. Senna italica é conhecida por seus excelentes valores medicinais como planta medicinal tradicional, mas pouco se sabe sobre sua comunidade bacteriana endofítica associada em condições extremas. No presente estudo, o sequenciamento metagenômico de 16S rRNA foi usado para relatar a diversidade de comunidades bacterianas endofíticas associadas às folhas e raízes da planta medicinal do deserto Senna italica que foi coletada na região de Asfan no nordeste de Jeddah, Arábia Saudita. Análises das sequências de rRNA 16S no nível taxonômico do filo revelaram que as comunidades bacterianas nas amostras de raízes e folhas pertenciam a cinco filos, incluindo Cyanobacteria, Proteobacteria, Actinobacteria, Firmicutes e filos não classificados. Os resultados indicaram que os filos mais comuns foram Cyanobacteria/Cloroplast e Actinobacteria. A análise das sequências de rRNA 16S no nível taxonômico do filo revelou que as comunidades bacterianas nas amostras de raízes e folhas pertenciam a doze gêneros no nível taxonômico de gênero. Os mais abundantes foram destacados para análise posterior, incluindo Okibacterium e Streptomyces encontrados em Actinobacteria, que foram os gêneros dominantes nas amostras de raízes. No entanto, Streptophyta encontrado em Cyanobacteria/Chloroplast foi o gênero dominante nas amostras de folhas. A análise metagenômica

*e-mail: rsjalal@uj.edu.sa
Received: September 5, 2022 – Accepted: October 22, 2022

de plantas medicinais leva à identificação de novos organismos ou genes que podem ter um papel na resistência ao estresse abiótico na planta. O estudo da diversidade taxonômica, filogenética e funcional do microbioma endofítico conhecerá melhor os candidatos inovadores que podem ser selecionados como agentes biológicos para melhorar os processos agrícolas e industriais, especialmente para o melhoramento agrícola do deserto.

Palavras-chave: microbioma, endófitos, PGPEB, metagenômica, *Senna italica*.

1. Introduction

Like other so-called higher creatures, plants do not exist as beings in their own right. Instead, they are biotic systems made up of the plant and many microorganisms known as the plant microbiome (Harman et al. 2021). Endophytes are essential in promoting plant growth, development, and improving plant yields (Xia et al. 2019). This article focuses on plant-associated bacteria that colonize the plant's leaves and roots as microbial endophytes. The agronomic biotechnology of endophytic bacteria has been explored to address the global food security issue and apply sustainable solutions to food production problems. Using beneficial microorganisms in agricultural production increases productivity, plant development, and ecological system recovery (Le Cocq et al., 2017).

According to the United Nations Organization (UN), the present global population of 7.6 billion people is predicted to exceed 9.8 billion people by 2050 (UN, 2019). Additionally, global warming exacerbates abiotic pressures, resulting in decreased agricultural production and cultivatable land (Cerri et al., 2007; Mittler, 2006; Pandey et al., 2017). The leading cause of over 50% of losses in significant crop yield is abiotic stresses, such as salinity, extreme temperatures, nutrient deficiency, UV radiation, and drought. As a result, the need for an environmentally friendly, sustainable, and cost-efficient approach to ensuring food availability for a growing population has become an important, focused, and intensive research topic (Boyer, 1982; Eida et al., 2018).

Approximately one-third of the planet's biomass may be considered deserts (Makhalanyane et al., 2015). Although deserts appear to be uninhabitable to living beings, many organisms, including plants, have adapted to these severe circumstances by evolving mechanisms to adapt to this environment, such as extensive and deep root systems for exploiting soil at large depths (Ehleringer and Monson 1993; Hartwell, 2005; Yamori et al., 2014). Furthermore, the microbiome of plants is thought to be a significant element in the ability of plants to adapt to these environments (Friesen et al., 2011; Ortiz et al., 2015; Schlaeppi and Bulgarelli, 2015; Zelicourt et al., 2013).

Endophytes are a group of microbes that inhabit plants' tissues without harming their host (Fadiji and Babalola, 2020; Omomowo and Babalola, 2019). Endophytic microbes have various advantageous activities, including biocontrol activities, plant yield, phytoremediation development, and growth stimulation (Kumar et al., 2017; Pareek et al., 2017; Rana et al., 2020; Staniek et al., 2008; Yang et al., 2017), and they live surrounding the roots more than in the stem and leaves (Dudeja et al., 2012). Endophytic microbes produce several bioactive compounds for more stable symbiosis, influencing plants' growth and facilitating better adaptation to the environment (Das and Varma 2009). Endophytic bacteria could be used to produce a range of agricultural applications (biofertilizers and biocontrol agents), industrial-medical bioproduct production, and bioremediation (Andrews et al., 2010; Ryan et al., 2008). It is commonly accepted that beneficial bacteria, known as plant growth-promoting endophyte bacteria (PGPEB), may be used as biofertilizers to improve plant development. PGPEB, are a group of unrelated bacteria that live in symbiotic relationships with plants and have been developed as a sustainable agricultural production alternative. According to their strategy of colonization, PGPEB can be rhizosphere (live in a thin layer of roots in the rhizosphere), epiphytic (at the base of its colonizing tactics or the surface of the leaf), or endophytic (inside the plant body). PGPEB can directly or indirectly impact plant growth (Glick, 2012), PGPEB act to improve plant growth and stress resistance (Santoyo et al., 2016).

The plant Senna italica belongs to the Fabaceae family (subfamily Caesalpinaceae) (Adjou et al., 2021; Masoko et al., 2010; Dabai, 2012; Yagi et al., 2013). The Fabaceae or Leguminosae family, also known as the legumes, is the third biggest plant family and comprised of over 730 genera and over 19,000 species is widespread and commercially significant. Senna is an essential genus of flowering plants with about 350 species (Adjou et al., 2021; Khalaf et al., 2019; Rahman and Parvin, 2014). It has an essential effect on African folk medicine due to its therapeutic characteristics, also were considered it has significant antibacterial activities (Tshikalange et al., 2005).

The term metagenomics was initially used in 1998 and was defined as the evaluation of all genetic components isolated directly from environmental samples (Handelsman et al., 1998). The study and analysis of plant-associated microorganisms, their composition, and their activities and roles remain challenging (Azaroual et al., 2022). Nowadays, next-generation sequencing (NGS) molecular approaches are widely utilized to characterize microbial communities and their dynamics in various plants and plant compartments (Fadiji and Babalola, 2020), where NGS platforms mostly rely on amplicon sequencing methods that target the 16S rRNA gene for plant microbiome study (D'Amore et al., 2016). Using the metagenomics technique to examine bacterial communities will provide more than our prospects to those studied by traditional methods that do not target unstable bacteria known in laboratory conditions and will allow us to characterize these bacteria and identify the critical genes associated with soil and plant communities (Alves et al., 2018).

This study aimed to discover the characteristics, classification, and diversity of endophytic bacterial communities associated with the leaves and roots of the desert medicinal plant Senna italica by applying the metagenomics techniques, which may lead to a new strain beneficial for biotechnological applications. These methods

will raise the proportion and sustainability of agriculture, particularly under drought stress conditions. This study will better understand the interactions between the plant microbes, enhancing agriculture production and meeting the global food demand between 59% to 98% by 2050. Additionally, to gain knowledge of microbial diversity and to examine the relationship between the communities of microbial and their environments.

2. Materials and Methods

2.1. Study location

The study area was located in the Asfan, in northeast Jeddah, Saudi Arabia (Table 1). In Asfan region, the temperate in April ranges between (29-33°C) in April 2021, as it is classified as a hot, dry, sandy, and lower amount of rainfall. The Senna italica plant communities are growing significantly in this region despite all these characteristics.

2.2. Plant sampling

Sampling was carried out on April 2021 in the morning. The temperature was 31°C. A total of six samples, namely the Senna italica plant, were collected. Three Senna italica samples were taken from roots, where the drilling was done to reach the roots' hair area (18-25 cm depth). The other three Senna italica samples were collected from leaves. Until further analysis, each of the roots and leaves was cut into small pieces and then, stored with liquid Nitrogen (-196°C) and then kept at -20°C. The aim of taking three samples from roots and the others from leaves were to avoid statistical errors.

2.3. DNA extraction, PCR, 16S rRNA gene sequencing, and Illumina amplicon sequencing

Roots and leaves samples were shipped to Macrogen Inc. Company (Seoul, South Korea), and genomic DNA was extracted from the roots and the leaves samples. DNA purity and quantification were evaluated using the Picogreen (Invitrogen, cat. #P7589) fluorescence-based quantification method.

Bacterial V3-V4 16S rRNA gene segments were amplified by PCR using the universal primers (Bakt_341F: CCTACGGNGGCWGCAG) and (Bakt_805R: GACTACHVGGGTATCTAATCC). The program of PCR amplification was carried out by an initial denaturation at 95°C for 5 minutes. Then, followed by 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, followed by a final elongation at 72 °C for 10 minutes (Lorenz, 2012). The purified amplicons were utilized for library creation and deep sequencing using an Illumina SBS technology, and then 300 bp pair-end reads of the V3 and V4 sections were extracted and selected from the Illumina-recommend (Klindworth et al., 2013). Since Illumina announced a suggested library preparation procedure for sequencing on the MiSeq technology, the V3 and V4 regions have become the most used amplicon targets in microbiota investigations (Wu et al., 2020).

2.4. 16S dataset processing and statistical analysis

Raw sequence data derived from the sequencing process was transferred as FASTA files for each sample, and sequencing quality files. Files were accessible using the bioinformatics program Quantitative Insights Into Microbial Ecology (QIIME), where they were processed and analyzed following general procedures recommended by (Caporaso et al., 2010a). QIIME software is an open-source bioinformatics tool for microbiome analysis from raw DNA sequencing data supplied by Illumina or other sequencing programs. In addition, QIIME provides raw read quality pretreatment, Operational Taxonomic Units (OTUs) picking, taxonomic assignment, phylogenetic reconstruction, diversity analysis, and graphical presentations. All the statistical analyses were performed using the QIIME tool (Macrogen, 2017).

2.5. OTU analysis

The CD-HIT-OTU program is a multi-step pipeline to generate OTU clusters for ribosomal ribonucleic acid (rRNA) tags from Illumina platforms, which were used to filtered and trimmed V3-V4 16S rRNA sequence readings. In addition, the CD-HIT-OTU-MiSeq can cluster the spliced Paired-End reference database together with samples; thus, the OTUs can be derived.

The FLASH program was used to merge paired-end reads from next-generation sequencing studies to exclude low-quality sequences. Sequences were de-replicated and assigned to specific samples, filtered by length and quality (length: 350–450 bp; quality threshold: 20). The obtained sequences were assigned and grouped into

Table 1. Senna italica sample codes and their origin.

Sample	Type of plant	Tissue	Temperature	Altitude	Latitude	Longitude	Location
Leaves.1	*Senna italica*	Leaf	31ºC	2.8 m above sea level	21.53.13.3" N	39.15.56.6" E	Asfan, in the region of Jeddah.
Leaves.2		Leaf					
Leaves.3		Leaf					
Roots.1		Root					
Roots.2		Root					
Roots.3		Root					

OTUs with UCLUST (is a novel clustering algorithm used for clustering sequences within a similarity threshold to a reference sequence that will cluster to an OTU) (Edgar, 2010; Macrogen, 2017) using 97% identified clustering. The most abundant sequence from each OTU was selected. The chimeric sequences were removed with Chimera Slayer. Based on the OTUs results, bacterial taxonomy was assigned using the Ribosomal Database Project Classifier.

2.6. Bacterial diversity, richness, and taxonomic distribution of taxa

OTUs were defined at the genus level (3% sequence divergence) using the Complete Linkage Clustering tool of RDP. Alpha diversity represents the diversity and richness of each sample calculated by rarefying a small percentage of randomly picked sequences. Using taxonomic classifications and a phylogenetic tree, the Shannon index, which indicates the diversity, and the Chao1 index, which indicates the richness of the microbial population, were estimated. Simpson index, which indicates quantifying biological diversity, and Goods coverage index, which indicates library coverage of each sample, were also estimated. A software package Mothur has been used to analyze the complexity of species to estimate indicators Shannon and Simpson's. Calculating the OTU numbers of the collected tags and identifying the most significant depth permitted to keep all samples were used to create a rarefaction curve, generated based on indices metrics of chao1, Shannon, and the Simpson by QIIME. The weighted and unweighted UniFrac distance matrix was calculated and shown using main coordinate analysis to discover beta diversity indicating the diversity across samples. For diversity analysis, the dissimilarity of bacterial communities was determined using principal component analysis (PCA) on weighted UniFrac distances among all samples. These were all based on a report from (Macrogen, 2017).

