Alswat AM *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 1, Article number 13053
DOI:10.15835/nbha51113053
Research Article

Investigating the metagenomics of the bacterial communities in the rhizosphere of the desert plant *Senna italica* and their role as plant growth promoting factors

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Abstract

Natural microbial communities associated with desert plants are found in soils that face nutrient deficiencies and extreme environments, including salinity and drought. In this study, 16S rRNA metagenomic sequencing was used to screen and identify bacterial assemblies associated with the desert plant *Senna italica*, obtained from diverse soil samples located in the Asfan region, northeast of Jeddah, Saudi Arabia. Several studies found *Senna italica* as a valuable medicinal plant for treating different diseases; however, a few studies were done on its association with bacterial communities under drought conditions. This study aimed to identify bacterial communities present in the drought soil environment of the *Senna italica* plants. To approach our goals, we applied metagenomic techniques, discovering a new bacterial strain beneficial for biotechnological applications. Our results showed that the analysis of the 16S rRNA sequences at the taxonomic phylum level detected 15 phyla of bacterial populations in the soil samples. The most prevalent was kept for further research. Our findings demonstrated that rhizospheric bacteria may be used as indicators of plant growth rate and survival ability in hostile environments. Studying the soil microbiome's taxonomic, phylogenetic, and functional diversity will facilitate identifying new candidates for biological agents that can be used to improve agricultural and industrial processes.

Keywords: metagenomics; microbiome; PGPR; rhizosphere; *Senna italica* plant

Received: 23 Dec 2022. Received in revised form: 30 Jan 2023. Accepted: 17 Feb 2023. Published online: 20 Feb 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Deserts are one of the harshest terrestrial ecosystems, with high amounts of solar radiation and low rainfall. Rainfall levels are low, while temperatures are very high. Desert soils are distinguished by reduced water retention, nutritional deficiency, and salinity (Eida *et al*., 2018). In addition, the soil has the most complex and dynamic properties for the growth and diversity of various organisms and microorganisms concerning soil pH, chemical and physical qualities, and geographic placements (Bui, 2013; Orozco-Mosqueda *et al*., 2018). These microbes control plant life and health by decomposing soil biomass, fertility, nitrogen, carbon, and other nutrient cycles. Each gram of soil contains thousands of taxonomic bacteria, archaea, and eukaryotes, which reflects the diversity of their biological compositions that may affect their functions. Furthermore, these diverse microbial populations could exist as free-living or symbiotic organisms, as their impact can range from pathogenic to beneficial to mutualistic (Chaparro *et al*., 2012; Fierer *et al*., 2012). However, studying the diversity and composition of microorganisms in soil is difficult due to the lack of non-cultured bacteria (Hill *et al*., 2000). Microorganism composition can vary dramatically between soil samples taken from different locations. Based on estimates, one gram of farmed soil may hold up to 2 x 10^9 microorganisms (Majeed *et al*., 2020).

Bacterial populations exist in an intimate association within and around the roots of plants, which include both rhizosphere (A narrow zone of the soil influenced by the roots' exudate release and associated organisms) and the endosphere (the interior root) (Berg *et al*., 2014). Plant health is closely associated with the activity of these microbes by determining the composition of their associated bacterial microbiomes (Berendsen, Pieterse and Bakker, 2012). This interconnection enhances the impact of the perturbations in the abiotic environment and their effects on plants, and their associated microbial populations(Wardle *et al*., 2004). Due to agriculture production, it has recently been reported that drought is a wide-scale disruption and the most effective natural disaster (Gornall *et al*., 2010; Al-Ashkar *et al*., 2021; Al-Suhaibani *et al*., 2021; Ding *et al*., 2021 Roy *et al*., 2021; Seleiman *et al*., 2021; Elshayb *et al*., 2022; Al-Selwey *et a*l., 2023). Environmental parameters, such as nutrient availability and uptake, rhizospheric soil ecosystem, mechanisms of plant protection, and microorganisms' reproduction, are all factors to consider (Varma, Tripathi, and Prasad n.d.; Hewedy *et al*., 2020; Mukhtar *et al*., 2020) The environmental models' prediction suggests the increasing frequency and intensity of drought due to global climate change (Battisti and Naylor, 2009; Lesk *et al*., 2016). The plant's microbiome may cause environmental variance; thus, understanding the impact of drought on root-associated bacterial communities is mandatory for developing strategies to combat drought (Naylor and Coleman-Derr, 2018). This interaction will display a wide range of microorganisms capable of promoting the growth of various crop plants under biotic and abiotic conditions, and serving as biological agents in different industrial and medical applications (Baeshen *et al*., 2020).

