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Comparative Metagenomic Analysis of Bacterial Communities Isolated from *Artemisia judaica* Cultivated in Central Region of Saudi Arabia Based on 16S rRNA

Thamer Albalawi

Department of Biology, College of Science and Humanities, Prince Sattam bin Abdulaziz University, Alkharj 11942, Saudi Arabia

RHIZOSPHERE, which is the soil region influenced by plant root exudates, is a critical interface for understanding the microbial communities associated with plant growth and health. Using high-throughput sequencing of 16S rRNA, we examined the taxonomic diversity and composition of the bacterial communities present in the rhizospheric soil of central Saudi Arabia. Our analysis revealed a rich and diverse bacterial community that has adapted to the arid conditions of desert ecosystems. These taxonomic assignments provided insights into the predominant phyla and genera, shedding light on the microbial ecology of the rhizosphere in this extreme environment. In this study, two soil samples (EC,1.1 dSm-1 and pH 7.4), collected from rhizospheric zone (TCR600) and lower shoot surface (TCS400) of *Artemisia Judaica* plants, were compared. The TCR600 sample had a GC (guanine cytosine) content of 54% and 61,489 operational taxonomic units (OTUs). The TCR600 sample displayed a broader spectrum of values along the first principal component, indicating higher bacterial diversity within this sample set than that of TCS400. 16sRNA (ribonucleic acid) sequencing revealed the presence of 17 phyla in each sample; however, the TCR600 samples showed pronounced differences of 63.24% based on taxonomic diversity and relative abundance. *Bacillus* (38.721%) was the dominant genus in TCR600, followed by *Pseudomonas* (20.251%). This study also elucidates the potential functional roles of these bacteria in nutrient cycling, stress tolerance, and plant-microbe interactions. These findings contribute to our understanding of ecological dynamics within the rhizospheric zone and its nearby regions and offer implications for sustainable agricultural practices in arid regions.

Keywords: Rhizospheric bacteria**, G**enomics diversity, 16S rRNA sequencing, Microbial communities.

1. Introduction

Plants contain a range of microbial communities within their structure such as in the roots, leaves, stems, flowers, fruit and seeds which may be able to sustain distinct microbial assemblages (**Berg *et al.*, 2014**). Microorganisms live as epiphytes on the plant surface or endophytes within the plant tissues (**Turner *et al.*, 2013**). Advancements in high throughput sequencing technologies have explored more of the plant microbiome, which is the structure and processes of the microorganisms associated with the plant, which has drawn a lot of scientific attention (**Müller *et al.*, 2016**).

An essential approach for studying the soil's biological wonders is employing the 16S rRNA-based metagenomic analysis. This method stands central in the domain of soil microbial ecology. By doing this technology, estonian scientists were able to categorize millions of bacteria that they found in their soil samples (**Oliveira *et al.*, 2017**). Soil is a complex ecosystem and a dynamic habitat containing multitude life forms including bacteria, archaea, fungi and viruses (**Vincent *et al.*, 2023**). Many microorganisms are vital for the maintenance of soils, nutrient balance, organic matter reduction, plant growth and metabolism. Mapping microbial communities and their functions within soil warrants intelligent managerial actions for agriculture, ecosystem conservation, climate change mitigation, land resources sustainability, and other global issues (**Reyes-Sánchez *et al.*, 2022**).

The 16S metagenomic analyses rest on executing several important actions. The first step is to collect a sample with desired characteristic and fold it into a tube. To barcode the sample, one single-barcoding step must be executed. Then, a single mix PCR (polymerase chain reaction) reaction is performed with double-barcoded primers, which generates unique primer pairs for every sample. In the end, each sample will have a unique set of primers (**Chakravorty *et al.*, 2007**). The last step is quantitative measurement of the target regions and secondary quantification of PCR products in order to generate the needed products.

*Corresponding author e-mail: t.albalawi@psau.edu.sa
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The process starts with collecting soil samples from different locations as each type of soil has its own microbial population. Afterward, DNA (deoxyribonucleic acid) is preserved from the samples to capture the microorganisms' genetic material. The following step includes Polymerase Chain Reaction (PCR) processes for amplifying particular regions of the16S rRNA gene, focusing on its variable regions to enrich the sequences that aid in classification (**Zhang *et al.,* 2021**). After amplification, high throughput sequencing systems are used to create enormous datasets containing the genetic information of the soil's microbial population. These sequences undergo intricate bioinformatics workflows in which software along with taxonomic databases are used to identify the microorganisms by their 16S rRNA sequences (**Lobanov *et al.,* 2022**).

An advantageous feature of 16S rRNA-based metagenomic analysis is its independence from bacterial cultures. As noted above, unlike other techniques employed in microbiology that involve growing and isolating microbial species from a sample, this method does not depend on the ability to culture microorganisms, especially since the vast majority of soil microorganisms are notoriously difficult to culture, or perhaps have never been cultivated (**Rizal *et al.,* 2020**). Thus, 16S rRNA-based metagenomic analysis captures a more accurate depiction of the soil microbial community structure without bias, providing information on abundant as well as rare taxa which can be crucial from an ecological perspective. In addition, such methodologies allow a high level of detail at which the organisms are identified, being able to assign them to a particular genus, and in some cases, even to a specific species. Understanding accurately the taxonomy of the microbial communities and the potential functions within them is critical to fathom the ecosystems and their associated landscapes (**Jacquiod *et al.,* 2016**).

Also, the in-depth metagenomic analysis that utilizes 16S rRNA is easily scalable, making it ideal for studies of larger magnitude. It is possible to analyze thousands of microbes from one soil sample, making it easy to study complicated and diverse places (**Pérez-Cobas *et al.,* 2020**). The information obtained from 16S rRNA based metagenomic analysis contains important data about the soil microbial community. Modern technology can analyze the sequences from the soil samples and elucidate their community composition and structure. This data can be presented through various visualization methods such as heat maps , bar graphs, or phylogenetic trees to show the taxonomic diversity, abundance, or microbial community composition (**Lapidus and Korobeynikov, 2021**).

Soil science widely utilizes 16S rRNA-based metagenomic analysis and has extended its application to numerous scientific disciplines. In agriculture, understanding the composition and dynamics of soil microbial communities is critical for evaluating soil health. Fertility enhancement and productivity optimization lie within researchers' grasp if agricultural practices like organic input, pesticide use and crop rotation are assessed (**Khangura *et al.,* 2023**). Besides, the identification of useful microorganisms that suppress soil-borne pathogens naturally is essential in the formulation of eco-friendly strategies for disease management in farming systems (**Ayaz *et al.,* 2023**).

In environmental science and ecology, 16S rRNA-based metagenomic analysis is a powerful tool for ecosystem studies. This allows researchers to investigate the roles of soil microbial communities in vital processes, such as nutrient cycling, organic matter decomposition, and soil carbon storage. It also helps to understand how microbial communities respond to changes in the environment, management of the land, and climate shifts (**Jansson and Hofmockel, 2018**) . This information is important for conservation and restoration efforts focusing on ecosystems under severe global environmental perturbations. Additionally, this approach aids in soil bioremediation exercises that seek to eradicate pollution from soils. By isolating and defining microorganisms capable of degrading certain pollutants and contaminants, appropriate procedures for the remediation of polluted environments can be developed. This is important for the environmental restoration and ecosystem recovery processes that have been subjected to industrial activities and pollution (**Purohit *et al.,* 2020**). As noted, in the area of soil science, the 16S rRNA gene based metagenomic analysis is one of the most useful innovative approaches (**Saleem *et al.,* 2022; Farhat *et al.,* 2021**).