2.7. 16S rRNA gene sequence-based phylogenetic analysis of the endophytic bacteria isolated from six samples

Nucleotide sequences of endophytic bacteria 16S rRNA gene were obtained from NCBI GenBank. The taxonomic level was selected for the entire phylum and genus were chosen, and the phylogenetic tree at the phylum and genus level was built using the program Phylogeny was used to perform phylogenetic analysis on these sequences (Dereeper et al., 2008, 2010). Sequences were saved in FASTA format, then copied and pasted into the space provided in the online tool's area. Maximum likelihood was used to conduct the phylogenetic analysis. We have utilized a one-click mode, which is an automated program that executes analysis step by step, starting with sequences alignment (MUSCLE 3.8.31) (Edgar, 2004), then alignment refinement (Gblocks 0.91b), phylogeny (PhyML 3.1/3.0 aLRT) (Anisimova and Gascuel, 2006; Guindon and Gascuel, 2003) to the tree rendering (TreeDyn 198.3) (Chevenet et al., 2006).

3. Results

In the present work analyses, we used the metagenomic technique as a potent tool to classify and diversity of endophytic bacterial communities associated with the leaves and roots Senna italica plant. The abundance and diversity of the endophytic bacteria were analyzed on the Illumina base sequencing platform based on the 16S rRNA.

3.1. Preliminary sequencing data statistics and 16S rRNA statistical analysis

Through the MiSeq sequencing the raw read statistics and sequence quality ratings were collected. The preliminary sequencing data statistics are shown in Table 2 and Supplementary Material Figure S1. The total number of sequence reads and the assembly results for the six samples were obtained using the FLASH software. Data showed a complete clean-read sequence, which reached 499,923 reads.

In the roots tissue samples, the highest reads were in the Roots.1 sample at 101,468 reads, and the lowest reads were at 74,842 reads in the Roots.3 sample. In contrast, the highest reads in the samples of the leaves tissues (Leaves.2 sample) reached 91,718 reads, while the lowest was at 69,799 reads found in the Leaves.3 sample. The GC content in the three samples of roots tissues and the three samples of leaf tissues was taken between 54.89% to 55% and 54.93% to 54.94%, respectively. The results indicate that the content of GC in the six different samples is relatively close together, with an increase in some roots samples. The results of higher reads of GC content in root tissues compared to the leaf tissues may indicate that

Table 2. The total number of sequences reads.

	FLASH Software					
Sample Name	**Total Bases**	**Read Count**	**N (%)**	**GC (%)**	**Q20 (%)**	**Q30 (%)**
Leaves.1	36,769,930	83,006	0	54.94	99.11	96.78
Leaves.2	40,620,614	91,718	0	54.94	99.21	97.05
Leaves.3	30,910,183	69,799	0	54.93	99.21	97.05
Roots.1	45,059,191	101,468	0	54.89	99.17	96.92
Roots.2	35,057,690	79,090	0	54.98	99.15	96.88
Roots.3	33,172,674	74,842	0	55	99.07	96.68
Total number	221,590,282	499,923				

roots are closer to soil microbial communities than other tissues, which may harbor more microbial communities. The percentage of reading quality for the six samples of roots and leaves is shown in Supplementary Material Figure S2.

This Table 2 indicates the total number of sequences reads of endophytic bacteria in *Senna italica*. FLASH Software includes Total Bases, Read Count, GC (%), Q20(%), and Q30(%). Total Bases: The total number of bases in reads identified; Read Count: The total number of sequences reads; GC (%): The GC percentage in sequence reads; Q20(%): The percentage of bases in which the phred score is above 20; Q30(%): The percentage of bases in which the phred score is above 30.

3.2. Operational Taxonomic Unit (OTU) analysis

The CD-HIT-OTU software and rDnaTools were used to remove any contamination from the sequences. The grouping of sequences relayed on the 97% identity threshold for data statistics and analysis using the software QIIME. The number of OTUs of the six samples was comparatively low compared with other published metagenomic sequence analyses indicating the host specificity of the endophytic population. The OTUs distribution pattern indicated that most endophytic bacteria were included under roots tissues samples where the Roots.1 sample had the most OTUs with 24, whereas the Leaves.1 sample had the fewest OTUs with 13. The clustering results of the six roots and leaves samples allocated to the OTU are shown in Figure 1A, and the number of OTUs on each sample is shown in Figure 1B.

3.3. Community richness and diversity (α-Diversity)

The alpha diversity and rarefaction analyses focus on the OTUs, defined as a group of sequences with a sequence identity on a certain threshold, often set to 97% (Kozich et al., 2013). Clustering based on 97% identity decreases the size of the raw 16S rRNA database (Schloss and Westcott, 2011) and reduces the errors of sequencing in downstream diversity estimations because invalid sequences are likely to be integrated with correct sequences (Eren et al., 2013; Mysara et al., 2017). According to (Macrogen 2017), the Chao1 value describes richness estimates for an OTU definition, the Shannon value describes the community's species diversity, which is influenced by both species richness and species evenness, and the inverse Simpson value measures the likelihood that two randomly chosen individuals belong to the same species in the environment. Therefore, the alpha diversity of the microbiota in each sample was analyzed by considering the Observed OTUs (richness), The Shannon (diversity), and the Inverse Simpson.

Higher Shannon and Inversed Simpson values indices indicated the bacterial community diversity of *Senna italica* leaves and roots samples. The several curves based on observed Shannon and Inversed Simpson values are shown in Figure 2. The results suggested that these libraries detected most of the endophytic bacterial diversity in the samples used in our study. A rarefaction curve is a valuable tool for determining the species composition of a sample characterization and predicting the number of species within it. Additionaly, it determines if the sample size in biodiversity and community surveys is sufficient to estimate species abundance (Budka et al., 2019).

Due to (Macrogen, 2017), if the alpha rarefaction curve becomes flattered to the right, it indicates that a reasonable number of reads used in the analysis was sufficient to identify species/OTUs; thus, additional sequencing is not necessary. In our results, the rarefaction curves have tended to approach the saturation plateau (curve flattered to the right), which indicates that a reasonable number of reads were used in the analysis and that OTU abundance was diverse in all six samples (Figure 3). The Roots samples microbiota showed the highest values in Good's coverage

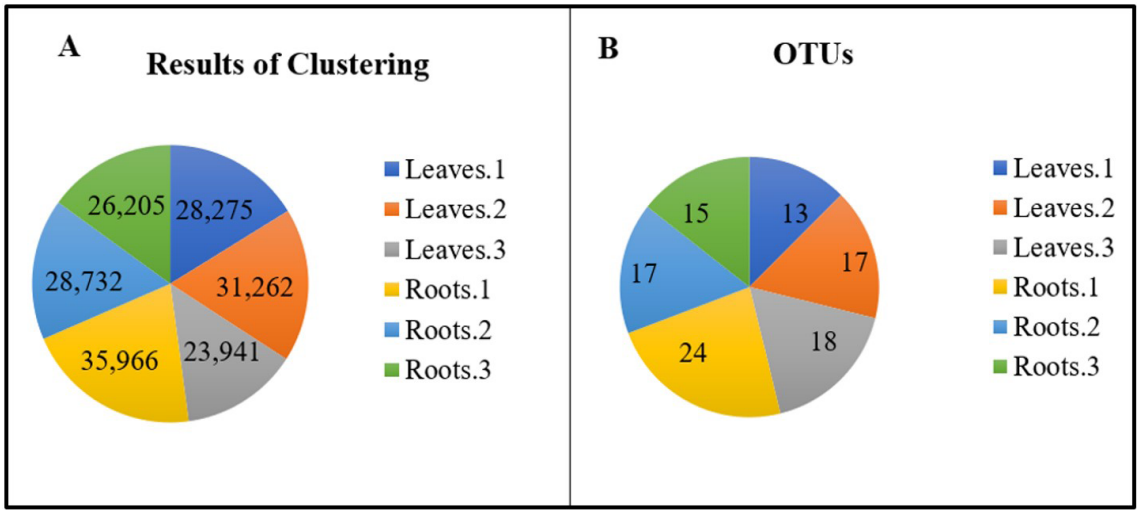

Figure 1. (A) Results of clustering: Assembling a group of organisms (The organisms in the same group are similar). (B) The number of OTUs generated for each sample. The Root.1 sample had the most OTUs of 24, while the Leave.1 sample had the fewest of 13. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with *Senna italica*.

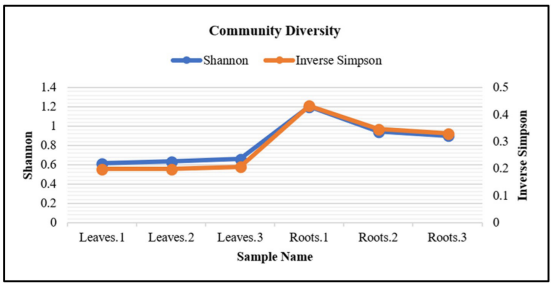

Figure 2. Different curve based on observed Shannon value and Inversed Simpson value. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with *Senna italica*.

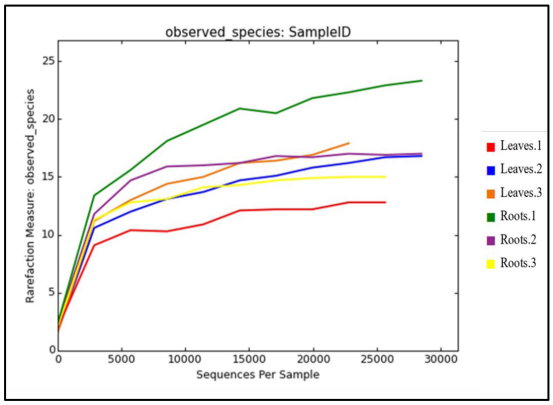

Figure 3. Alfa rarefaction curve observed based on observed species (OTUs) value. The curve has shown flatter to the right, which indicates the comparatively high species richness of the *senna italica* samples. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with *Senna italica*.

of all indices compared with the leaves samples, which was 99% for all leaves and roots samples except two, including Roots.2 and Roots.3 with 100% (Supplementary Material Table S1).

3.4. The diversity across samples (β-Diversity)

Principal coordinate analysis (PCoA) was done to obtain a clear picture of taxonomic clustering (Beta diversity) between the samples as per standard protocol (Caporaso et al., 2010b), and it was used to clarify the extent of variation of the endophytic bacteria population in the different samples to better understand the plant-related microbial population (Yang et al., 2017). The distribution of core genera indicated the existence of diverse endophytic bacteria in the leaves and roots of *Senna italica*, and the relative abundance of the same core genus differed in different tissue samples of *Senna italica*. Principal component analysis (PCoA) was used to clarify the extent of variation of the endophytic bacteria population in the different samples to better understand the microbial population that correlates with the leaves and roots of *Senna italica*. Data are presented as a 2D plot to better

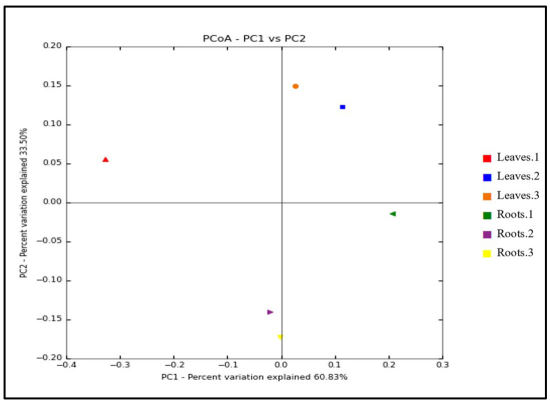

Figure 4. Beta diversity analysis. Unweighted PCoA of UniFrac distances, Principal coordinate analysis illustrates differences between bacterial communities in *senna italica* roots and leaves. Two first components (PC1 and PC2) were plotted and represented 94.33% of whole inertia. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with *Senna italica*. The red triangle indicates Leaves.1. The green triangle indicates Root.1. The purple triangle indicates Root.2. The yellow square indicates Root.3. The blue square indicates Leaves.2. The orange circle indicates Leaves.3.

illustrate the relationship. PCoA (Figure 4) identified OTU abundance in the samples and the unweighted UniFrac, which refers to unique species identified with two principal component factors which are (PC1 and PC2) in relation to the percentage abundance of groups, explaining the variation of 60.83 and 33.50%, respectively.