Microbes found in desert plant roots promote plant development and stress resistance in crop species (Marasco *et al*., 2012)(Mengual *et al*., 2014). In addition, bacteria and fungi play an essential role in nutrient cycling in desert habitats (Morgan *et al*., 2005; Bonfante and Anca, 2009; Makhalanyane *et al*., 2015). Plant growth-promoting rhizobacteria (PGPR), a rhizosphere-colonizing bacterium, is thought to be the most effective choice for improving plant health and soil fertility. Furthermore, implementing PGPR for sustainable agriculture can aid in the reduction of environmental pollution caused by agricultural run-off, which leads to groundwater contamination. How the role and effectiveness of rhizosphere bacteria in plant growth promotion, and their efficacy in improving soil health and microbial diversity (Vandenkoornhuyse *et al*., 2015)(Functions, no date).

Environmentally damaging practices, such as deforestation and abusing chemical fertilizers and pesticides in agriculture are accompanied by a rapidly growing human population (). Furthermore, due to greenhouse gas emissions, global warming exacerbates abiotic pressures and reduces cultivable land and agricultural production (Cerri *et al*., 2007; Mittler, 2006; Pandey *et al*., 2017). Drought, salt, severe

temperatures, UV radiation, nutrient shortage, and inaccessibility are abiotic factors that cause more than half of all crop losses (Khan *et al*., 2015). Finding ecologically acceptable, cost-effective, and long-term solutions to ensure food availability for a growing population have become a focus of significant research (Godfray *et al*., 2010). As a result, there is a pressing demand for biological agents that are widely accepted. A better solution to this problem is to use the PGPR, as they are critical in increasing soil productivity, enhancing plant growth, and suppressing phytopathogens in developing environmentally friendly and long-term agriculture. Studies present an environmentally benign strategy for increasing crop output and health, as well as the development of sustainable agriculture and commercialization through plant growth-boosting rhizobacteria (Shailendra Singh, 2015).

Since prehistory, plants have been used as a cure for treating diseases, as people from different continents follow this ancient tradition (Adjou, Koudoro and Nonviho, 2021). The plant *Senna italica*, a species of the Fabaceae family (subfamily: Caesalpinaceae), has an important impact on African folk medicine due to its therapeutic properties. In Venda, South Africa, *S. italica* has grown in popularity as a way to improve health and prevent or treat ailments (Kawo, 2011). For example, several Senna species were examined effectively in treating sexually transmitted infections (STIs), and others have significant antibacterial activities (Tshikalange *et al*., 2005). This indicates the pharmacological importance of *S. italica,* which justifies its use in treating several diseases (Dabai, 2012). Additionally, *S. italica* may grow throughout the year at a warm temperature, as this plant is the host of the root-lesion nematode. .This interaction will expose a vast array of microbes capable of promoting the growth of a wide range of crop plants under various abiotic and biotic conditions, as well as serving as biological agents in many industrial and medical applications (Majeed *et al*., 2020).

Severe termite attacks are observed during perennial cultivation. The world's population is expected to grow from nearly 7 billion to 8 billion by 2030 and to more than 9 billion by 2050. By 2050, the global agri-food system will be subjected to unprecedented stresses (Serraj and Pingali, 2019). Metagenomics techniques have been employed in many studies to understand better the microbial communities of various ecosystems from soils collected in deserts (hot and cold), forests, meadows, and tundra (Baeshen *et al*., 2020). Thus, metagenomic techniques would be more accurate in investigating bacterial communities than the classical methods targeting dangerous bacteria.

Additionally, studying uncultured bacteria has provided valuable insight into previously unknown catalysts that could not be detected using traditional methods (Orsini *et al*., 2017). Metagenomic techniques will also identify bacteria and their essential genes associated with soil and plants (Didelot *et al*., 2012; Alves *et al*., 2018) .