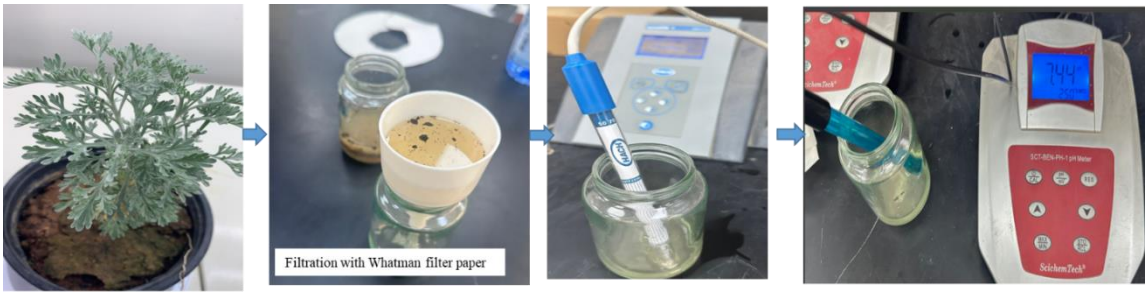
Now our understanding of microbial diversity, the composition of different groups within ecosystems, and the functioning of ecosystems in soil environments has changed completely. This is as a result of high throughput sequencing, certainly as far as the soil microbiome is concerned (**Fierer, 2017**). Metagenomic sequencing and analysis is likely to further shape strategies for land management, conservation of the environment, restoration of ecosystems, and providing solutions to other global problems. Such advanced technology techniques will help resolve many challenges and enhance knowledge in science (**Albalawi and Alam, 2015**).

The primary objective of this study was to characterize bacterial communities using metagenomic techniques. Therefore, we aimed to identify novel bacterial strains that hold promise for various biotechnological applications. Furthermore, this study aims to enhance our understanding of the interactions between bacteria and their hosts, which may ultimately contribute to improved agricultural productivity. Additionally, our findings could shed light on the relationship between microbial diversity and the dynamics of contemporary ecosystems.

2. Materials and Methods

2.1. Sample collection

In Alkharj Province, Saudi Arabia, variety of plant species have been cultivated generaly in winter season, and among that, *Artemisia judaica* was the most abundant plant found in this region. In order to conduct the isolation of microorganisms, soil samples (2 g) with EC (1.1 dS m^{-1}) and pH (7.4) harbouring root and lower shoot surface were collected from a nursery (altitude: 24.177214242359053; longitude 47.28827954944128) (https://maps.app.goo.gl/Ke8X57E9PHRbj3ov8). The samples were homogenized and introduced into sterile Erlenmeyer flasks (150 mL) for further use. Soil samples isolated from both samples were further sent for metagenomic studies.



Flow Chart for soil analyses

2.2. Molecular characterization

Soil samples associated with the roots and stems of *A. judaica* were collected and sent to Biokart India Pvt. Ltd. for DNA isolation and subsequent analyses, including PCR and soil metagenomics, to screen the bacterial communities present in these samples following the kit instruction provided by biokark India.. The samples were mixed with C1 cell lysis buffer, vortexed for 10 min, and centrifuged. The supernatant was collected in a clean tube, and C2 buffer solution was added to remove proteins and RNA (ribosenucleic acid). Samples were incubated, centrifuged, and incubated again with C3 buffer solution to remove residues. The supernatant was collected in a clean tube and washed with C5 buffer. Finally, a C6 buffer solution was added to the center of a white filter membrane, and the eluted DNA was collected in a clean tube using the Xploregen kit (Xploregen DNA extraction kit (Xploregen Discoveries, India) for the isolation and purification of genomic DNA).

Samples were analyzed for DNA (deoxyribonucleic acid) quality using a NanoDrop 260/280 spectrophotometer with a value of 1.8 to 2 prior to polymerase chain reaction (PCR) amplification. The 16S rRNA gene was amplified using universal bacterial primer sets, 16sF (5'-AGAGTTTGATGMTGGCTCAG-3') and 16sR (5'-TTACCGCGGCMGCSGGCAC-3'). The PCR reaction was carried out in a volume of 25 µl, containing DNA template, 1× PCR Enzyme Buffer, 3.2 mM $MgCl_2$, 0.5 mM dNTPs, 10 pM of each primer, and 2 U of high fidelity DNA Polymerase. The PCR conditions were as follows: a 2-minute initial denaturation at 95 °C; 25 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 60 °C; 2 minutes of elongation at 72 °C; and a final extension for 10 minutes at 72 °C. A thermocycler (Bio-Rad Laboratories, Inc., USA) was used to perform PCR. A negative control was included in the PCR process to confirm the absence of reagent contamination, and the PCR product was purified using PCR cleanup.

Ampure beads were used to purify amplicons from the samples to eliminate any remaining primers. Subsequently, eight PCR cycles were performed to create sequencing libraries, including Illumina barcoded adapters. The libraries were then analyzed using a Qubit dsDNA (double stranded deoxyribonucleic acid) High Sensitivity Assay Kit and purified using amplicon beads. A 2 × 300 PE v3-v4 sequencing kit and an Illumina MiSeq platform were used for sequencing.

2.3. OTU analysis

In the subsequent PCR, a volume of 1 µL of the amplicon was utilized. The V3 hypervariable region of the 16S rRNA gene, proposed by Bartram et al. (2011), was selected because of its optimal taxonomic resolution (Huse et al., 2008), conserved flanking regions (**Muyzer *et al.*, 1993**), and a length of approximately 170–190 nucleotides (Gloor et al., 2010). This length is suitable for paired-end 125-base read assembly. The database utilized for the 16S V3-V4 region was the NCBI. The raw data obtained from the sequencer were demultiplexed into the FASTQ format using bcl. The quality of the demultiplexed data was assessed using Multiqc (Version

1.10.1) and Fastqc (Version 0.11.9). Samples that cleared quality control were considered suitable for additional examination, and 16S metagenomic analysis was conducted using our metagenomics pipeline, the Biokart Pipeline. Analytical visualization was started once the run was finished, and the final raw OTU table was obtained. Microsoft Excel (2016) was used to create the top ten species in each sample and abundance feature tables (Supplementary Material). Other analyses, such as heatmap, core microbiome, Dendrogram, Alpha diversity, beta diversity, PCA plot, and rarefaction curve analyses, were performed using Microbiome Analyst (https://www.microbiomeanalyst.ca).

3. Results

3.1. α-Diversity and bacterial richness

The total number of amplicon sequences reads obtained from TCR600 (accession no. SAMN37576526) and TCS400 (accession no. SAMN37576525) samples of *Artemisia judaica* were 0.2 and 0.4 million with a GC content of 54% and 53.5%, respectively (Table 1). Similarly, TCR600 had 61489 operational taxonomic units (OTU), while TCS400 was more diverse with 99029 OTUs.

Table 1. RAW reads statistics of 16 sRNA from *Artemesia judaica* root (TCR600) and shoot surface (TCS400).

S. No.	Sample	GC Content (%)	Read Count (in Millions)	OTUs
1	TCR600	54	0.2	61489
2	TCS400	53.5	0.4	99029

3.2. Diversity among TCR600 samples (β-Diversity)

Distribution analysis of the core genera revealed diverse bacterial communities in the root (TCR600) samples of *Artemisia judaica*, showing different relative abundances of the core genera across various tissue samples. Most of the sample reads reached saturation points, as seen in the rarefaction curve in Fig. 1, indicative of full coverage of the sampling efforts (Fig. 2). To better understand the variations in the bacterial population, Principal component analysis (PCA) was performed, the results of which were visualized as a 2D plot and effectively illustrated the relationships between the samples. PCA was performed using the unweighted UniFrac method to identify the abundance of OTUs in the samples, which were represented by two principal components (PC1 and PC2). These factors explained 100% and 0% of the variation, respectively, as shown in Fig. 3.