The PCoA analysis revealed that roots samples had a significantly higher PC1 value, except for Roots.2. Leaves samples had a higher PC2 value, except for Leaves.1. When samples are close to each other, this indicates a high degree of similarity. Our results showed that Roots.2 and Roots.3 in roots samples were relatively comparable. Likewise, Leaves.2 and Leaves.3 in leaves samples were relatively similar.

This Table 3 indicates the number of OUTs and Alpha diversity of endophytic bacteria in *Senna italica.* Alpha diversity includes Chao1 and Shannon. Chao1: Species richness estimators evaluate the total number of species present in a community using the frequency of occurrence of rarer OTUs. If a sample contains many singletons or doubletons, more undetected OTUs likely exist, and the Chao 1 index will estimate higher species richness than it would for a sample without rare OTUs. **Shannon:** A quantitative measure that reflects the number of different types (species) present within a dataset. It also simultaneously takes into account how evenly the basic entities (individuals) are distributed among those types.

3.5. Taxonomic classification at the phyla and genera levels

The phylogenetic tree based on 16S rRNA depicts the diversity and taxonomy of bacteria isolated from the six samples of roots and leaves at both the phylum levels (Figure 5), and genus levels (Supplementary Material

Table 3. Number of OUTs and Alpha diversity of endophytic bacteria in Senna italica.

Samples	Number of sequences	Observed OTUs	Alpha diversity	
			Chao1	Shannon
Leaves.1	83,006	13.0	13.5	0.615718226422
Leaves.2	91,718	17.0	23.0	0.635846981097
Leaves.3	69,799	18.0	28.0	0.660643836755
Roots.1	101,468	24.0	25.0	1.20627592565
Roots.2	79,090	17.0	17.0	0.944092778437
Roots.3	74,842	15.0	15.0	0.902923313288

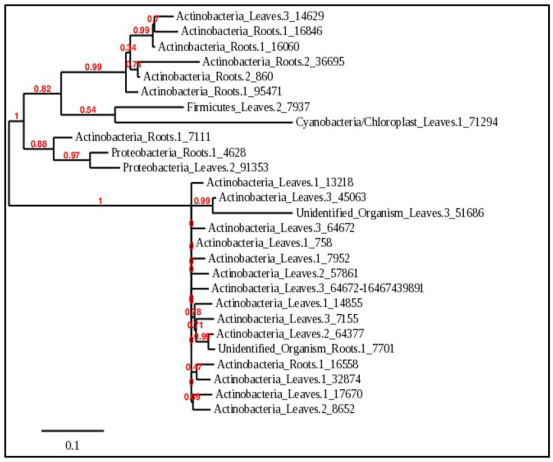

Figure 5. Phylogenetic tree based on 16S rRNA gene sequences representing the diversity of endophytic bacterial communities associated with the leaves and roots from the desert medicinal plant *Senna italica* "at the Phylum level". The tree was constructed using the "one-click" mode in Phylogeny.fr. (Dereeper et al., 2008).

Figure S3). A *phylogenetic tree* is a branching schema that depicts the predicted evolutionary relationships among various biological species based on physical or genetic similarities and differences (Dees et al., 2014). The closed evolution distance between taxa, the shorter the length of the branch. In addition to taxonomic makeup and abundance analyses, phylogenetic trees can explain species evolution relationships.

3.6. Taxonomic composition analysis

All sequences were classified from phylum to genus according to the program QIIME using the default setting. The sequences were classified into five different phyla, five classes, five orders, 11 families, and 12 genera. The overall bacterial composition of the different samples was similar, while the distribution of each phylum varied in all samples. The bacterial communities in the six roots and leaves samples at the phylum-level taxonomic distribution showed that they belong to five phyla (Figure 6A). These bacteria include *Actinobacteria*, *Cyanobacteria/Chloroplast*, *Proteobacteria*, *Firmicutes*, and unclassified phyla. The most abundant ones were highlighted for further analysis. To identify the taxa associated with the *Senna italica* samples and analyze their distribution in the different species and organs, two taxonomic levels (phylum and genus) were considered. The results indicated the two most dominant phyla: *Actinobacteria* (6 genera), *Cyanobacteria/Chloroplast* (one genus). The remaining phyla involving *Proteobacteria* (3 genera), *Firmicutes* (one genus), and unclassified phyla (one genus) were low in abundance.

In the root endophytic communities, sequences assigned to *Actinobacteria* (22.84%) were more abundant than leaves samples. Sequences assigned to *Cyanobacteria* (88.91%) were more abundant than root samples in the leaf endophytic communities. Analysis of the bacterial commonalities at the phylum classification showed that *Cyanobacteria/Chloroplast* was the most abundant division compared to the rest of the other samples. *Cyanobacteria* appeared significantly shown in the six samples, especially those collected from Leaves (Leaves.1, Leaves.2, and Leaves.3), followed by Roots.3, Roots.2, and Roots.1) (Figure 6F). *Actinobacteria* were the most present phylum in the sample collected from Roots.1 of the roots samples and the most abundant in the samples collected from Leaves.3 of the leaves samples (Figure 6B). *Proteobacteria* were significantly shown in the samples collected from both Roots.1 and Roots.3 of the roots samples and were found in leaves samples but were relatively infrequent (Figure 6C). Similarly, the unclassified phyla were relatively infrequent, except in the Roots.1 sample (Figure 6D). The *Firmicutes* phylum was identified in the reading of the sequences but almost negligible proportions (Figure 6E).

The five bacteria observed in Figure 6 were found at the phylum level, estimated at 12 genera. *Cellulominas, Okibacterium, Arthobacter, Kocuria, Pseudonocardia*, and *Streptomyces* bacteria are found in the *Actinobacteria*. *Microvirga*, *Ensifer*, and an unclassified genus were found in the *Proteobacteria*. *Streptophyta* were present in *Cyanobacteria/Chloroplast*, whereas *Bacillus* and one unclassified genus were found in the *Firmicutes* and in the unclassified phyla, respectively (Figure 7A). *Okibacterium* and *Streptomyces* were the most abundant genera found in the phylum of *Actinobacteria* (Figure 7B-7C). However, *Streptophyta* were the most abundant found in the phylum of *Cyanobacteria/Chloroplast* (Figure 7D), and *Ensifer* was the most abundant found in the phylum of *Proteobacteria*.

According to the lowest abundance at the genus level, *Cellulomonas*, *Arthrobacter*, *Kocuria* (*Actinobacteria* phylum),

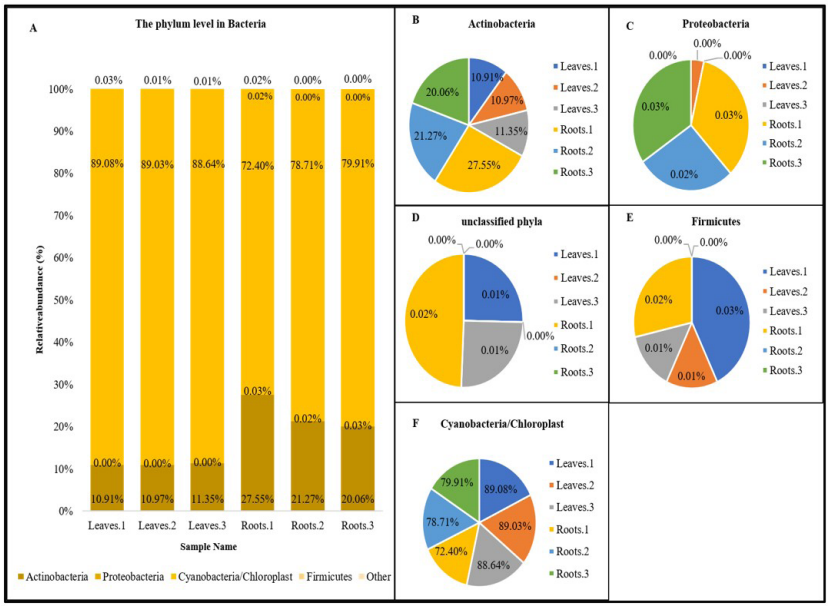

Figure 6. A. The phylum level in Bacteria (bar chart), the bacterial composition of the different samples was similar, while the distribution of each phylum varied in all samples. Based on the V3-V4 region of the 16S rRNA region. Bacterial communities at the phylum classification among the samples (pie chart), as a percentage of the total bacteria isolated from roots and leaves endophyte region. Based on the full-length 16S rRNA sequences. (B) The number of Actinobacteria among the samples. (C) The number of Proteobacteria among the samples. (D) The number of unclassified phyla among the samples. (E) The number of Firmicutes phyla among the samples. (F) The number of Cyanobacteria/Chloroplast among the samples. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with *Senna italica*.

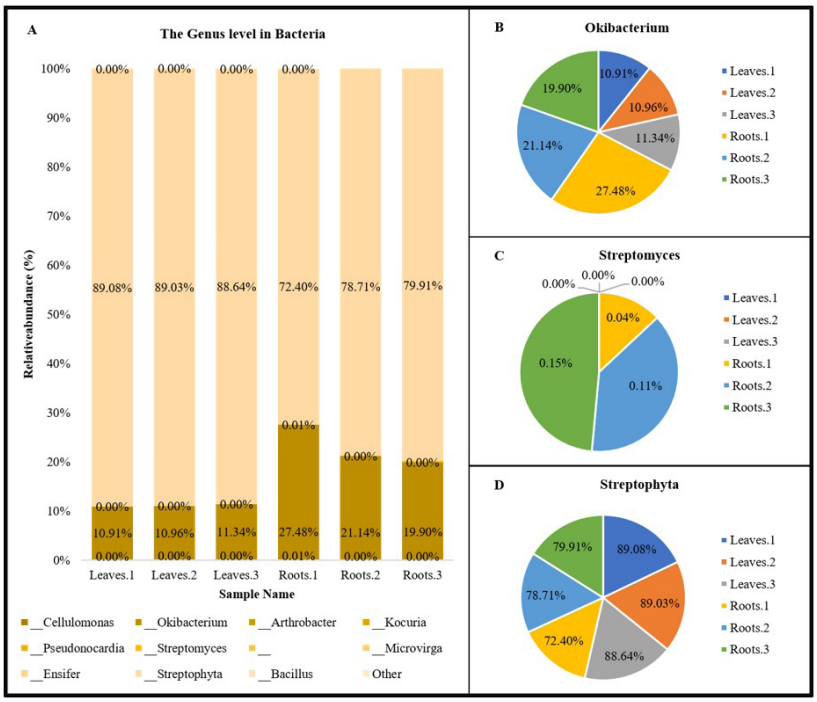

Figure 7. A. The Genus level in Bacteria (bar chart), the 12genera of the five bacteria were detected at the level of the phylum. Based on the V3-V4 region of the 16S rRNA region. The relative most abundance in the taxonomic composition distribution in samples of Genus -level (pie chart) as a percentage of the total bacteria isolated from roots and leaves endophyte region. Based on the full-length 16S rRNA sequences. (B) and (C) The most abundant genera found in the phylum of *Actinobacteria*. (D) The most abundant genus found in the phylum of *Cyanobacteria*. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with *Senna italica*.

Microvirga, unclassified genus (*Proteobacteria* phylum), and one unclassified genus (unclassified phyla) existed only in Roots.1 sample, while there were not present in the rest samples. *Pseudonocardia* (*Actinobacteria* phylum) presented only in Roots.2 and Roots.3 samples; however, *Ensifer* (*Proteobacteria*) was found in all roots samples, but these genera were not found in the leaves samples.

4. Discussion

We aimed in this study to characterize and classify the endophytic bacterial communities associated with the leaves and roots of the desert medicinal plant *Senna italica*. Using metagenomics techniques, we collected the *Senna italica* plant from the Asfan region in northeast Jeddah, Saudi Arabia. With several biotechnological applications, we hypothesize that these techniques may identify beneficial strains that promote crop survival under various environmental stresses, particularly under drought stress conditions. Based on our observations of bacterial endophyte diversity, we also hypothesized that the leaves and the roots of the *Senna italica* plant are colonized by several beneficial endophyte bacteria, which help the plants adapt and support their ability to resist drought conditions. Our findings confirmed other previous observations (Adeleke et al., 2022; Kuźniar et al., 2019), which supported our goals, indicating production of several agricultural applications from bacterial endophyte diversity, including biofertilizers and biocontrol agents, industrial-medical bioproduct production, and bioremediation . Therefore, bacteria were isolated from the roots and leaves of the *Senna italica* desert plant and studied further. This study will better understand the interactions between the plant microbes, enhancing agriculture production and meeting the global food demand between 59% to 98% by 2050.