In this study, we highlight numbers of beneficial bacteria isolated from the rhizosphere of *S. italica* using metagenomic approaches and biotechnological applications. Due to the next green revolution and sustainable crop production, it is essential to understand soil complexity, plant microbiomes, and fuel innovations. In addition, the increasing need for alternative experimental approaches and the development of new tools have provided new insights into our understanding of microbiome dynamics and their interaction with host organisms. Hence, we aim to identify bacterial communities present in the drought environment of the soil of *S. italica* plants by applying metagenomics techniques, which may lead to the discovery of a new bacterial strain beneficial for biotechnological applications. We hypothesize that these isolated bacteria could be drought-resistant, enhancing agriculture production and meeting the global food demand.

Materials and Methods

Study site

The study was performed at Asfan, Northeast Jeddah, Saudi Arabia (latitude: 21.53'13.3" N, longitude: 39.15'06.6" E, and altitude: 2.8m) above sea level. The Asfan climate is hot, dry, and sandy, with a decreased amount of rainfall.

Sample collection

Sampling was carried out on April 11, 2021, at 11 am. The temperature was 31 °C. A total of four samples were collected from the area of the desert plant, namely the Senna plant was collected. Three samples were taken from the rhizosphere of each plant with a depth of 18-25 cm below the first layer. The fourth sample, a free-soil sample, was used as a control, which was accumulated from a close area of non-plant growth. The control sample was used to compare microbes in the rhizosphere, where *S. italica* has grown and immediately stored at -20 °C.

DNA Extraction, PCR amplification, and sequencing of the 16S rRNA Gene

The collected soil samples were delivered to Macrogen Inc. Company in Seoul, South Korea, where genomic DNA was extracted. The Picogreen fluorescence-based quantification method (Invitrogen, cat. #P7589) was used to assess DNA purity and quantity. The universal primers (Bakt 341F: (Bakt_341F: CCTACGGNGGCWGCAG) and (Bakt_805R: GACTACHVGGGTATCTAATCC): were used to amplify bacterial V3-V4 16S rRNA gene segments by PCR. An initial denaturation (94 °C-5 minutes), followed by 30 cycles (denaturation at 94 °C-30 seconds, annealing at 57 °C-40 s, and extension at 72 °C-1.30 s, with a final elongation at 72 °C-10 min). The 300 bp pair-end reads of the V3 and V4 sections were extracted using the purified amplicons for library creation and deep sequencing on an Illumina SBS technology (Baeshen *et al*., 2020).

Processing and statistical analysis of the 16S Dataset

The raw sequencing data were analysed using Quantitative Insights into Microbial Ecology (QIIME) (*http://qiime.org*). QIIME is an open-source bioinformatics tool for analysing the raw DNA sequencing data of microbiomes resulting from Illumina or other sequencing programs. QIIME provides quality pretreatment, operational taxonomic units (OTUs) selection, taxonomic classification, phylogenetic reconstruction, diversity analysis, and graphical displays (Macrogen, 2017).

The CD-HIT-OTU *http://weizhongli-lab.org/cd-hit-otu/* filter and trim V3-V4 16S rRNA sequence readings. The Fast Length Adjustment of SHort reads (FLASH) software *http://ccb.jhu.edu/software/FLASH/* was used to merge paired-end reads from next-generation sequencing experiments to exclude low-quality sequences. With a cutoff of 97% identity, OTUs were employed to link and classify a unique sequence set. The Ribosomal Database Project (RDP) Classifier was utilized for taxonomic composition.

The alpha diversity was estimated using Chao1 based on reported from Macrogen (Chao and Bunge, 2002). The Shannon and Simpson indices were estimated using the Mothur software tool to quantify species complexity *http://www.mothur.org*. The rarefaction curve was created by calculating the OTU numbers of the gathered tags and determining the most significant depth at which all samples could be kept. The weighted and unweighted UniFrac distances were calculated and displayed using primary coordinate analysis (PCoA) to detect beta diversity, as UniFrac is a system of evolution data to compare bacterial species between different samples. Finally, the Interactive Tree of Life (ITOL) generated a phylogenetic tree at the genus level.