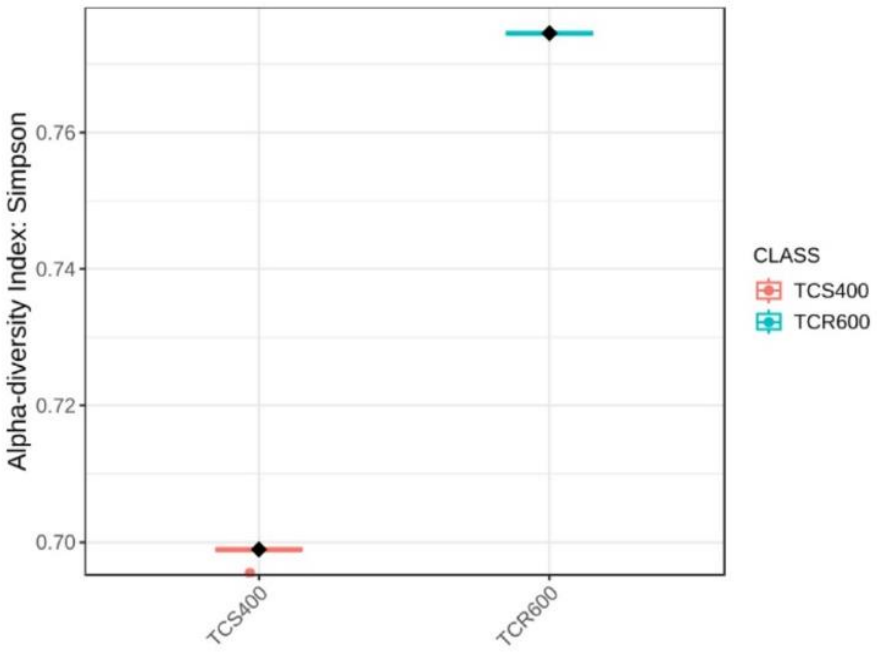


Fig. 1. The showing Curves based on observed Shannon and Simpson values for the samples TCR600 (root) and TCS400 (shoot surface) of *Artemisia judaica*.

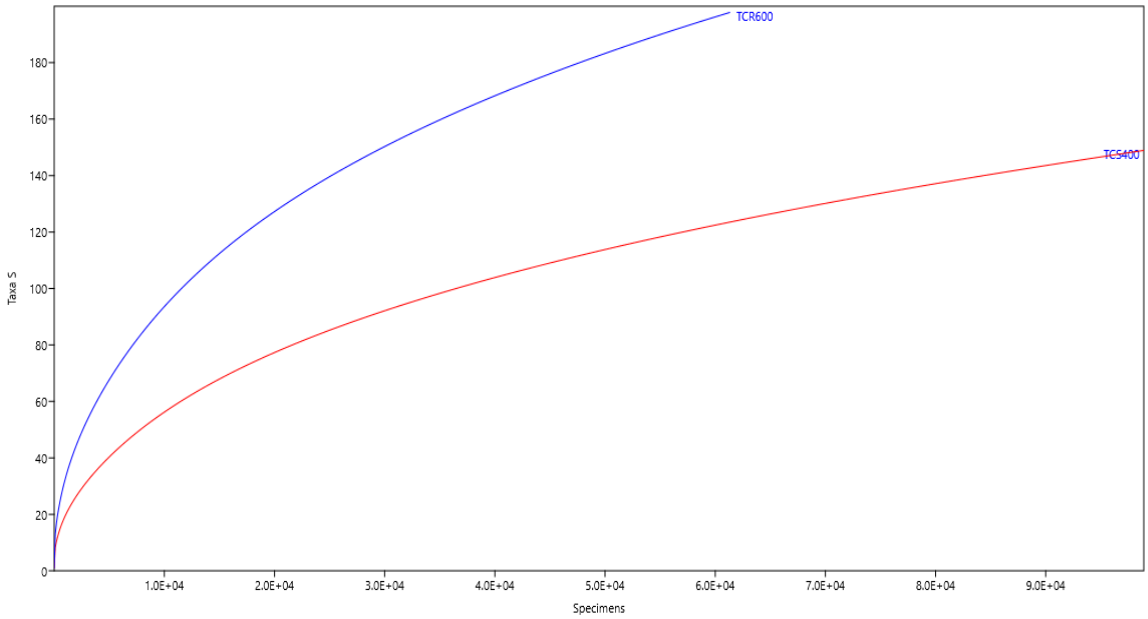


Fig. 2. Alpha rarefaction curve, derived from observed species (OTUs) values, exhibits a rightward flattening, signifying a comparatively elevated species richness in both TCR600 (root) and TCS400 (shoot surface) samples of *Artemisia judaica*.

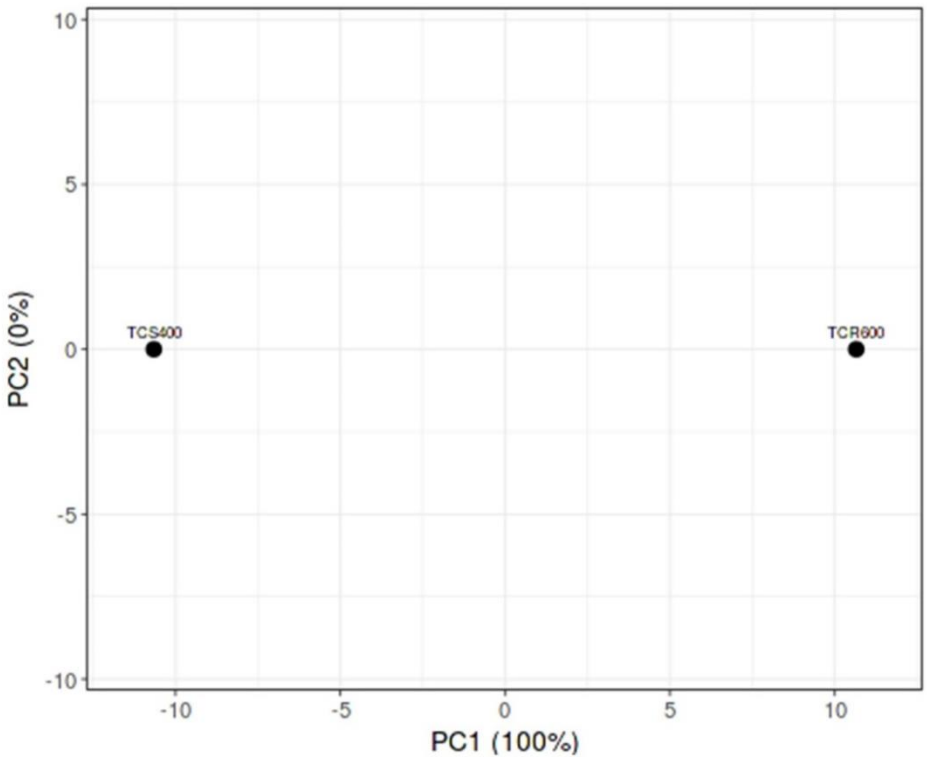


Fig. 3. Beta diversity test was performed through an Unweighted Principal Coordinate Analysis (PCA) of UniFrac distances, which visually depicted distinctions in bacterial communities between TCR600 (root) and TCS400 (shoot surface) samples of *Artemisia judaica*. The plotting of the two primary components (PC1 and PC2) captured 97% of the total inertia.