Abiotic stresses like drought, heat, and salinity are key limiting variables that reduce agricultural output globally due to their detrimental effects on all plant activities. Endophytic bacteria colonize the tissues of the host without causing harm to the host (Reinhold-Hurek and Hurek, 2011). In recent studies, endophytes have been proven vital in increasing plant growth and yield, controlling pathogens, removing pollutants, and helping to plant nitrogen absorption (Chen et al. 1995). Furthermore, endophytic bacteria have recently been revealed to help the host plant cope with the negative consequences of abiotic stress (Li et al., 2017b).

Bacterial cultivation-based isolation techniques, which are widely employed, are ineffective owing to several constraints, including medium compositions, nutritional and environmental needs of microbial populations (Fierer et al., 2012). Our microbiome analysis involves sampling collection, processing, NGS sequencing, and bioinformatics analysis to provide the composition of those microbiota populations associated with desert plants.

Six samples were collected from the same plant, including the roots and leaves of the desert medicinal plant *Senna italica*. Amplification of the v1-v3 bacterial 16S rRNA genes regions by PCR detected bacterial biodiversity in these extreme conditions. Therefore, 499,923 high-quality sequences were obtained and classified at the phylum and genus levels, and the differences between bacterial combinations were studied in the six samples. Bacterial richness and diversity were examined in each sample. A slight change was found among the samples (from 13 to 24 OTUs) in the six samples from the same plant.

Bacterial lineages dominate the plant root endosphere which is inhabited by complex microbial groups and microorganisms (Compant et al., 2021). The sequencing results showed that the taxonomic distribution of the bacterial communities at the phylum level indicated five phyla. More importantly, multiple studies have revealed that these bacteria, notably PGPEB, offer various environmental and ecological advantages (Shailendra Singh 2015). Endophytic bacteria's involvement in agricultural yield improvement is now well established. Endophytic bacteria, which live and thrive inside plant tissue, have been intensively explored for disease control and stress reduction in many plants. PGPEB can thus be a beneficial tool for enhancing crop growth and production under both non-stressed and stressful environmental situations, such as low soil fertility (Singh et al., 2021). A diverse spectrum of bacterial endophytes has been isolated from various plant species, like rice, including genera *Pseudomonas*, *Corynebacterium*, *Sphingomonas*, and *Bacillus* (Adhikari et al. 2001), tomato, including genera *Enterobacter*, *Pseudomonas*, and *Micrococcus* (Samish et al., 1961), and soybean root nodules, including genera *Bacillus* (Bai et al. 2002). Endophytic bacteria are not evenly distributed throughout plant organs, and certain dominating phyla appear to be tissue- or organ-specific. Numerous investigations using various plant organs indicated considerable variances in the plant endophytic microbiota's composition (Akinsanya et al., 2015; Bodenhausen et al., 2013; Li et al., 2017a; Maropola et al., 2015; Romero et al., 2014; Sarria-Guzmán et al., 2016; Tian and Zhang, 2017; Yang et al., 2017).

Our Illumina-based analyses revealed that the dominance of several most dominant phyla among the six different samples was Cyanobacteria. This phylum was the most abundant in all six samples when compared to the bacterial communities of roots and leaves at a rate ranging between 89.08% to 72.40%. The highest percentage was found in the Leaves.1 sample, and the lowest percentage was found in the Roots.1 sample. Similarly, a study by (Zhang et al. 2019) analyzing endophytic bacterial communities associated with roots and leaves of plants growing in extreme environments, such as the Atacama desert and Patagonia, has also shown great dominance by the Cyanobacteria. Our analyses revealed the abundance of Cyanobacteria members in the roots and leaves of the desert *Senna italica* plants. High abundances of this phylum have also been reported in the endospheres. Lower abundances of cyanobacteria have been observed in the endosphere and other regions (rhizosphere and rhizoplane) of the medicinal perennial plant *Stellera chamaejasme* L. (Jin et al. 2014). Cyanobacteria are a varied collection of photosynthetic bacteria (some of which are nitrogen-fixing) that thrive in a wide range of severe settings, including rocks, soils, and deserts (Azua-Bustos et al., 2012; Mackenzie et al., 2013; Patzelt et al., 2014). To the gap of our knowledge, there have been no previous reports of Cyanobacteria

associated with the endosphere of the desert medicinal plant *Senna italica*.

The Gram-negative bacteria, including cyanobacteria, are the most diverse photosynthetic bacteria with chlorophyll and photosystems I and II that allow them to carry out oxygenic photosynthesis (Issa et al., 2014), (Canfora et al. 2014). Cyanobacteria can maintain photosynthetic metabolism in desert-like environments (high radiation, drought, and salt stress, and so on) (Chen et al., 2013; Harel et al., 2004; Singh et al., 2013; Singh et al., 2010). Cyanobacteria exist in leaves, stems, and roots, and are well-known and essential because they contribute significantly to nitrogen fixation by synthesizing nitrogen-containing cellular components from elemental nitrogen (Issa et al., 2014). For instance, the study (Mishra and Pabbi, 2004) has reported that based on the ability of cyanobacteria on nitrogen fixation, its application in rice crops as bio-fertilizer could prove eco-friendly and cost-effective measures for improving rice productivity. Furthermore, cyanobacteria act as plant growth promoters as a source of bio-energy and food supplements. Cyanobacteria species have many applications in bioremediation, bio-fertilizers and bio-control agents (Singh et al., 2016). Based on our findings, this is consistent with the hypotheses and objective of this study, thus, maximum use of cyanobacteria as bio-fertilizers will minimize dependency on fertilizers, and might improve efforts for a more sustainable environment and ecosystem.

In this study, the phylum *Actinobacteria* were found the second most abundant in all six samples when compared to the other bacterial communities of roots and leaves. This phylum is common at a rate ranging between 10.91% and 27.55%, with the highest percentage observed in the Roots.1 sample, and the lowest percentage was in the leaves.1 sample. Thus, our results showed the predominance of *Actinobacteria* in the endophytic communities of the roots than in the endophytic communities of the leaves, which was also observed by other studies for a number of yield plants, including barley, maize, grapevine, and rice (Bulgarelli et al., 2013; Hernández et al., 2015; Niu et al., 2017; Zarraonaindia et al., 2015). Additionally, endophytic Actinobacteria were found to influence nutrient absorption and plant growth (Rajkumar et al., 2006), and to promote plant development in grains and legumes (Mano et al., 2007, Desriac et al., 2013).

According to a (Madhurama et al., 2014) study on three medicinal plants, the endophytic *Actinobacteria* are plentiful in roots, moderately in stems, and found in minor quantities in leaves. Likewise, our findings showed higher proportions of *Actinobacteria* in the roots samples of the *Senna italica* plant more than in the leaves samples. Endophytic *Actinobacteria* have a logical distribution pattern since the roots have the most exposure to interactions with the microbial community in the rhizosphere. (Huang, 2012) has reported the occurrence of some genera of the phylum *Actinobacteria* in desert plants, including *Streptomyces, Micromonospora*, *Nocardia*, *Nonomuraea*, and *Amycolatopsis*. Similar to our results, where we found that the

roots of the desert *Senna italica* plant contained *Actinobacteria* that include some genera, such as *Okibacterium*, *Arthrobacter*, *Kocuria*, *Pseudonocardia*, and *Streptomyces*.

Despite abiotic stress and nutritional deficiency, desert plants were colonized by several endophytic *Actinobacteria*, most notably *Streptomyces*, followed by other rare genera and new species, such as *Okibacterium*, *Arthrobacter*, *Kocuria*, and *Pseudonocardia* (Singh and Dubey, 2018). However, studies on desert plant endophytes are still unexplored. *Actinobacteria* contain significant amounts of bioactive chemicals and are frequently employed as a source of antibacterial biomaterials (Elbendary et al., 2018). *Actinobacteria* build many secondary metabolites with considerable medicinal and economic significance. They are also crucial in reintroducing unregulated biomaterials through plant and animal breakdown. Moreover, several antibiotics are derived from *Actinobacteria*, particularly some species of *Streptomyces*. In addition, they have a significant function in resisting ultraviolet (UV) radiation and dehydration (Barka et al., 2016; Rodríguez et al., 1989; Zhao et al., 2018). It has been suggested that secondary metabolites generated by endophytic *Actinobacteria* are predominantly pharmaceutically relevant groups, such as quinones, alkaloids, flavonoids, terpenoids, steroids, phenolics, and peptides (Yu et al., 2010).

Our results showed that *Proteobacteria* frequently exist in the root samples, specifically in Roots.1 and Roots.3, with a proportion of 0.03%. Whereas in the Roots.2 samples, they were estimated as 0.02%. Comparable to our results, in a study conducted by (Singha, Singh, and Pandey 2021) on scented black rice, the relative abundance of *Proteobacteria* was higher in the plants' roots than in the shoot. Similarly, (Hallmann et al. 1997; Sessitsch et al. 2012) reported that *Proteobacteria* were the endophytic dominant phylum in the roots of crops, such as wheat and rice. Additionally, (Mano et al., 2007) suggested a higher abundance of endophytic *Proteobacteria* in roots than in shoots. *Proteobacteria* include antibacterial and antifungal properties, which promote plant development. They are engaged in the bioremediation of numerous toxic compounds and are a source of naturally occurring bioactive products (Bodenhausen et al., 2013; Desriac et al., 2013; Mukhtar et al., 2018). *Proteobacteria* are sensitive to climate change and impact the soil biosphere, owing to their participation in the global carbon, nitrogen, and sulfur cycles (Zhao et al., 2018). The current study found the predominance of endophytic bacteria, including *Actinobacteria* and *Proteobacteria* in all root samples. This is consistent with (Hong et al. 2019), where they observed the prevalence of *Proteobacteria* and *Actinobacteria* in all roots of *Panax ginseng* plant. Moreover, (Robinson et al. 2016) demonstrated that the roots are suitable sites for endophyte colonization because they are a store for photosynthetic carbon and are sheltered from temperature, solar radiation, and moisture changes.

We observed unclassified bacteria at the phylum level with a proportion of 0.02% in the Roots.1 sample. Their occurrence may refer to a lack of a reference sequence in the database, and these bacteria may contain an unidentified potential filter. According to the sequencing results, *Firmicutes* bacteria were identified in all six samples but in negligible proportions. *Firmicutes* have a variety of roles in

stress tolerance, e.g., drought and bioremediation (Dai et al. 2019). For example, *Firmicutes* presented by *Bacillus* species can create salt-stress compounds to withstand salt, resulting in osmotic pressures. It is worth mentioning that most studies has demonstrated that *Bacillus* species may grow in relative quantities in desert soil following stress (Abo-Aba et al., 2015; Meena et al., 2017).

Our results from the diverse phyla exhibited their ability to resist abiotic stress, such as salinity and drought. On the genus level, numerous studies have demonstrated the benefits of bacteria in environmental, medical, industrial, and agricultural applications. A comparison of the communities associated with the leaves and roots reveals both ubiquitous and organ-specific groups. For example, *Okibacterium* was the most abundant genus in root and leaf-associated communities, which fall under *Actinobacteria*. *Okibacterium* genus is found in all six samples, where it was found in leaves samples with an average (16.95% ±0.02832), and percentages were (11.34%, 10.96%, 10.91%) in samples leaves.3, leaves.2, and leaves.1 respectively. While Okibacterium genus was found in roots samples at relatively higher rates, where the percentages were as follows (27.48%, 21.14%, 19.90%) for the Roots.1, Roots.2, and Roots.3 samples respectively. But no reports on this endophyte are known.

Okibacterium is a newly discovered plant-associated bacterial genus which have two species. *Okibacterium* species have been isolated from the roots and seeds of plants (Fay et al. 2021). According to (Wang et al. 2015), endophytic *Okibacterium* species are Gram-positive, catalase-positive, oxidase-positive, aerobic, and non-motile and irregular rods. Their strains involve glycine, homoserine, lysine, glutamate, and alanine in their peptidoglycan cell wall, distingueshing them from the other *Plantibacter* genus members. Studies on *Okibacterium*-plant-interaction remain; therefore this aspect deserves attention for future studies.