Results

Statistical Analysis of 16S rRNA

The current investigation utilized the metagenomic technique to explore the microbial community structure and diversity of four soil samples linked with *S. italica* plant. The Illumina SBS was used using the 16S rRNA gene to analyse different soil samples. Figure S1 shows the percentage of each read for each soil sample. FLASH software was used to calculate the total amount of sequence reads and the assembly results for the four samples, as shown in Figure S2 and Table 1. A total of 340,319 clean sequence reads were identified, with the highest value observed in the soil.1 crust sample and the lowest in the control sample as 92,112 and 76,899, respectively.

Table 1. Total numbers of sequences read

Sample Name	Total Bases	Read Count	N (%)	GC (%)	Q20 (%)	Q30 (%)
Control	34,464,868	76,899	0	56.91	99.1	96.64
Soil.1	41,350,103	92,112	0	56.91	99.11	96.72
Soil.2	39,952,302	88,958	0	56.73	99.16	96.86
Soil.3	36,979,641	82,350	0	57.43	99.13	96.72
Total number	152,746,914	340,319				

Total bases: Are the total amount of bases found in reads. Count of pages read: The total numbers of reads in a sequence. GC (%): The percentage of sequence reads that contain GC. Q20 (%): The numbers of bases with a phred score > 20. Q30 (%): The percentage of bases with a phred score of 30 or higher.

Analysis of Operational Taxonomic Units (OTUs)

The CD-HIT-OTU software and rDnaTools were employed to screen the potential contamination on the sequences. The results of clustering for the four soil samples allocated to the OTUs are shown in Figure 1.

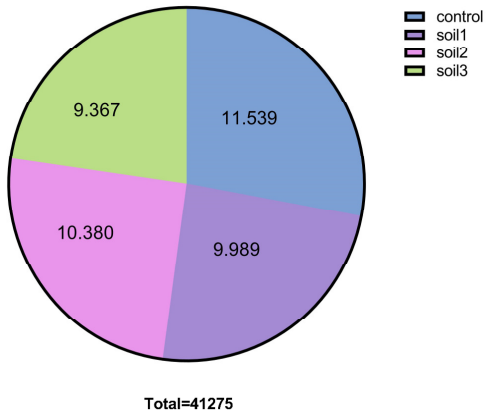

Figure 1. Result of clustering (soil. 1, soil. 2, soil. 3, and control sample) associated to *Senna italica*
The control sample contains 11,539. Soil. 2 contains 10,380. However, soil. 1 and soil. 3 contain 9,989 and 9,367, respectively.

Community diversity and richness

Several indices were used to investigate the complexity of species. A. Chao1 value represents richness estimates for an OTU definition. The B. Shannon value defines the diversity of species, which is influenced by their richness and evenness. C. The inverse Simpson value measures the likelihood that two randomly picked individuals in the environment are of the same species. The OTUs and alpha diversity measures (Chao1, Shannon, Simpson) for each sample are shown in Table 2. The numbers of OTUs for each sample are shown in Figure 2. The highest value of OTUs was estimated as 283 for the soil. 3 samples, while the lowest number

was found for the control sample as 235. Figure 3 displays several curves based on observed Shannon and Inversed Simpson values; however, Figure 4 represents the observed alpha rarefaction curve.

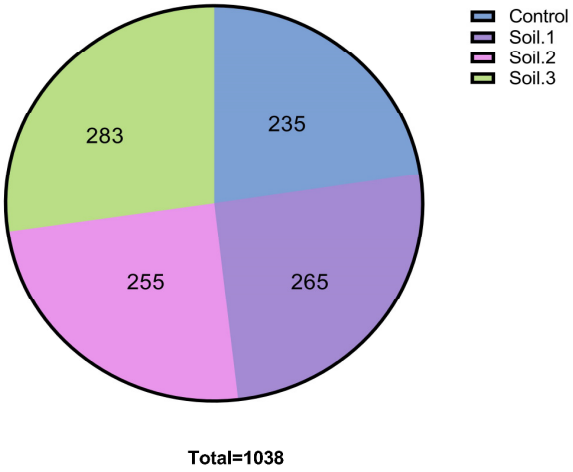

Figure 2. The number of OTUs for each sample (soil. 1, soil. 2, soil. 3, and control sample) associated to *Senna italica*
The highest number of OTUs was 283 belongs to soil. 3, while the lowest was 235 belongs to control sample.