The PCA graph shows the relationship between two variables, TC200 and TC400. TC200 and TC400 are two measures of the abundance of certain types of bacteria in a sample. The PCA graph shows that the TCR600 samples were separated along the first principal component (PC1). This indicates that the two samples had different values for the first principal component. The first principal component was a linear combination of the original variables TC200 and TC400, which captured most of the variation in the data. The PCA graph also showed that the TCR600 samples were more spread out than the TCS400 samples. This indicates that the TCR600 samples had a wider range of values for the first principal component, which suggests that the TCR600 samples had a greater diversity of bacteria than the TCS400 samples. The first principal component (PC1) accounted for 97% of the variation in the data. This means that PC1 captured most of the information regarding the relationship between the TC200 and TC400 variables. The TCR600 samples were located on the positive side of PC1, whereas the TCS400 samples were located on the negative side of PC1. This suggests that the TCR600 samples had higher TC200 values and lower TC400 values than the TCS400 samples. The TCR600 samples were also more widespread than the TCS400 samples along PC1. This suggests that the TCR600 samples had a wider range of TC200 values than the TCS400 samples. This is consistent with the conclusion that the TCR600 samples had a greater diversity of bacteria than the TCS400 samples. Overall, the PCA graph showed that the TCR600 and TCS400 samples had different bacterial communities. The TCR600 samples had a wider range of values for the first principal component, suggesting that they contained a greater diversity of bacteria.

3.3. Phyla associated with bacterial samples

Analysis of 16S rRNA gene amplicon reads resulted in the identification of a total of seventeen classifiable phyla. Analysis of the bacterial compositions of the TCR600 samples revealed substantial differences in the distribution of the major phyla. Firmicutes, the predominant phylum, displayed a considerable increase from 63.236% in TCR600. In contrast, Proteobacteria population was around 33.761% suggesting dynamic interplay between these dominant phyla. Actinobacteria, although constituted a relatively small proportion 2.568% in TCR600. Other phyla, such as Bacteroidetes, Planctomycetes, Acidobacteria, Verrucomicrobia, Chloroflexi, Fusobacteria, Spirochaetes, Nitrospirae, Cyanobacteria, Candidatus Saccharibacteria, Armatimonadetes, Chlamydiae, Deinococcus-Thermus, and Gemmatimonadetes, also showed varying degrees of abundance between the two samples, reflecting the complexity and diversity of the microbial communities. The observed fluctuations in the phylum composition underscore the dynamic nature of the bacterial populations in these samples and highlight the potential ecological adaptations or shifts occurring within the respective microbial ecosystems of TCR600 (Fig. 4, Table 2S).

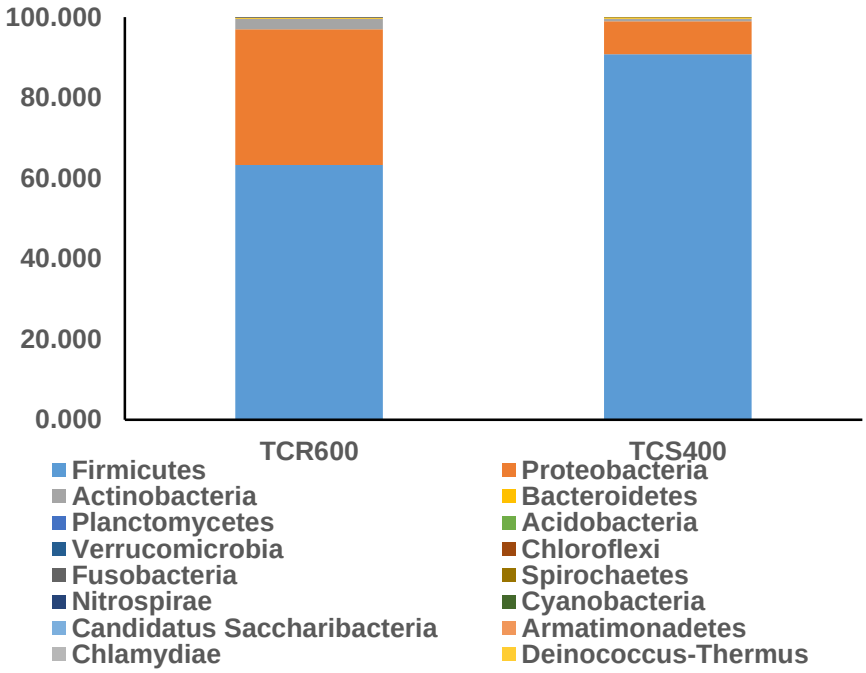


Fig. 4. Study of taxonomic diversity and Relative Abundance of Bacterial phyla in both TCR600 (root) and TCS400 (shoot surface) samples of *Artemisia judaica*.

Table 2S. Taxonomic diversity and Relative Abundance of Bacterial phyla in the of *Artemisia judaica* TCR600 (root) and TCS400 (shoot surface) Samples.

S. No.	Phyla	Bacterial Samples (%)	
		TCR600	TCS400
1	Firmicutes	63.24	90.79
2	Proteobacteria	33.76	8.16
3	Actinobacteria	2.57	0.69
4	Bacteroidetes	0.28	0.33
5	Planctomycetes	0.04	0.01
6	Acidobacteria	0.03	0.01
7	Verrucomicrobia	0.02	0.01
8	Chloroflexi	0.01	0.00
9	Fusobacteria	0.01	0.00
10	Spirochaetes	0.01	0.00
11	Nitrospirae	0.01	0.00
12	Cyanobacteria	0.01	0.00
13	Candidatus Saccharibacteria	0.00	0.00
14	Armatimonadetes	0.00	0.00
15	Chlamydiae	0.00	0.00
16	Deinococcus-Thermus	0.00	0.00
17	Gemmatimonadetes	0.00	0.00

3.4. Bacterial population at class and order level

A study of the class-level distribution of bacterial communities in TCR600 revealed notable variations in abundance. Bacilli, a significant class, showed a substantial population of 38,646 while Gammaproteobacteria was having a population of 12,738 in TCR600 suggesting a positive trend in the abundance of these classes. Alphaproteobacteria also demonstrated its presence as 6,872 emphasizing the dynamic nature of bacterial community at the class level. Actinobacteria, Betaproteobacteria, Clostridia, and Bacteroidia displayed varying degrees of abundance in the samples, reflecting the intricate changes in microbial composition. Furthermore, several classes, such as Planctomycetia, Coriobacteriia, Acidobacteriia, Deltaproteobacteria, Flavobacteriia, Fusobacteriia, Verrucomicrobiae, Sphingobacteriia, Spirochaetia, Anaerolineae, Chitinophagia, Nitrospira, Cytophagia, Unclassified Cyanobacteria, Epsilonproteobacteria, Tepidiformia, Unclassified Candidatus Saccharibacteria, Chlamydia, Fimbriimonadia, Spartobacteria, Candidatus Brocadiae, Deinococci, Erysipelotrichia, Gemmatimonadetes, Negativicutes, Oligoflexia, Opitutae, Phycisphaerae, Thermoleophilia, Zetaproteobacteria, Chloroflexia, Rubrobacteria, and Vicinamibacteria, exhibited varying degrees of presence or absence in the samples, highlighting the intricate and diverse nature of bacterial communities at the class level in TCR600 (Fig. 5).