A recent study (Yang et al. 2017) on the tree peony found *Streptomyces* in roots, where no detection was observed in the leaf. This supports our findings, as *Streptomyces* of *Actinobacteria* phylum has the highest prevalence in the root samples (Roots.1: 0.04%, Roots.2: 0.11%, and Roots.3: 0.15%), with an average of (0.05% ± 0.00026). Several physiological studies have indicated that *Streptomyces* can create bioactive secondary metabolites, such as antifungals, antivirals, antitumors, antihypertensives, immunosuppressants, and antibiotics (Kong et al., 2019; de Lima Procópio et al., 2012; Seipke et al., 2012). About 53 *Actinobacteria* were isolated from the Qinghai-Tibet plateau and classified as *Streptomyces* and *Cellulomonas* (Ding et al., 2013). Those results are consistent with ours, where we detected *Streptomyces* and *Cellulomonas* as species of *Actinobacteria*. Most of these strains could produce active chemicals (Ding et al. 2013). The metagenomic analysis of bioactive secondary metabolites can be evaluated in the future (Schofield and Sherman, 2013; Wilson and Piel, 2013).

Between *Cyanobacteria* and *Chloroplast*, the *Streptophyta* were found significantly among the six samples, with a higher proportion in the leaves (Average, 88.92%, ± 0.00138) than in the roots (Average, 77.01% ± 0.0233). *Streptophyte* is one of the most important extant green algae lineages, allowing the speculation of the DNA content modifications that have occurred during their history (Kapraun, 2007). *Streptophyta* is critical in primary production, nitrogen fixation, and nutrient cycling. Although data on *Streptophyta* is few, its functional significance is well acknowledged. The activities of these communities have been ascribed to worldwide carbon fixation comparable to 6% of terrestrial vegetation and around 40% of global biological N fixing. *Streptophyta* and other green algae and cyanobacteria can create a correlated relationship with soil particles. They play significant ecological functions in primary production, water retention, and soil stability (Glaser et al., 2017). However, studies on the interaction between *Streptophyta* and plants are still unknown. *Ensifer* of *Proteobacteria* phylum was found in root-and leaf-associated communities. *Ensifer* comprises symbiotic and nonsymbiotic N2-fixation of leguminous plants (Fagorzi et al., 2020).

Our study demonstrates that some microbial populations, such as *Okibacterium* and *Streptophyta* were not identified as endophytes inhabiting *Senna italica* plant, which could have biotechnological applications, particularly in the production of novel microbial inoculants. On the other hand, a number of genera have been recorded representing the least abundant bacterial community among the phyla that have been found, and include the following genera Pseudonocardia, Cellulomonas, Arthrobacter, and Kocuria which of the Actinobacteria phylum. Microvirga and unclassified genus which of Proteobacteria phylum. And one unclassified genus which of the unclassified phylum. Noting that, all the above-mentioned genera were found only in the roots samples, while they were not found in the leaves samples. Some low-abundance microbial taxa are referred to as "satellite taxa" (Hanski 1982; Magurran and Henderson 2003) they can be determined mostly by their local abundance and habitat specialization (Jousset et al. 2017). It was recently proposed that low-abundance taxa could be able to fend off invasions of unwanted microbial in soil ecosystems (Mallon et al. 2015) and significantly contributed to the formation of volatile antifungal chemicals, which ultimately protected plants against soil-borne phytopathogens (Hol et al., 2015).

5. Conclusion

This study explores the diversity of endophytic bacterial communities associated with the leaves and roots of the desert medicinal plant *Senna italica*. This plant was collected from the Asfan region, in northeast Jeddah, SA. Illumina Miseq using the 16S rRNA gene as the biomarker was conducted to examine the bacterial diversity of *Senna italica* samples to get a broader overview of endophytic bacteria. To the best of our knowledge, this study is the first to use the PCR-based Illumina Miseq technology for investigating the diversity of endophytic bacteria communities associated with the leaves and roots of the desert medicinal plant *Senna italica* using cultivation-independent methods. This will lead to a better knowledge of novel candidates for biological agents that may be utilized to improve agricultural operations. According to our findings, endophytic bacteria can be studied as indicators of plant growth rate as well as their ability

to survive under harsh environmental circumstances. In addition to identifying endophytic bacterial communities using high-throughput molecular tools for taxonomic and phylogenetic characterization. In the future, there is a need for detailed representation and comparative functional and biochemical studies for the diversity of the endophytic microbiome are required to highlight different metabolic pathways. The studies in this regard will help to enhance the use of beneficial endophytes in sustainable agricultural methods. where there is still much to learn about desert endophytes, which can be revealed with more research.

Acknowledgements

Authors acknowledge receipt of funding via University of Jeddah, Jeddah, Saudi Arabia, under grant No. (UJ-22-DR-32). The authors, therefore, acknowledge with thanks the University of Jeddah technical and financial support.

References

ABO-ABA, S.E.M., SABIR, J.S., BAESHEN, M.N., SABIR, M.J., MUTWAKIL, M.H., BAESHEN, N.A., D'AMORE, R. and HALL, N., 2015. Draft genome sequence of Bacillus species from the rhizosphere of the desert plant Rhazya stricta. *Genome Announcements*, vol. 3, no. 6, p. e00957-15. http://dx.doi.org/10.1128/genomeA.00957-15. PMid:26543104.

ADELEKE, B.S., FADIJI, A.E., AYILARA, M.S., IGIEHON, O.N., NWACHUKWU, B.C. and BABALOLA, O.O., 2022. Strategies to enhance the use of endophytes as bioinoculants in agriculture. *Horticulturae*, vol. 8, no. 6, p. 498. http://dx.doi.org/10.3390/horticulturae8060498.

ADHIKARI, T.B., JOSEPH, C.M., YANG, G., PHILLIPS, D.A. and NELSON, L.M., 2001. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. *Canadian Journal of Microbiology*, vol. 47, no. 10, pp. 916-924. http://dx.doi.org/10.1139/w01-097. PMid:11718545.

ADJOU, E.S., KOUDORO, A.Y. and NONVIHO, G., 2021. Phytochemical profile and potential pharmacological properties of leaves extract of Senna italica Mill. *American Journal of Pharmacological Sciences*, vol. 9, no. 1, pp. 36-39.

AKINSANYA, M.A., GOH, J.K., LIM, S.P. and TING, A.S.Y., 2015. Metagenomics study of endophytic bacteria in Aloe vera using next-generation technology. *Genomics Data*, vol. 6, pp. 159-163. http://dx.doi.org/10.1016/j.gdata.2015.09.004. PMid:26697361.

ALVES, L.F., WESTMANN, C.A., LOVATE, G.L., SIQUEIRA, G.M.V., BORELLI, T.C. and GUAZZARONI, M.E., 2018. Metagenomic approaches for understanding new concepts in microbial science. *International Journal of Genomics*, vol. 2018, p. 2312987. http://dx.doi.org/10.1155/2018/2312987. PMid:30211213.

ANDREWS, M., HODGE, S. and RAVEN, J.A., 2010. Positive plant microbial interactions. *Annals of Applied Biology*, vol. 157, no. 3, pp. 317-320. http://dx.doi.org/10.1111/j.1744-7348.2010.00440.x.

ANISIMOVA, M. and GASCUEL, O., 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Systematic Biology*, vol. 55, no. 4, pp. 539-552. http://dx.doi.org/10.1080/10635150600755453. PMid:16785212.

AZAROUAL, S.E., KASMI, Y., AASFAR, A., ARROUSSI, H., ZEROUAL, Y., KADIRI, Y., ZRHIDRI, A., ELFAHIME, E., SEFIANI, A. and KADMIRI, I.M., 2022. Investigation of bacterial diversity using 16S rRNA sequencing and prediction of its functionalities in Moroccan phosphate mine ecosystem. *Scientific Reports*, vol. 12, no. 1, p. 3741. http://dx.doi.org/10.1038/s41598-022-07765-5. PMid:35260670.

AZUA-BUSTOS, A., URREJOLA, C. and VICUÑA, R., 2012. Life at the dry edge: microorganisms of the Atacama Desert. *FEBS Letters*, vol. 586, no. 18, pp. 2939-2945. http://dx.doi.org/10.1016/j.febslet.2012.07.025. PMid:22819826.

BAI, Y., D'AOUST, F., SMITH, D.L. and DRISCOLL, B.T., 2002. Isolation of plant-growth-promoting Bacillus strains from soybean root nodules. *Canadian Journal of Microbiology*, vol. 48, no. 3, pp. 230-238. http://dx.doi.org/10.1139/w02-014. PMid:11989767.

BARKA, E.A., VATSA, P., SANCHEZ, L., GAVEAU-VAILLANT, N., JACQUARD, C., MEIER-KOLTHOFF, J.P., KLENK, H.-P., CLÉMENT, C., OUHDOUCH, Y. and VAN WEZEL, G.P., 2016. Taxonomy, physiology, and natural products of actinobacteria. *Microbiology and Molecular Biology Reviews*, vol. 80, no. 1, pp. 1-43. http://dx.doi.org/10.1128/MMBR.00019-15. PMid:26609051.

BODENHAUSEN, N., HORTON, M.W. and BERGELSON, J., 2013. Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. *PLoS One*, vol. 8, no. 2, p. e56329. http://dx.doi.org/10.1371/journal.pone.0056329. PMid:23457551.

BOYER, J.S., 1982. Plant productivity and environment. *Science*, vol. 218, no. 4571, pp. 443-448. http://dx.doi.org/10.1126/science.218.4571.443. PMid:17808529.

BUDKA, A., ŁACKA, A. and SZOSZKIEWICZ, K., 2019. The use of rarefaction and extrapolation as methods of estimating the effects of river eutrophication on macrophyte diversity. *Biodiversity and Conservation*, vol. 28, no. 2, pp. 385-400. http://dx.doi.org/10.1007/s10531-018-1662-3.

BULGARELLI, D., SCHLAEPPI, K., SPAEPEN, S., VAN THEMAAT, E.L. and SCHULZE-LEFERT, P., 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, vol. 64, no. 1, pp. 807-838. http://dx.doi.org/10.1146/annurev-arplant-050312-120106. PMid:23373698.

CANFORA, L., BACCI, G., PINZARI, F., PAPA, G., DAZZI, C. and BENEDETTI, A., 2014. Salinity and bacterial diversity: to what extent does the concentration of salt affect the bacterial community in a saline soil? *PLoS One*, vol. 9, no. 9, p. e106662. http://dx.doi.org/10.1371/journal.pone.0106662. PMid:25188357.

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., HUTTLEY, G.A., KELLEY, S.T., KNIGHTS, D., KOENIG, J.E., LEY, R.E., LOZUPONE, C.A., MCDONALD, D., MUEGGE, B.D., PIRRUNG, M., REEDER, J., SEVINSKY, J.R., TURNBAUGH, P.J., WALTERS, W.A., WIDMANN, J., YATSUNENKO, T., ZANEVELD, J. and KNIGHT, R., 2010a. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, vol. 7, no. 5, pp. 335-336. http://dx.doi.org/10.1038/nmeth.f.303. PMid:20383131.

CAPORASO, J.G., LAUBER, C.L., WALTERS, W.A., BERG-LYONS, D., LOZUPONE, C.A., TURNBAUGH, P.J., FIERER, N. and KNIGHT, R., 2010b. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, suppl. 1, pp. 4516-4522. http://dx.doi.org/10.1073/pnas.1000080107. PMid:20534432.

CERRI, C.E.P., SPAROVEK, G., BERNOUX, M., EASTERLING, W.E., MELILLO, J.M. and CERRI, C.C., 2007. Tropical agriculture and global warming: impacts and mitigation options. *Scientia Agrícola*, vol. 64, no. 1, pp. 83-99. http://dx.doi.org/10.1590/S0103-90162007000100013.

CHEN, C., BAUSKE, E.M., MUSSON, G., RODRIGUEZKABANA, R. and KLOEPPER, J.W., 1995. Biological control of Fusarium Wilt on cotton by use of endophytic bacteria. *Biological Control*, vol. 5, no. 1, pp. 83-91. http://dx.doi.org/10.1006/bcon.1995.1009.

CHEN, L., DENG, S., PHILIPPIS, R., TIAN, W., WU, H. and WANG, J., 2013. UV-B resistance as a criterion for the selection of desert microalgae to be utilized for inoculating desert soils. *Journal of Applied Phycology*, vol. 25, no. 4, pp. 1009-1015. http://dx.doi.org/10.1007/s10811-012-9906-1.