Table 2. Community richness and diversity

Sample Name	OTUs	Chao1	Shannon	Gini-Simpson	Good's Coverage
Control	235	313.12	5.06690179	0.92266147	0.994476591
Soil.1	265	322.6	5.41719838	0.941282189	0.993594876
Soil.2	255	328.7	5.29433893	0.936824728	0.993563263
Soil.3	283	303.788	5.73210289	0.950355062	0.994898513

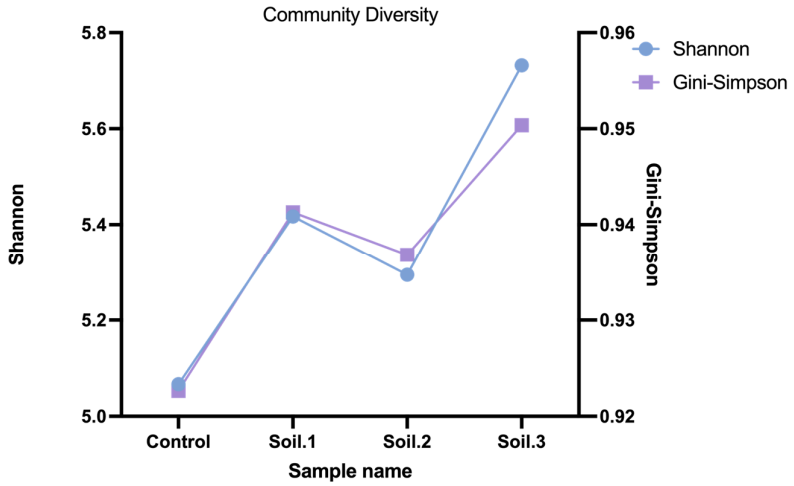

Figure 3. Different curve based on observed Shannon and inversed Simpson values. Soil. 1, soil. 2, soil. 3, and control sample associated to *Senna italica*

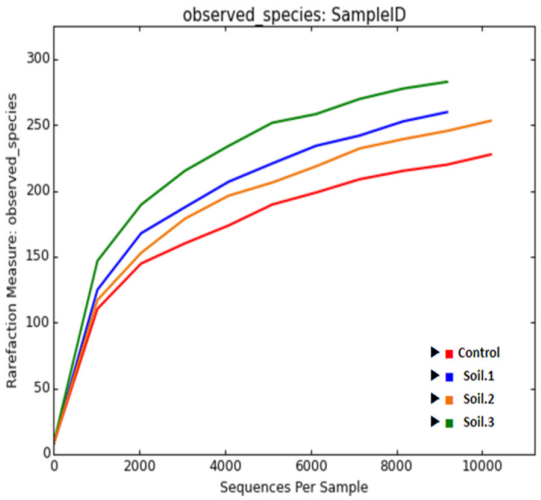

Figure 4. Alfa rarefaction curve based on observed species (OTUs) (Soil. 1, soil. 2, soil. 3, and control sample) associated to *Senna italica*. As shown, the curve takes a rightward direction, and this indicates the use of a reasonable number of readings in the analysis

Principal coordinate analysis (PCoA) was used to determine the diversity and variation in the composition of the four soil samples. Figure 5 displays the PCoA based on the abundance of OTUs for all the samples, including the control and the unweighted UniFrac belongs to certain species. The red triangle indicates the control sample, and the blue square indicates the soil. 1. The orange circle and the green rectangle refer to soil. 2 and soil. 3 samples, respectively. The degree of similarity between samples increases with closeness.