Analysis of the order-level distribution of bacterial taxa in TCR600, expressed as a percentage of the total composition, revealed significant variations in the abundance of various orders. Bacillales, a prominent order, exhibited substantial inhabitants of 38,502 at TCR600, indicating a pronounced prevalence of this bacterial order. Conversely, the abundance of Pseudomonadales appeared only 12,545. Several other orders, including Rhizobiales, Micrococcales, Burkholderiales, and Clostridiales, also demonstrated varying degrees of abundance between the two samples, emphasizing the dynamic nature of bacterial communities at the order level. Streptomycetales, Bacteroidales, and Lactobacillales exhibited differences in their respective percentages, highlighting the intricate changes in microbial composition at this taxonomic level. Moreover, numerous orders, such as Enterobacterales, Rhodospirillales, Sphingomonadales, Xanthomonadales, Bifidobacteriales, Corynebacteriales, Rhodobacterales, Flavobacteriales, Caulobacterales, Coriobacteriales, Acidobacteriales, Gemmatales, Vibrionales, Fusobacteriales, Verrucomicrobiales, Micromonosporales, Rhodocyclales, Sphingobacteriales, Spirochaetales, Anaerolineales, Bryobacterales, Eggerthellales, Isosphaerales, Myxococcales, Propionibacteriales, Aeromonadales, Cellvibrionales, Chitinophagales, Nitrospirales, Cytophagales, Desulfobacterales, Neisseriales, Oscillatoriales, Campylobacterales, Pirellulales, Tepidiformales, Unclassified Candidatus Saccharibacteria, Chromatiales, Desulfovibrionales, Desulfuromonadales, Fimbriimonadales, Nitrosomonadales, Parachlamydiales, Planctomycetales, Unclassified Spartobacteria, Actinomycetales, Bdellovibrionales, Candidatus Brocadiales, Deinococcales, Erysipelotrichales, Frankiales, Gemmatimonadales, Legionellales, Mariprofundales, Methylococcales, Opitutales, Orbales, Pasteurellales,

Sedimentisphaerales, Solirubrobacterales, Syntrophobacterales, Thiotrichales, Veillonellales, Cardiobacteriales, Chloroflexales, Oceanospirillales, Pseudonocardiales, Rubrobacterales, and Unclassified Vicinamibacteria, either exhibited varying degrees of presence or were absent in the samples, underscoring the complexity and diversity of bacterial communities at the order level in TCR600 (Fig. 6).

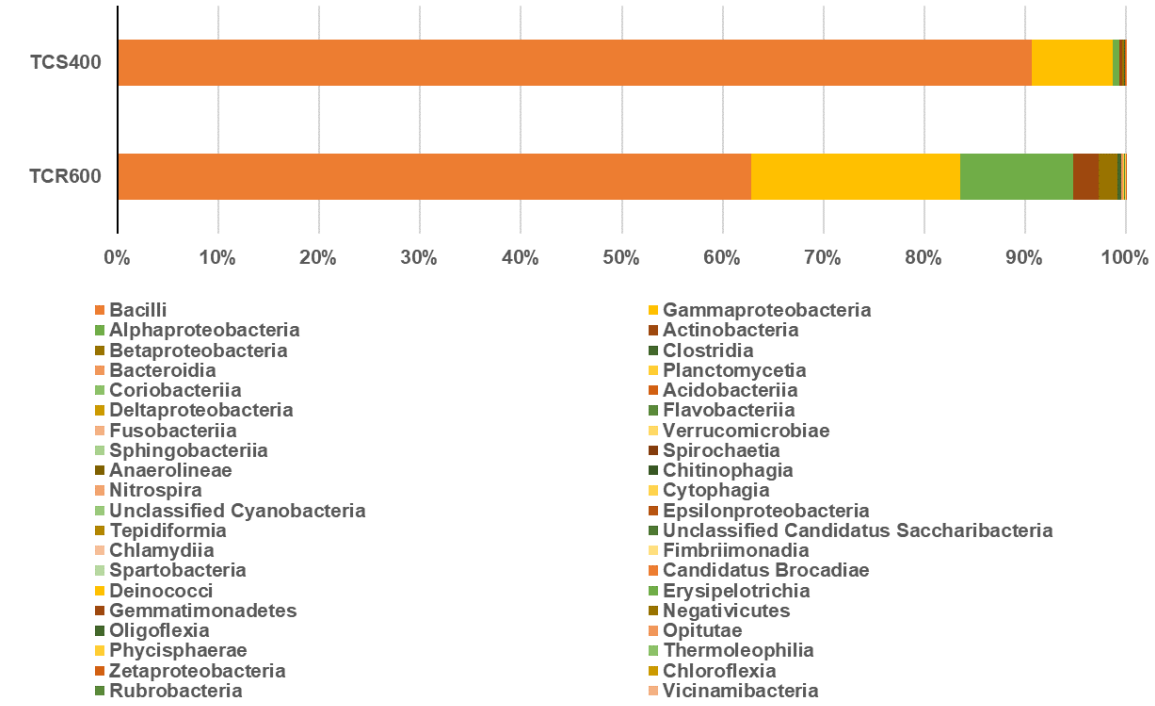


Fig. 5. Percentage of bacterial diversity based on classes associated with TCR600 (root) and TCS400 (shoot surface) samples of *Artemisia judaica*.

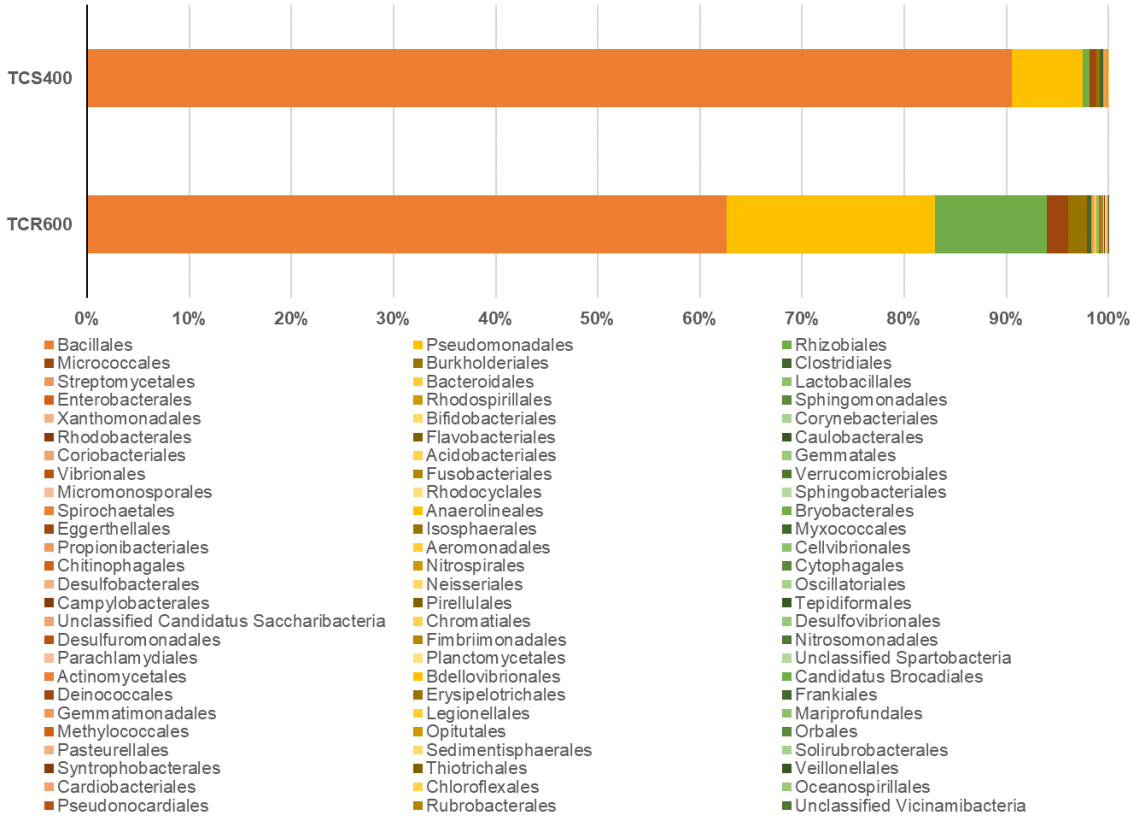


Fig. 6. Percentage of bacterial order associated with TCR600 (root) and TCS400 (shoot surface) samples of *Artemisia judaica*.