CHEVENET, F., BRUN, C., BAÑULS, A.L., JACQ, B. and CHRISTEN, R., 2006. TreeDyn: towards dynamic graphics and annotations for analyses of trees. *BMC Bioinformatics*, vol. 7, no. 1, p. 439. http://dx.doi.org/10.1186/1471-2105-7-439. PMid:17032440.

COMPANT, S., CAMBON, M., VACHER, C., MITTER, B., SAMAD, A. and SESSITSCH, A., 2021. The plant endosphere world – bacterial life within plants. *Environmental Microbiology*, vol. 23, no. 4, pp. 1812-1819. PMid:32955144.

D'AMORE, R., IJAZ, U.Z., SCHIRMER, M., KENNY, J.G., GREGORY, R., DARBY, A.C., SHAKYA, M., PODAR, M., QUINCE, C. and HALL, N., 2016. A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics*, vol. 17, no. 1, p. 55. http://dx.doi.org/10.1186/s12864-015-2194-9. PMid:26763898.

DABAI, Y.U., 2012. Phytochemical screening and antibacterial activity of the leaf and root extracts of Senna italica. *African Journal of Pharmacy and Pharmacology*, vol. 6, no. 12, pp. 914-918. http://dx.doi.org/10.5897/AJPP11.852.

DAI, L., ZHANG, G., YU, Z., DING, H., XU, Y. and ZHANG, Z., 2019. Effect of drought stress and developmental stages on microbial community structure and diversity in peanut rhizosphere soil. *International Journal of Molecular Sciences*, vol. 20, no. 9, p. 2265. http://dx.doi.org/10.3390/ijms20092265. PMid:31071918.

DAS, A. and VARMA, A., 2009. Symbiosis: the art of living. In: A. VARMA and A.C. KHARKWAL, eds. *Symbiotic fungi: principles and practice*. Berlin: Springer, pp. 1-28. http://dx.doi.org/10.1007/978-3-540-95894-9_1.

DEES, J., MOMSEN, J.L., NIEMI, J. and MONTPLAISIR, L., 2014. Student interpretations of phylogenetic trees in an introductory Biology student interpretations of phylogenetic trees in an introductory Biology course. *CBE Life Sciences Education*, vol. 13, no. 4, pp. 666-676. http://dx.doi.org/10.1187/cbe.14-01-0003. PMid:25452489.

DEREEPER, A., AUDIC, S., CLAVERIE, J.M. and BLANC, G., 2010. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evolutionary Biology*, vol. 10, no. 1, p. 8. http://dx.doi.org/10.1186/1471-2148-10-8. PMid:20067610.

DEREEPER, A., GUIGNON, V., BLANC, G., AUDIC, S., BUFFET, S., CHEVENET, F., DUFAYARD, J.F., GUINDON, S., LEFORT, V., LESCOT, M., CLAVERIE, J.M. and GASCUEL, O., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, vol. 36, no. Web Server, pp. W465-W469. http://dx.doi.org/10.1093/nar/gkn180. PMid:18424797.

DESRIAC, F., JÉGOU, C., BALNOIS, E., BRILLET, B., CHEVALIER, P. and FLEURY, Y., 2013. Antimicrobial peptides from marine proteobacteria. *Marine Drugs*, vol. 11, no. 10, pp. 3632-3660. http://dx.doi.org/10.3390/md11103632. PMid:24084784.

DING, D., CHEN, G., WANG, B., WANG, Q., LIU, D., PENG, M. and SHI, P., 2013. Culturable actinomycetes from desert ecosystem in northeast of Qinghai-Tibet Plateau. *Annals of Microbiology*, vol. 63, no. 1, pp. 259-266. http://dx.doi.org/10.1007/s13213-012-0469-9.

DUDEJA, S.S., GIRI, R., SAINI, R., SUNEJA-MADAN, P. and KOTHE, E., 2012. Interaction of endophytic microbes with legumes. *Journal of Basic Microbiology*, vol. 52, no. 3, pp. 248-260. http://dx.doi.org/10.1002/jobm.201100063. PMid:21953403.

EDGAR, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, vol. 32, no. 5, pp. 1792-1797. http://dx.doi.org/10.1093/nar/gkh340. PMid:15034147.

EDGAR, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, vol. 26, no. 19, pp. 2460-2461. http://dx.doi.org/10.1093/bioinformatics/btq461. PMid:20709691.

EHLERINGER, J.R. and MONSON, R.K., 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics*, vol. 24, no. 1, pp. 411-439. http://dx.doi.org/10.1146/annurev.es.24.110193.002211.

EIDA, A.A., ZIEGLER, M., LAFI, F.F., MICHELL, C.T., VOOLSTRA, C.R., HIRT, H. and SAAD, M.M., 2018. Desert plant bacteria reveal host influence and beneficial plant growth properties. *PLoS One*, vol. 13, no. 12, p. e0208223. http://dx.doi.org/10.1371/journal.pone.0208223. PMid:30540793.

ELBENDARY, A.A., HESSAIN, A.M., EL-HARIRI, M.D., SEIDA, A.A., MOUSSA, I.M., MUBARAK, A.S., KABLI, S.A., HEMEG, H.A. and JAKEE, J.K., 2018. Isolation of antimicrobial producing Actinobacteria from soil samples. *Saudi Journal of Biological Sciences*, vol. 25, no. 1, pp. 44-46. http://dx.doi.org/10.1016/j.sjbs.2017.05.003. PMid:29379355.

EREN, A.M., MAIGNIEN, L., SUL, W.J., MURPHY, L.G., GRIM, S.L., MORRISON, H.G. and SOGIN, M.L., 2013. Oligotyping: differentiating between closely related microbial taxa using 16S rRNA gene data. *Methods in Ecology and Evolution*, vol. 4, no. 12, pp. 1111-1119. http://dx.doi.org/10.1111/2041-210X.12114. PMid:24358444.

FADIJI, A.E. and BABALOLA, O.O., 2020. Metagenomics methods for the study of plant-associated microbial communities: a review. *Journal of Microbiological Methods*, vol. 170, p. 105860. http://dx.doi.org/10.1016/j.mimet.2020.105860. PMid:32027927.

FAGORZI, C., ILIE, A., DECOROSI, F., CANGIOLI, L., VITI, C., MENGONI, A. and DICENZO, G.C., 2020. Symbiotic and nonsymbiotic members of the genus Ensifer (syn. Sinorhizobium) are separated into two clades based on comparative genomics and high-throughput phenotyping. *Genome Biology and Evolution*, vol. 12, no. 12, pp. 2521-2534. http://dx.doi.org/10.1093/gbe/evaa221. PMid:33283865.

FAY, M., SALAZAR, J.K., RAMACHANDRAN, P. and STEWART, D., 2021. Microbiomes of commercially-available pine nuts and sesame seeds. *PLoS One*, vol. 16, no. 6, p. e0252605. http://dx.doi.org/10.1371/journal.pone.0252605. PMid:34153055.

FIERER, N., LEFF, J.W., ADAMS, B.J., NIELSEN, U.N., BATES, S.T., LAUBER, C.L., OWENS, S., GILBERT, J.A., WALL, D.H. and CAPORASO, J.G., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 52, pp. 21390-21395. http://dx.doi.org/10.1073/pnas.1215210110. PMid:23236140.

FRIESEN, M.L., PORTER, S.S., STARK, S.C., VON WETTBERG, E.J., SACHS, J.L. and MARTINEZ-ROMERO, E., 2011. Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics*, vol. 42, no. 1, pp. 23-46. http://dx.doi.org/10.1146/annurev-ecolsys-102710-145039.

GLASER, K., DONNER, A., ALBRECHT, M., MIKHAILYUK, T. and KARSTEN, U., 2017. Habitat-specific composition of morphotypes with low genetic diversity in the green algal genus Klebsormidium (Streptophyta) isolated from biological soil crusts in Central European grasslands and forests. *European*

Journal of Phycology, vol. 52, no. 2, pp. 188-199. http://dx.doi.org/10.1080/09670262.2016.1235730.

GLICK, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, vol. 2012, p. 963401. http://dx.doi.org/10.6064/2012/963401. PMid:24278762.

GUINDON, S. and GASCUEL, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, vol. 52, no. 5, pp. 696-704. http://dx.doi.org/10.1080/10635150390235520. PMid:14530136.

HALLMANN, J., QUADT-HALLMANN, A., MAHAFFEE, W.F. and KLOEPPER, J.W., 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, vol. 43, no. 10, pp. 895-914. http://dx.doi.org/10.1139/m97-131.

HANDELSMAN, J., RONDON, M.R., BRADY, S.F., CLARDY, J. and GOODMAN, R.M., 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chemistry & Biology*, vol. 5, no. 10, pp. R245-R249. http://dx.doi.org/10.1016/S1074-5521(98)90108-9. PMid:9818143.

HANSKI, I., 1982. Dynamics of regional distribution: the core and satellite species hypothesis. *Oikos*, vol. 38, no. 2, p. 210. http://dx.doi.org/10.2307/3544021.

HAREL, Y., OHAD, I. and KAPLAN, A., 2004. Activation of photosynthesis and resistance to photoinhibition in cyanobacteria within biological desert crust. *Plant Physiology*, vol. 136, no. 2, pp. 3070-3079. http://dx.doi.org/10.1104/pp.104.047712. PMid:15466226.

HARMAN, G., KHADKA, R., DONI, F. and UPHOFF, N., 2021. Benefits to plant health and productivity from enhancing plant microbial symbionts. *Frontiers in Plant Science*, vol. 11, p. 610065. http://dx.doi.org/10.3389/fpls.2020.610065. PMid:33912198.

HARTWELL, J., 2005. *The co-ordination of central plant metabolism by the circadian clock*. London: Portland Press Ltd.

HERNÁNDEZ, M., DUMONT, M.G., YUAN, Q. and CONRAD, R., 2015. Different bacterial populations associated with the roots and rhizosphere of rice incorporate plant-derived carbon. *Applied and Environmental Microbiology*, vol. 81, no. 6, pp. 2244-2253. http://dx.doi.org/10.1128/AEM.03209-14. PMid:25616793.

HOL, W.H.G., GARBEVA, P., HORDIJK, C., HUNDSCHEID, P.J., GUNNEWIEK, P.J., VAN AGTMAAL, M., KURAMAE, E.E. and BOER, W., 2015. Non-random species loss in bacterial communities reduces antifungal volatile production. *Ecology*, vol. 96, no. 8, pp. 2042-2048. http://dx.doi.org/10.1890/14-2359.1. PMid:26405729.

HONG, C.E., KIM, J.U., LEE, J.W., BANG, K.H. and JO, I.H., 2019. Metagenomic analysis of bacterial endophyte community structure and functions in Panax ginseng at different ages. *3 Biotech*, vol. 9, no. 8, p. 300. http://dx.doi.org/10.1007/s13205-019-1838-x. PMid:31355109.

HUANG, X.L., 2012. Isolation and bioactivity of endophytic filamentous actinobacteria from tropical medicinal plants. *African Journal of Biotechnology*, vol. 11, no. 41, pp. 9855-9864. http://dx.doi.org/10.5897/AJB11.3839.

ISSA, A.A., ABD-ALLA, M.H. and OHYAMA, T., 2014. Nitrogen fixing cyanobacteria: future prospect. In: T. OHYAMA, ed. *Advances in biology and ecology of nitrogen fixation*. Rijeka: IntechOpen, pp. 24-48.

JIN, H., YANG, X.Y., YAN, Z.Q., LIU, Q., LI, X.Z., CHEN, J.X., ZHANG, D.H., ZENG, L.M. and QIN, B., 2014. Characterization of rhizosphere and endophytic bacterial communities from leaves, stems and roots of medicinal Stellera chamaejasme L. *Systematic and Applied Microbiology*, vol. 37, no. 5, pp. 376-385. http://dx.doi.org/10.1016/j.syapm.2014.05.001. PMid:24958606.

JOUSSET, A., BIENHOLD, C., CHATZINOTAS, A., GALLIEN, L., GOBET, A., KURM, V., KÜSEL, K., RILLIG, M.C., RIVETT, D.W., SALLES, J.F., VAN DER HEIJDEN, M.G., YOUSSEF, N.H., ZHANG, X., WEI, Z. and HOL, W.H., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. *The ISME Journal*, vol. 11, no. 4, pp. 853-862. http://dx.doi.org/10.1038/ismej.2016.174. PMid:28072420.