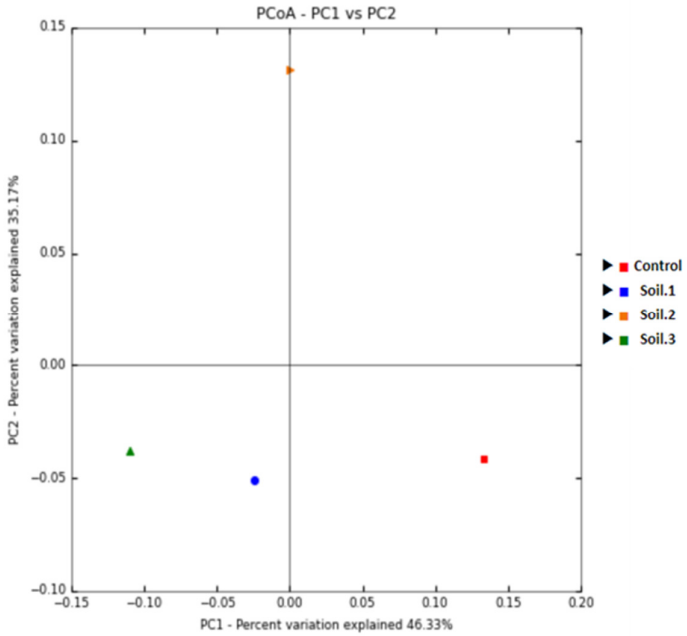

Figure 5. Beta diversity analysis. Unweighted PCoA of UniFrac distances. (Soil. 1, soil. 2, soil. 3, and control sample) associated to *Senna italica*, Principal coordinate analysis shows the differences between bacterial communities in the soil sample of *Senna italica*

Species richness estimators use the frequent occurrence of rarer OTUs to estimate the overall number of species present in a community. If a sample comprises a lot of singletons or doubletons, there are probably more undetected OTUs. Hence, the Chao 1 index was used to estimate more species richness than if the sample did not contain any unusual OTUs. Shannon index, a quantitative measurement, evaluates the diversity of different species in a dataset. It also observed the basic entities (individuals) on how they are divided into different categories. When individuals are classified into species, Simpson indicates the number of the existing species, their distribution, and the measurements of their concentration.

A phylogenetic tree based on 16S rRNA displays the taxonomy and diversity of the isolated bacteria for each soil sample at both the phylum and genus levels (Figures 6 - 7). A phylogenetic tree is a diagram displays the projected evolutionary relationships between distinct biological taxa based on physical or genetic similarities and differences. For example, the shorter the branch length, the closer the evolutionary distance between taxa is. Additionally, a phylogenetic tree elucidates the development links of different species, taxonomic makeup, and abundance analysis.