3.5. Family and genus-level bacterial community distribution

This table 4S (supplementary file**)** provides the abundance percentages of different bacterial families in the TCR600 samples. The Bacillaceae family was the most abundant in root samples, accounting for 56.32% of the TCR600 samples. The Pseudomonadaceae and Rhizobiaceae families were also relatively prevalent, accounting for 20.319% and 9.867% of the TCR600 sample. The Alcaligenaceae, Micrococcaceae, and Unclassified Rhizobiales families were more abundant in the TCR600 sample, accounting for 1.766%, 1.706%, and 1.036%. Conversely, the Enterobacteriaceae, Rhodospirillaceae, Unclassified Enterobacterales, Moraxellaceae, and Ruminococcaceae families were almost 0.035%, 0.026%, 0.022%, 0.020%, and 0.020% in the TCR600 sample. Overall, the bacterial community compositions in the were similar; however, there were some notable differences in the abundances of certain families in the vein diagram (Fig. 7 and 8).

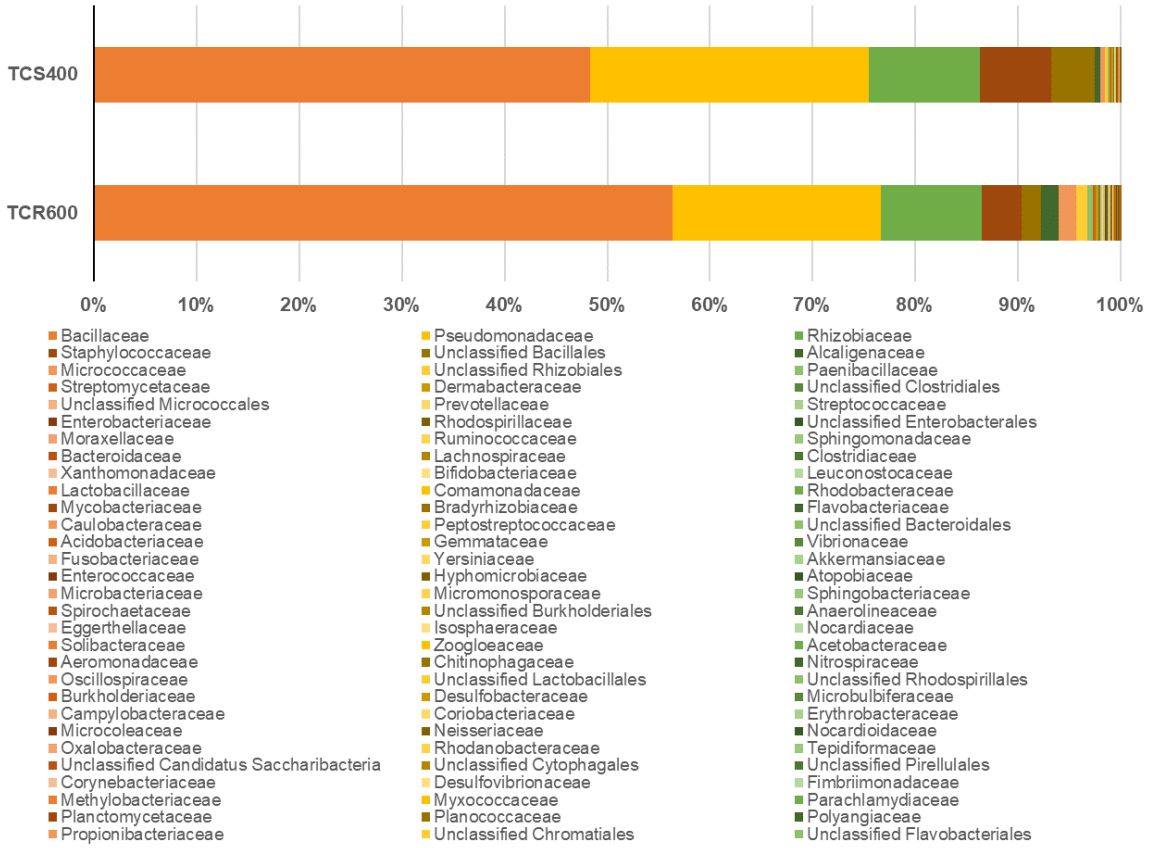


Fig. 7. Distribution of families found in TCR600 (root) and TCS400 (shoot surface)) samples of *Artemisia judaica*.

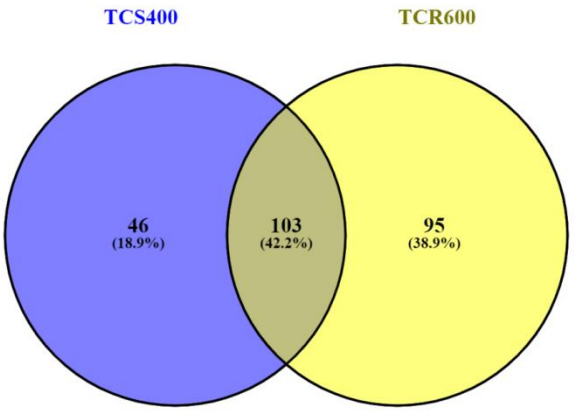


Fig. 8. Venn diagram describing the OUT distribution across tissue sample different tissue samples TCR600 (root) and TCS400 (shoot surface) of *Artemisia judaica*.

While comparing the samples at the genus level, Table 3S shows that the OTUs (operational taxonomic units) of TCR600 and TCS400 showed noteworthy differences in the genomic content of specific genera. OTUs are groups of closely related bacteria defined by their DNA sequences. Table 3S shows that the TCR600 sample contained a wider diversity of OTUs than the TCS400 sample. There were 61,489 OTUs in the TCR600 sample, and only 99,029 OTUs in the TCS400 sample (Table 1). This indicates that there were more types of bacteria in the TCR600 sample than in the TCS400 sample. The first sample, TCR600, had a higher average number of genes per genus (48.334%) than the second sample, TCS400 (38.721%; Table 3S). The top five gene-rich genera in both samples were *Bacillus*, *Staphylococcus*, *Pseudomonas*, Unclassified *Bacillaceae*, and *Rhizobium* (Fig. 9). However, the order of these genera differed slightly between the samples; the most common core microbiomes are listed in Fig. 10. For TCR600, *Bacillus* was the most gene-rich genus, followed by *Staphylococcus*, *Pseudomonas*, Unclassified *Bacillaceae*, and *Rhizobium*, while in TCS400, *Staphylococcus* was the most gene-rich genus, followed by *Bacillus*, *Pseudomonas*, *Rhizobium*, and Unclassified *Bacillaceae*. There were also notable differences in the gene counts of certain genera between the two samples. In the TCR600 treatment, several genera had a higher number of genes than in the TCS400 treatment, indicating potential genomic richness and complexity. *Bacillus* exhibited 38.721% of genes in TCR600, in contrast to 5.628% of genes in TCS400, illustrating a considerable disparity in genomic content. *Staphylococcus*, *Pseudomonas*, Unclassified *Bacillaceae*, and *Rhizobium* also followed this pattern, showing elevated gene counts at TCR600. Conversely, TCS400 exhibited a distinct set of genera with high gene counts, suggesting the presence of unique genomic characteristics in this sample. Notable examples included Alcaligenaceae (1,766 vs. 1,067 genes), Micrococcaceae (1,706 vs. 1,036 genes), Unclassified Rhizobiales (1.036 vs. 0.577 genes), Paenibacillaceae (0.569 vs. 0.265 genes), and Streptomycetaceae (0.307 vs. 0.167 genes). Overall, the bacterial genomes in the two samples were similar in terms of their overall gene content; however, some notable differences were observed in the gene counts of certain genera (Table 3S, Fig. 11).