KAPRAUN, D.F., 2007. Nuclear DNA content estimates in green algal lineages: chlorophyta and streptophyta. *Annals of Botany*, vol. 99, no. 4, pp. 677-701. http://dx.doi.org/10.1093/aob/mcl294. PMid:17272304.

KHALAF, O.M., GHAREEB, M.A., SAAD, A.M., MADKOUR, H.M.F., EL-ZIATY, A.K. and ABDEL-AZIZ, M.S., 2019. Phenolic constituents, antimicrobial, antioxidant, and anticancer activities of ethyl acetate and n-butanol extracts of senna italica. *Acta Chromatographica*, vol. 31, no. 2, pp. 138-145. http://dx.doi.org/10.1556/1326.2018.00412.

KLINDWORTH, A., PRUESSE, E., SCHWEER, T., PEPLIES, J., QUAST, C., HORN, M. and GLÖCKNER, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, vol. 41, no. 1, p. e1. http://dx.doi.org/10.1093/nar/gks808. PMid:22933715.

KONG, D., WANG, X., NIE, J. and NIU, G., 2019. Regulation of antibiotic production by signaling molecules in streptomyces. *Frontiers in Microbiology*, vol. 10, p. 2927. http://dx.doi.org/10.3389/fmicb.2019.02927. PMid:31921086.

KOZICH, J.J., WESTCOTT, S.L., BAXTER, N.T., HIGHLANDER, S.K. and SCHLOSS, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, vol. 79, no. 17, pp. 5112-5120. http://dx.doi.org/10.1128/AEM.01043-13. PMid:23793624.

KUMAR, M., SAXENA, R. and TOMAR, R.S., 2017. Endophytic microorganisms: promising candidate as biofertilizer. In: D.G. PANPATTE, Y.K. JHALA, R.V. VYAS and H.N. SHELAT, eds. *Microorganisms for green revolution*. Singapore: Springer, vol. 1, pp. 77-85. http://dx.doi.org/10.1007/978-981-10-6241-4_4.

KUŹNIAR, A., WŁODARCZYK, K. and WOLIŃSKA, A., 2019. Agricultural and other biotechnological applications resulting from trophic plant-endophyte interactions. *Agronomy*, vol. 9, no. 12, p. 779. http://dx.doi.org/10.3390/agronomy9120779.

LE COCQ, K., GURR, S.J., HIRSCH, P.R. and MAUCHLINE, T.H., 2017. Exploitation of endophytes for sustainable agricultural intensification. *Molecular Plant Pathology*, vol. 18, no. 3, pp. 469-473. http://dx.doi.org/10.1111/mpp.12483. PMid:27559722.

LI, O., XIAO, R., SUN, L., GUAN, C., KONG, D. and HU, X., 2017a. Bacterial and diazotrophic diversities of endophytes in Dendrobium catenatum determined through barcoded pyrosequencing. *PLoS One*, vol. 12, no. 9, p. e0184717. http://dx.doi.org/10.1371/journal.pone.0184717. PMid:28931073.

LI, Y., CHENG, C. and AN, D., 2017b. Characterisation of endophytic bacteria from a desert plant lepidium perfoliatum L. *Plant Protection Science*, vol. 53, no. 1, pp. 32-43. http://dx.doi.org/10.17221/14/2016-PPS.

LORENZ, T.C., 2012. Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments*, vol. 63, p. e3998. http://dx.doi.org/10.3791/3998. PMid:22664923.

MACKENZIE, R., PEDRÓS-ALIÓ, C. and DÍEZ, B., 2013. Bacterial composition of microbial mats in hot springs in Northern Patagonia: variations with seasons and temperature.

Extremophiles, vol. 17, no. 1, pp. 123-136. http://dx.doi.org/10.1007/s00792-012-0499-z. PMid:23208511.

MACROGEN, 2017. *NGS analysis manual – OUT*. Seoul: Macrogen.

MADHURAMA, G., SONAM, D., URMIL, P.G. and RAVINDRA, N.K., 2014. Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. *African Journal of Microbiological Research*, vol. 8, no. 2, pp. 184-191. http://dx.doi.org/10.5897/AJMR2012.2452.

MAGURRAN, A.E. and HENDERSON, P.A., 2003. Explaining the excess of rare species in natural species abundance distributions. *Nature*, vol. 422, no. 6933, pp. 714-716. http://dx.doi.org/10.1038/nature01547. PMid:12700760.

MAKHALANYANE, T.P., VALVERDE, A., GUNNIGLE, E., FROSSARD, A., RAMOND, J.-B. and COWAN, D.A., 2015. Microbial ecology of hot desert edaphic systems. *FEMS Microbiology Reviews*, vol. 39, no. 2, pp. 203-221. http://dx.doi.org/10.1093/femsre/fuu011. PMid:25725013.

MALLON, C.A., POLY, F., ROUX, X., MARRING, I., VAN ELSAS, J.D. and SALLES, J.F., 2015. Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. *Ecology*, vol. 96, no. 4, pp. 915-926. http://dx.doi.org/10.1890/14-1001.1. PMid:26230013.

MANO, H., TANAKA, F., NAKAMURA, C., KAGA, H. and MORISAKI, H., 2007. Culturable endophytic bacterial flora of the maturing leaves and roots of rice plants (Oryza sativa) cultivated in a paddy field. *Microbes and Environments*, vol. 22, no. 2, pp. 175-185. http://dx.doi.org/10.1264/jsme2.22.175.

MAROPOLA, M.K.A., RAMOND, J.-B. and TRINDADE, M., 2015. Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in Sorghum bicolor (L. Moench). *Journal of Microbiological Methods*, vol. 112, pp. 104-117. http://dx.doi.org/10.1016/j.mimet.2015.03.012. PMid:25775938.

MASOKO, P., GOLOLO, S.S., MOKGOTHO, M.P., ELOFF, J.N., HOWARD, R.L. and MAMPURU, L.J., 2010. Evaluation of the antioxidant, antibacterial, and antiproliferative activities of the acetone extract of the roots of Senna italica (Fabaceae). *African Journal of Traditional, Complementary, and Alternative Medicines*, vol. 7, no. 2, pp. 138-148. http://dx.doi.org/10.4314/ajtcam.v7i2.50873. PMid:21304625.

MEENA, K.K., SORTY, A.M., BITLA, U.M., CHOUDHARY, K., GUPTA, P., PAREEK, A., SINGH, D.P., PRABHA, R., SAHU, P.K., GUPTA, V.K., SINGH, H.B., KRISHANANI, K.K. and MINHAS, P.S., 2017. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Frontiers in Plant Science*, vol. 8, p. 172. http://dx.doi.org/10.3389/fpls.2017.00172. PMid:28232845.

MISHRA, U. and PABBI, S., 2004. Cyanobacteria: a potential biofertilizer for rice. *Resonance*, vol. 9, no. 6, pp. 6-10. http://dx.doi.org/10.1007/BF02839213.

MITTLER, R., 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, vol. 11, no. 1, pp. 15-19. http://dx.doi.org/10.1016/j.tplants.2005.11.002. PMid:16359910.

MUKHTAR, S., MIRZA, B.S., MEHNAZ, S., MIRZA, M.S., MCLEAN, J. and MALIK, K.A., 2018. Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome. *World Journal of Microbiology & Biotechnology*, vol. 34, no. 9, p. 136. http://dx.doi.org/10.1007/s11274-018-2509-5. PMid:30128756.

MYSARA, M., NJIMA, M., LEYS, N., RAES, J. and MONSIEURS, P., 2017. From reads to operational taxonomic units: an ensemble processing pipeline for MiSeq amplicon sequencing data. *GigaScience*, vol. 6, no. 2, pp. 1-10. http://dx.doi.org/10.1093/gigascience/giw017. PMid:28369460.

NIU, B., PAULSON, J.N., ZHENG, X. and KOLTER, R., 2017. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 12, pp. E2450-E2459. http://dx.doi.org/10.1073/pnas.1616148114. PMid:28275097.

OMOMOWO, O.I. and BABALOLA, O.O., 2019. Bacterial and fungal endophytes: tiny giants with immense beneficial potential for plant growth and sustainable agricultural productivity. *Microorganisms*, vol. 7, no. 11, p. 481. http://dx.doi.org/10.3390/microorganisms7110481. PMid:31652843.

ORTIZ, N., ARMADA, E., DUQUE, E., ROLDÁN, A. and AZCÓN, R., 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. *Journal of Plant Physiology*, vol. 174, pp. 87-96. http://dx.doi.org/10.1016/j.jplph.2014.08.019. PMid:25462971.

PANDEY, P., IRULAPPAN, V., BAGAVATHIANNAN, M.V. and SENTHIL-KUMAR, M., 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Frontiers in Plant Science*, vol. 8, p. 537. http://dx.doi.org/10.3389/fpls.2017.00537. PMid:28458674.

PAREEK, S., SAGAR, N.A., SHARMA, S., KUMAR, V., AGARWAL, T., GONZÁLEZ-AGUILAR, G.A. and YAHIA, E.M., 2017. Chlorophylls: chemistry and biological functions. In: E.M. YAHIA, ed. *Fruit and vegetable phytochemicals: chemistry and human health*. 2nd ed. Hoboken: John Wiley & Sons Ltd, vol. 1, pp. 269-284. http://dx.doi.org/10.1002/9781119158042.ch14.

PATZELT, D.J., HODAČ, L., FRIEDL, T., PIETRASIAK, N. and JOHANSEN, J.R., 2014. Biodiversity of soil cyanobacteria in the hyper-arid Atacama Desert, Chile. *Journal of Phycology*, vol. 50, no. 4, pp. 698-710. http://dx.doi.org/10.1111/jpy.12196. PMid:26988453.

PROCÓPIO, R.E.L., SILVA, I.R., MARTINS, M.K., AZEVEDO, J.L. and ARAÚJO, J.M., 2012. Antibiotics produced by Streptomyces. *The Brazilian Journal of Infectious Diseases*, vol. 16, no. 5, pp. 466-471. http://dx.doi.org/10.1016/j.bjid.2012.08.014. PMid:22975171.

RAHMAN, A.H.M.M. and PARVIN, M.I.A., 2014. Study of medicinal uses on Fabaceae family at Rajshahi, Bangladesh. *Research in Plant Sciences*, vol. 2, no. 1, pp. 6-8.

RAJKUMAR, M., NAGENDRAN, R., LEE, K.J., LEE, W.H. and KIM, S.Z., 2006. Influence of plant growth promoting bacteria and Cr6+ on the growth of Indian mustard. *Chemosphere*, vol. 62, no. 5, pp. 741-748. http://dx.doi.org/10.1016/j.chemosphere.2005.04.117. PMid:15982703.

RANA, K.L., KOUR, D., KAUR, T., DEVI, R., YADAV, A.N., YADAV, N., DHALIWAL, H.S. and SAXENA, A.K., 2020. Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie van Leeuwenhoek*, vol. 113, no. 8, pp. 1075-1107. http://dx.doi.org/10.1007/s10482-020-01429-y. PMid:32488494.

REINHOLD-HUREK, B. and HUREK, T., 2011. Living inside plants: bacterial endophytes. *Current Opinion in Plant Biology*, vol. 14, no. 4, pp. 435-443. http://dx.doi.org/10.1016/j.pbi.2011.04.004. PMid:21536480.

ROBINSON, R.J., FRAAIJE, B.A., CLARK, I.M., JACKSON, R.W., HIRSCH, P.R. and MAUCHLINE, T.H., 2016. Endophytic bacterial community composition in wheat (Triticum aestivum) is determined by plant tissue type, developmental stage and soil nutrient availability. *Plant and Soil*, vol. 405, no. 1, pp. 381-396. http://dx.doi.org/10.1007/s11104-015-2495-4.

RODRÍGUEZ, E., ARQUÉS, J.L., RODRÍGUEZ, R., NUÑEZ, M., MEDINA, M., TALARICO, T.L., CASAS, I.A., CHUNG, T.C., DOBROGOSZ, W.J., AXELSSON, L., LINDGREN, S.E., DOBROGOSZ, W.J., KERKENI, L., RUANO, P., DELGADO, L.L., PICCO, S., VILLEGAS, L., TONELLI, F., MERLO, M., RIGAU, J., DIAZ, D. and MASUELLI, M., 1989. We are IntechOpen, the world ' s leading publisher of open

access books built by scientists, for scientists TOP 1 %. *Intech*, vol. 32, pp. 137-144.