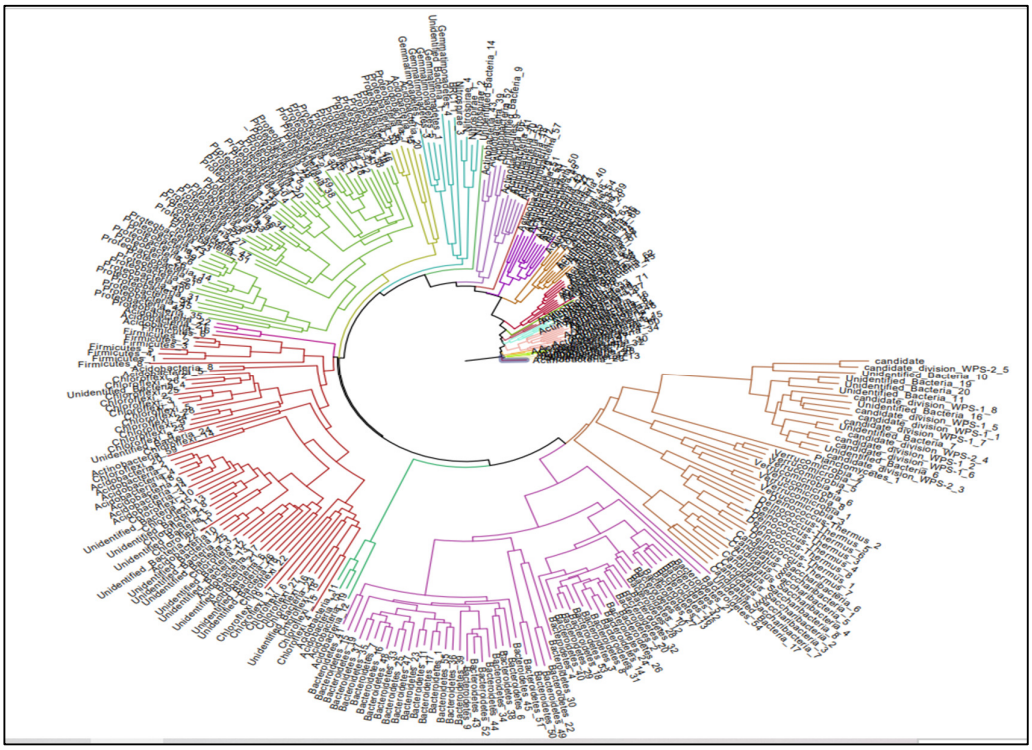

Figure 6. Un-rooted phylogenetic tree based on 16S rRNA gene sequence representing the diversity of bacteria isolated from the soil samples at booth the phylum level

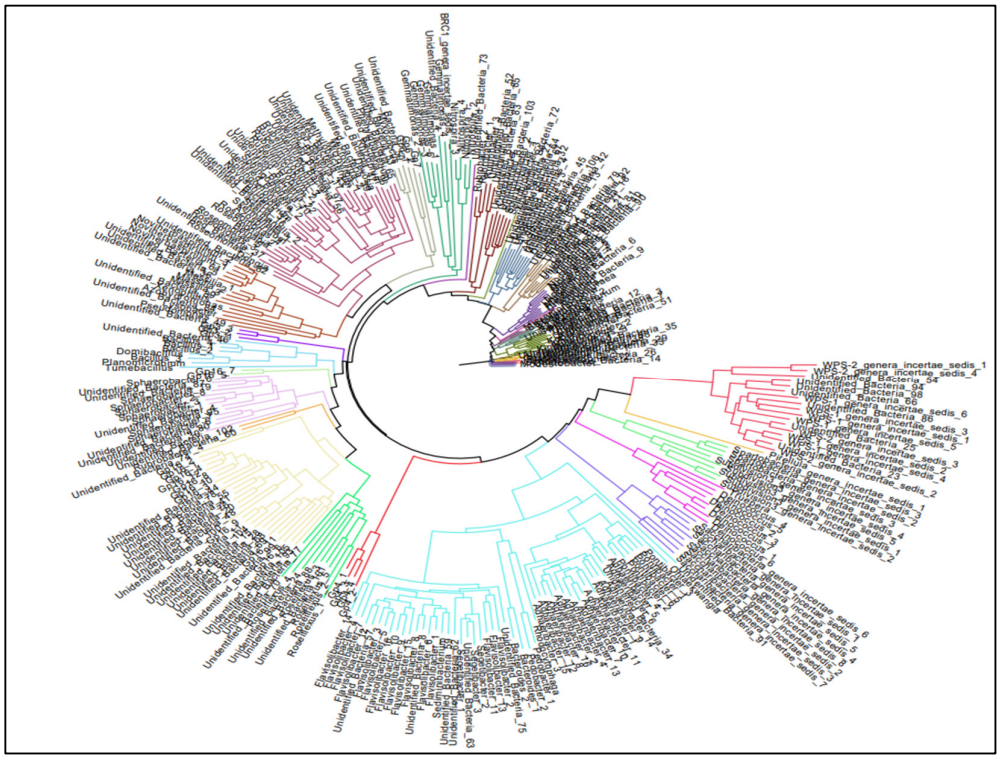

Figure 7. Un-rooted phylogenetic tree based on 16S rRNA gene sequence representing the diversity of bacteria isolated from the various soil samples at booth the genus level

The results of the phylum-level taxonomic distribution of the bacterial communities in all four soil samples revealed their classification into 17 phyla, with the most abundant being highlighted for further investigation. These phyla include Proteobacteria (35 genera), Actinobacteria (35 genera), Bacteroidetes (12 genera), Firmicutes (6 genera), Chloroflexi (7 genera), Acidobacteria (5 genera), Deinococcus-Thermus (two genera), and unclassified phyla (one genus).