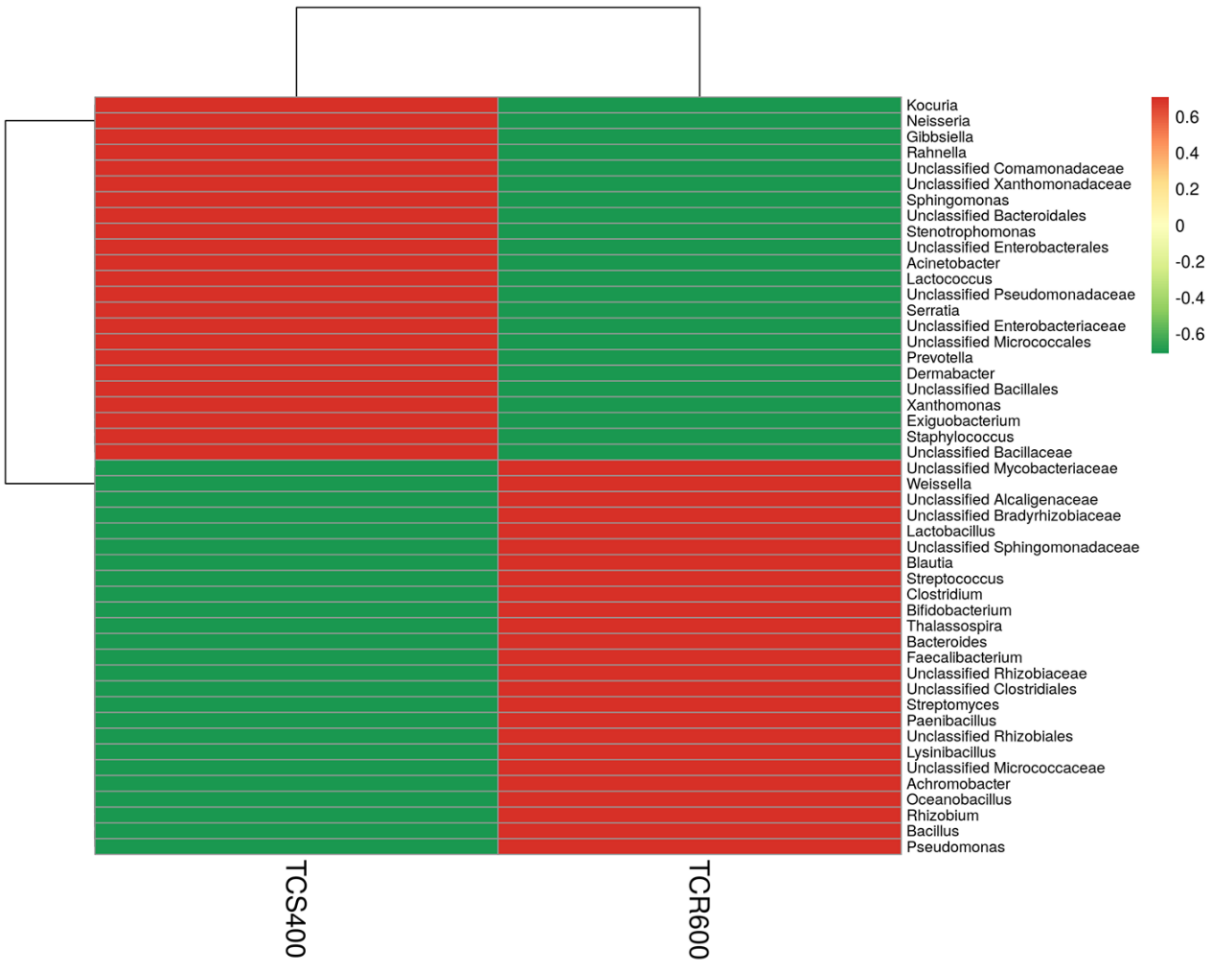


Fig. 9. A heat map showing the maximum abundance of microbes in different tissue samples TCR600 (root) and TCS400 (shoot surface) of *Artemisia judaica*.

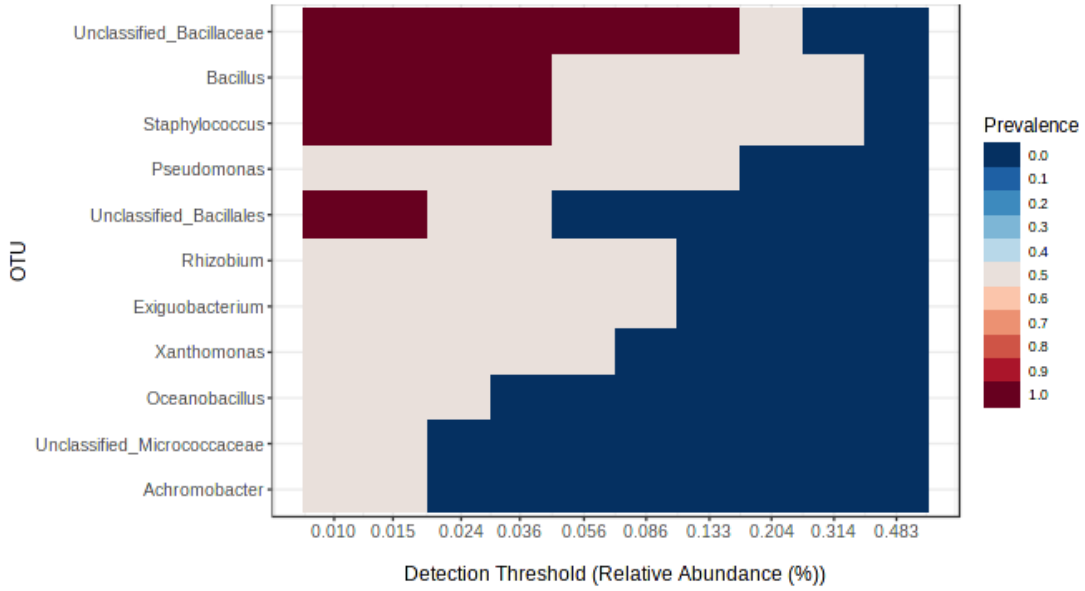


Fig. 10. Core microbiome obtained from different tissue samples TCR600 (root) and TCS400 (shoot surface) of *Artemisia judaica.*

S. No.	Genus	OTUs of TCR600 (%)	Genus	OTUs of TCS400 (%)
1	Bacillus		Staphylococcus	
2	Pseudomonas		Unclassified Bacillaceae	
3	Unclassified Bacillaceae		Exiguobacterium	
4	Rhizobium		Xanthomonas	
5	Staphylococcus		Bacillus	
6	Oceanobacillus		Unclassified Bacillales	
7	Unclassified Bacillales			
8	Achromobacter			
9	Unclassified Micrococcacea			
10	Lysinibacillus			
11	Unclassified Rhizobiales			

Fig. 11. Relative Genus Abundance level in TCR600 (root) and TCS400 (shoot surface) of *Artemisia Judaica.*

Table 3S. Percentage distribution of different Genus in TCR600 (root) and TCS400 (shoot surface) of Artemisia Judaica from central region of Saudi Arabia.