ROMERO, F.M., MARINA, M. and PIECKENSTAIN, F.L., 2014. The communities of tomato (Solanum lycopersicum L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene pyrosequencing. *FEMS Microbiology Letters*, vol. 351, no. 2, pp. 187-194. http://dx.doi.org/10.1111/1574-6968.12377. PMid:24417185.

RYAN, R.P., GERMAINE, K., FRANKS, A., RYAN, D.J. and DOWLING, D.N., 2008. Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters*, vol. 278, no. 1, pp. 1-9. http://dx.doi.org/10.1111/j.1574-6968.2007.00918.x. PMid:18034833.

SAMISH, Z., ETINGER-TULCZYNSKA, R. and BICK, M., 1961. Microflora within healthy tomatoes. *Applied Microbiology*, vol. 9, no. 1, pp. 20-25. http://dx.doi.org/10.1128/am.9.1.20-25.1961.

SANTOYO, G., MORENO-HAGELSIEB, G., OROZCO-MOSQUEDA, M.C. and GLICK, B.R., 2016. Plant growth-promoting bacterial endophytes. *Microbiological Research*, vol. 183, pp. 92-99. http://dx.doi.org/10.1016/j.micres.2015.11.008. PMid:26805622.

SARRIA-GUZMÁN, Y., CHÁVEZ-ROMERO, Y., GÓMEZ-ACATA, S., MONTES-MOLINA, J.A., MORALES-SALAZAR, E., DENDOOVEN, L. and NAVARRO-NOYA, Y.E., 2016. Bacterial communities associated with different Anthurium andraeanum L. plant tissues. *Microbes and Environments*, vol. 31, no. 3, pp. 321-328. http://dx.doi.org/10.1264/jsme2.ME16099. PMid:27524305.

SCHLAEPPI, K. and BULGARELLI, D., 2015. The plant microbiome at work. *Molecular Plant-Microbe Interactions*, vol. 28, no. 3, pp. 212-217. http://dx.doi.org/10.1094/MPMI-10-14-0334-FI. PMid:25514681.

SCHLOSS, P.D. and WESTCOTT, S.L., 2011. Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Applied and Environmental Microbiology*, vol. 77, no. 10, pp. 3219-3226. http://dx.doi.org/10.1128/AEM.02810-10. PMid:21421784.

SCHOFIELD, M.M. and SHERMAN, D.H., 2013. Meta-omic characterization of prokaryotic gene clusters for natural product biosynthesis. *Current Opinion in Biotechnology*, vol. 24, no. 6, pp. 1151-1158. http://dx.doi.org/10.1016/j.copbio.2013.05.001. PMid:23731715.

SEIPKE, R.F., KALTENPOTH, M. and HUTCHINGS, M.I., 2012. Streptomyces as symbionts: an emerging and widespread theme? *FEMS Microbiology Reviews*, vol. 36, no. 4, pp. 862-876. http://dx.doi.org/10.1111/j.1574-6976.2011.00313.x. PMid:22091965.

SESSITSCH, A., HARDOIM, P., DÖRING, J., WEILHARTER, A., KRAUSE, A., WOYKE, T., MITTER, B., HAUBERG-LOTTE, L., FRIEDRICH, F., RAHALKAR, M., HUREK, T., SARKAR, A., BODROSSY, L., VAN OVERBEEK, L., BRAR, D., VAN ELSAS, J.D. and REINHOLD-HUREK, B., 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Molecular Plant-Microbe Interactions*, vol. 25, no. 1, pp. 28-36. http://dx.doi.org/10.1094/MPMI-08-11-0204. PMid:21970692.

SINGH, G.G.S., 2015. Plant Growth Promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *Journal of Microbial & Biochemical Technology*, vol. 7, no. 2, pp. 96-102. http://dx.doi.org/10.4172/1948-5948.1000188.

SINGH, H., ANURAG, K. and APTE, S.K., 2013. High radiation and desiccation tolerance of nitrogen-fixing cultures of the cyanobacterium Anabaena sp. strain PCC 7120 emanates from genome/proteome repair capabilities. *Photosynthesis Research*, vol. 118, no. 1, pp. 71-81. http://dx.doi.org/10.1007/s11120-013-9936-9. PMid:24122300.

SINGH, H., FERNANDES, T. and APTE, S.K., 2010. Unusual radioresistance of nitrogen-fixing cultures of Anabaena strains. *Journal of Biosciences*, vol. 35, no. 3, pp. 427-434. http://dx.doi.org/10.1007/s12038-010-0048-9. PMid:20826952.

SINGH, J.S., KUMAR, A., RAI, A.N. and SINGH, D.P., 2016. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Frontiers in Microbiology*, vol. 7, p. 529. http://dx.doi.org/10.3389/fmicb.2016.00529. PMid:27148218.

SINGH, R. and DUBEY, A.K., 2018. Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. *Frontiers in Microbiology*, vol. 9, p. 1767. http://dx.doi.org/10.3389/fmicb.2018.01767. PMid:30135681.

SINGH, R.K., SINGH, P., GUO, D., SHARMA, A., LI, D.P., LI, X., VERMA, K.K., MALVIYA, M.K., SONG, X.P., LAKSHMANAN, P., YANG, L.T. and LI, Y.R., 2021. Root-derived endophytic diazotrophic bacteria Pantoea cypripedii AF1 and Kosakonia arachidis EF1 promote nitrogen assimilation and growth in sugarcane. *Frontiers in Microbiology*, vol. 12, p. 774707. http://dx.doi.org/10.3389/fmicb.2021.774707. PMid:34975800.

SINGHA, K.M., SINGH, B. and PANDEY, P., 2021. Host specific endophytic microbiome diversity and associated functions in three varieties of scented black rice are dependent on growth stage. *Scientific Reports*, vol. 11, no. 1, p. 12259. http://dx.doi.org/10.1038/s41598-021-91452-4. PMid:34112830.

STANIEK, A., WOERDENBAG, H.J. and KAYSER, O., 2008. Endophytes: exploiting biodiversity for the improvement of natural product-based drug discovery. *Journal of Plant Interactions*, vol. 3, no. 2, pp. 75-93. http://dx.doi.org/10.1080/17429140801886293.

TIAN, X.-Y. and ZHANG, C.-S., 2017. Illumina-based analysis of endophytic and rhizosphere bacterial diversity of the coastal halophyte Messerschmidia sibirica. *Frontiers in Microbiology*, vol. 8, p. 2288. http://dx.doi.org/10.3389/fmicb.2017.02288. PMid:29209296.

TSHIKALANGE, T.E., MEYER, J.J.M. and 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*, vol. 96, no. 3, pp. 515-519. http://dx.doi.org/10.1016/j.jep.2004.09.057. PMid:15619572.

UNITED NATIONS – UN, 2019. *United Nations world population prospects 2019*. New York: UN.

WANG, H.F., ZHANG, Y.G., LI, L., LIU, W.H., HOZZEIN, W.N., CHEN, J.Y., GUO, J.W., ZHANG, Y.M. and LI, W.J., 2015. Okibacterium endophyticum sp. nov., a novel endophytic actinobacterium isolated from roots of Salsola affinis C. A. Mey. *Antonie van Leeuwenhoek*, vol. 107, no. 3, pp. 835-843. http://dx.doi.org/10.1007/s10482-014-0376-0. PMid:25566956.

WILSON, M.C. and PIEL, J., 2013. Metagenomic approaches for exploiting uncultivated bacteria as a resource for novel biosynthetic enzymology. *Chemistry & Biology*, vol. 20, no. 5, pp. 636-647. http://dx.doi.org/10.1016/j.chembiol.2013.04.011. PMid:23706630.

WU, W., CHEN, C., SHEEN, L. and WU, M., 2020. Evaluation of compatibility of 16S rRNA V3V4 and V4 amplicon libraries for clinical microbiome profiling. *BioRxiv*. In press.

XIA, Y., SAHIB, M.R., AMNA, A., OPIYO, S.O., ZHAO, Z. and GAO, Y.G., 2019. Culturable endophytic fungal communities associated with plants in organic and conventional farming systems and their effects on plant growth. *Scientific Reports*, vol. 9, no. 1, p. 1669. http://dx.doi.org/10.1038/s41598-018-38230-x. PMid:30737459.

YAGI, S., TIGANI, S., ALI, M., ELKHIDIR, I. and MOHAMMED, A.M.A., 2013. Chemical constituents and insecticidal activity of *Senna italica* Mill. from the Sudan. *International Letters of Chemistry,*

Physics and Astronomy, vol. 14, pp. 146-151. http://dx.doi.org/10.18052/www.scipress.com/ILCPA.14.146.

YAMORI, W., HIKOSAKA, K. and WAY, D.A., 2014. Temperature response of photosynthesis in C3, C4, and CAM plants: temperature acclimation and temperature adaptation. *Photosynthesis Research*, vol. 119, no. 1-2, pp. 101-117. http://dx.doi.org/10.1007/s11120-013-9874-6. PMid:23801171.

YANG, R., LIU, P. and YE, W., 2017. Illumina-based analysis of endophytic bacterial diversity of tree peony (Paeonia Sect. Moutan) roots and leaves. *Brazilian Journal of Microbiology*, vol. 48, no. 4, pp. 695-705. http://dx.doi.org/10.1016/j.bjm.2017.02.009. PMid:28606427.

YU, H., ZHANG, L., LI, L., ZHENG, C., GUO, L., LI, W., SUN, P. and QIN, L., 2010. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. *Microbiological Research*, vol. 165, no. 6, pp. 437-449. http://dx.doi.org/10.1016/j.micres.2009.11.009. PMid:20116229.

ZARRAONAINDIA, I., OWENS, S.M., WEISENHORN, P., WEST, K., HAMPTON-MARCELL, J., LAX, S., BOKULICH, N.A., MILLS, D.A., MARTIN, G., TAGHAVI, S., VAN DER LELIE, D. and GILBERT, J.A., 2015. The soil microbiome influences grapevine-associated microbiota. *mBio*, vol. 6, no. 2, p. e02527-14. http://dx.doi.org/10.1128/mBio.02527-14. PMid:25805735.

ZELICOURT, A., AL-YOUSIF, M. and HIRT, H., 2013. Rhizosphere microbes as essential partners for plant stress tolerance. *Molecular Plant*, vol. 6, no. 2, pp. 242-245. http://dx.doi.org/10.1093/mp/sst028. PMid:23475999.

ZHANG, Q., ACUÑA, J.J., INOSTROZA, N.G., MORA, M.L., RADIC, S., SADOWSKY, M.J. and JORQUERA, M.A., 2019. Endophytic bacterial communities associated with roots and leaves of plants growing in Chilean extreme environments. *Scientific Reports*, vol. 9, no. 1, p. 4950. http://dx.doi.org/10.1038/s41598-019-41160-x. PMid:30894597.

ZHAO, Y., SONG, C., DONG, H., LUO, Y., WEI, Y., GAO, J., WU, Q., HUANG, Y., AN, L. and SHENG, H., 2018. Community structure and distribution of culturable bacteria in soil along an altitudinal gradient of Tianshan Mountains, China. *Biotechnology, Biotechnological Equipment*, vol. 32, no. 2, pp. 397-407. http://dx.doi.org/10.1080/13102818.2017.1396195.

Supplementary Material

Supplementary material accompanies this paper.

Table S1. Number of Inversed Simpson and Good's coverage of Senna italica samples.

Figure S1. The total number of sequences reads among the six roots and leaves samples. Data showed a total of clean sequences read 499,923 with the highest value found in the roots.1 sample and the lowest value found in the leaves.3 sample. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with Senna italica.

Figure S2. The percentage of read quality of the six roots and leaves samples. Q20(%): The percentage of bases in which the phred score is above 20. Q30(%): The percentage of bases in which the phred score is above 30. Roots samples: Roots.1, Roots.2, and Roots.3. Leaves samples: Leaves.1, Leaves.2, and Leaves.3 are associated with Senna italica.

Figure S3. Phylogenetic tree based on 16S rRNA gene sequences representing the diversity of endophytic bacterial communities associated with the leaves and roots from the desert medicinal plant Senna italica "at the Genus level". The tree was constructed using the "one-click" mode in Phylogeny.fr. (Dereeper et al., 2008)

This material is available as part of the online article from https://doi.org/10.1590/1519-6984.267584