S. No.	Genus	OTUs of TCR600 (%)	Genus	OTUs of TCS400 (%)
1.	Bacillus	38.721	Staphylococcus	48.334
2.	Pseudomonas	20.251	Unclassified Bacillaceae	21.529
3.	Unclassified Bacillaceae	13.586	Exiguobacterium	10.765
4.	Rhizobium	9.748	Xanthomonas	6.883
5.	Staphylococcus	3.867	Bacillus	5.628
6.	Oceanobacillus	2.950	Unclassified Bacillales	4.251
7.	Unclassified Bacillales	1.851		
8.	Achromobacter	1.735		
9.	Unclassified Micrococcaceae	1.706		
10.	Lysinibacillus	1.067		
11.	Unclassified Rhizobiales	1.036		

4. Discussion

Bacterial communities associated with *Artemisia judaica* have been characterized globally using metagenomic approaches. The 16S rRNA gene, widely recognized as a reliable phylogenetic marker, has proven effective for profiling the microbiome of this desert-adapted plant in Saudi Arabia. It facilitates the exploration of microbial assemblages linked to the plant's roots and stems under extreme arid conditions. Unlike previous studies that primarily focus on rhizospheric microbes, the present investigation maps the internal bacterial communities inhabiting the roots and stems of *A. judaica*, offering novel insights into its endophytic microbiome.

Alpha diversity analysis, including observed OTUs (richness), Shannon (diversity), and inverse Simpson's tests, was conducted on each sample to assess the diversity of the microbiota. The analysis was based on operational taxonomic units (OTUs), defined as groups of sequences sharing 97% identity. Richness was assessed using the Chao1 index and OTU counts, while the Shannon and Inverse Simpson's indices evaluated diversity and species dominance (Fig. 1)

Another operational principal component analysis (PCA) to study taxonomic clustering (˘-diversity) of samples and followed step by step the method described by **Caporaso *et al.* (2011)**. This method offers deep insights into how bacterial populations change in different samples, and sheds light on the microbial populations found alongside plants (**Yang *et al.*, 2017**). The Chao1 index evaluates the total species estimate in a community. Higher values of Chao1 indicate greater probability of missing OTUs in the sample, especially those expected in high-singleton or doubleton samples (**Gwinn *et al.*, 2016**). A less limiting measure of diversity is the Shannon index which counts different species in a dataset as well as their proportional representation within it (**Kumar *et al.*, 2022**). The findings revealed that 99% of the bacterial communities in both samples belonged to five major phyla: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Planctomycetes are also consistent with the findings of **Janssen (2006; Ding *et al.*, 2013; Lagerlöf *et al.*, 2014**). As previously reported, **Khan and Khan (2020)** and **Sansupa *et al.* (2021)** found that the microbial communities of arid and forest soils, respectively, displayed a notable abundance of Firmicutes, Actinobacteria, and Proteobacteria, which are characterized as tolerant bacteria that play important roles in dealing with drought and contribute to bioremediation (**Dai *et al.*, 2019**). Additionally, studies by have consistently shown an increased prevalence of *Bacillus* spp. in desert soils following stress, highlighting their ability to resist abiotic stresses, such as salinity and drought (**Abo-Aba *et al.*, 2015; Meena *et al.*,2017; Yadav *et al.*, 2015**). The high number of OTUs representing Bacilli and Clostridia within the Firmicutes class and pH and phosphate may explain their dominance in the Arabian soils which are well known for their high level of phosphates resulting from excessive use of fertilizers, particularly between 1950s and 1980s (**Van Bruchem *et al.*, 1999**).

Other studies have indicated that Actinobacteria promote nutrient absorption and plant growth in legumes and grains (**Desriac *et al.*, 2013; Rajkumar *et al.*, 2006; Mano *et al.*, 2007**). **Huang *et al.* (2012)** reported the presence of Actinobacteria in desert plants, which aligns with our findings from *Artemisia judaica*, where root-associated communities in both TCR600 and TCS400 samples included Actinobacteria and several of its genera, such as *Streptomyces*, *Bifidobacterium*, and *Corynebacterium*. However, *Rhodococcus*, *Frankia*, and *Nocardia* were only observed in the TCR600 sample. Additionally, a small group of cyanobacteria was discovered in both TCS400 and TCR600 samples. They also found cyanobacteria in *Senna* (**Alsaedi *et al.*, 2022**). Similarly, **Zhang *et al.* (2019)** reported that cyanobacteria were the most abundant bacteria living inside plants in extreme environments. These are a diverse group of bacteria which photosynthesize and can survive in unfriendly conditions (**Azua-Bustos *et al.*, 2012; Patzelt *et al.*, 2014**). They fix nitrogen and support photosynthesis even under harsh desert conditions (**Harel *et al.*, 2004**).

These bacteria are found in the leaves, stems, and roots of plants, where they play crucial roles in nitrogen fixation (**Issa *et al.*, 2014**). In addition, they promote plant growth and serve as valuable sources of bioenergy and food supplements. Moreover, both our study and Alsaedi's study (**Alsaedi *et al.*, 2022**) noted a considerable presence of bacterial endophytes associated with the Proteobacteria phylum in the *Artemisia judaica* plants from the deserts of Saudi Arabia. Likewise, in rice fields of scented black rice, **Singha *et al.* (2021)** described endophytes in the roots as predominantly belonging to Proteobacteria, which are likely to enhance plant growth because of their antibacterial and antifungal activities. These bacteria are important in the global cycles of carbon, nitrogen, and sulfur as well as in bioremediation and in the formation of bioactive substances. They are also affected by climate change and its impacts on the soil biosphere. From our study, we attributed the capability of nitrogen mineralization and organic matter decomposition to Pseudomonas and Rhizobium, which belong to the class of Alphaproteobacteria. This is in line with the findings of **Gazdag *et al.* (2018) with a**ctinobacteria was observed asa third most abundant phylum in both samples.

Despite the harsh environmental conditions, desert plants are frequently colonized by diverse Actinobacteria, particularly members of the genus *Streptomyces*. Although the endophytic communities of desert plants remain relatively understudied, Actinobacteria are recognized for their ability to produce a wide array of bioactive

compounds. These bacteria play a vital role in the biosynthesis of economically important secondary metabolites and contribute to plant resilience by enhancing resistance to ultraviolet radiation and dehydration (**Elbendary *et al.*, 2018; Barka *et al.*, 2015; Zhao *et al.*, 2015**). Our study supports the prevalence of bacterial endophytes, including Actinobacteria and Proteobacteria, in line with the observations of **Hong *et al.* (2019)** in *Panax ginseng* plants and reports from **Robinson *et al.* (2016)**, which emphasized the suitability of roots as sites for endophyte colonization, attributed to their role as carbon stores and protection from environmental fluctuations.

5. Conclusion

This study focused on various bacteria found in the shoot and roots of *Artemisia judaica* plants in the Alkharj region of Southern Riyadh, Saudi Arabia. Using Illumina MiSeq technology and the 16S rRNA gene as a marker, a comprehensive analysis of the bacterial diversity in the TCR600 and TCS400 samples was performed. Our findings provide a better understanding of the bacterial diversity of *Artemisia judaica* and new perspectives on potential biological agents that could enhance agricultural practices. Our research suggests that examining these bacterial communities can provide insights into a plant's growth rate and ability to thrive under challenging environmental conditions. Additionally, our high-throughput molecular tools allowed us to identify, classify, and characterize the endophytic bacterial communities. Further research is required to understand the diverse metabolic pathways within the endophytic microbiome, including a more detailed representation and comparative functional and biochemical studies. Such investigations will contribute significantly to the use of beneficial bacterial populations in sustainable agriculture. Further research is required to uncover the full potential of desert endophytes.

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