Analyses of the bacterial populations at the phylum level demonstrated that Proteobacteria were the most abundant phylum in the soil. 2 samples. In contrast, Actinobacteria were the most phylum that existed in soil. 3 samples (Figure 8 A and B). Bacteroidetes significantly dominated the samples collected from the soil. 2 (Figure 8 C). Firmicutes and Chloroflexi were significantly observed in the control sample of the associated Senna italica plant (Figures 8D and E). However, the unclassified phyla were found in all soil samples, with a significant abundance in the soil. 3 of the Senna italica plant (Figure 8 F). Moreover, Acidobacteria were found in the soil. 3 samples of the *S. italica* plant (Figure 8 G), whereas Deinococcus-Thermus occurred in the soil. 1 (Figure 8 H).

The previously mentioned eight highly abundant bacteria found at the phylum level include large numbers of genera, estimated at 113. At the genus level, Microvirga, Rubellimicrobium, Roseomonas, Azospirillum, Skermanella, Sphingomonas, Massilia, Noviherbaspirillum, Devosia and five unclassified genera were found belonging to Proteobacteria. Aciditerrimonas, Cellulomonas, Blastococcus, Geodermatophilus, Modestobacter, Phycicoccus, Kineococcus, Arthrobacter, Kocuria, Nocardioides, Amycolatopsis, Saccharothrix, Streptomyces, Solirubrobacter and six unclassified genera were found associated with Actinobacteria. Bacillus, Domibacillus, and Planomicrobium were found in Firmicutes. Adhaeribacter, Pontibacter, Flavisolibacter, Segetibacter, Rhodocytophage, Pedobacter, and one unclassified genus of Bacteroidetes, Deinococcus, and Truepera, exist in Deinococcus Thermus. Gp16 was found in Acidobacteri, whereas Roseiflexus, Sphaerobacter, and one unclassified genus were found in Chloroflexi (Figure 9).

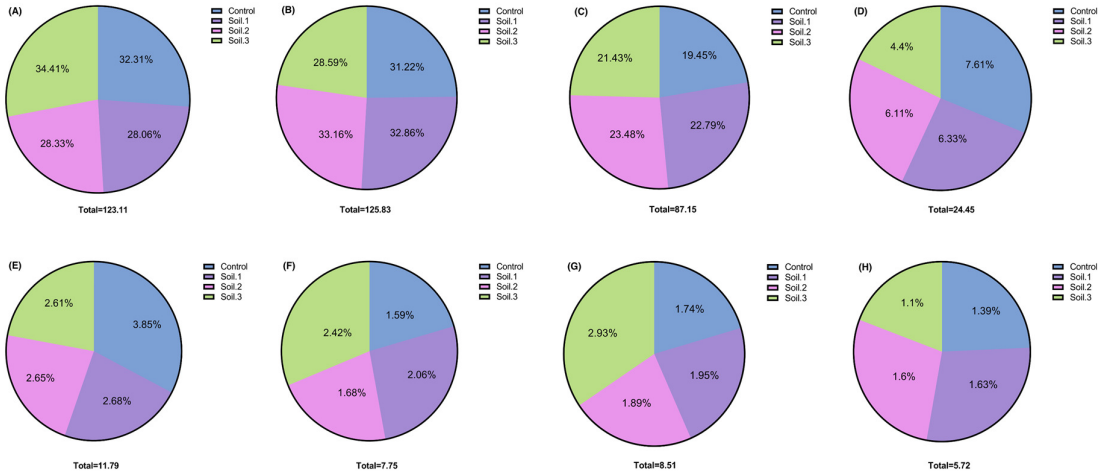

Figure 8. Pie charts of bacterial communities at the phylum classification among all the samples: Soil. 1, soil. 2, soil. 3, and control samples associated with *Senna italica* plant

(A) The amount of Actinobacteria among all the samples. (B) The amount of Proteobacteria among all the samples. (C) The amount of Bacteroidetes among all the samples. (D) The amount of Firmicutes among all the samples. (E) The amount of Chlolrofleix among all the samples. (F) The amount of the unclassified phyla among all the samples. (G) The amount of Acidobacteria among the samples. (H) The amount of the Deinococcus Thermus among all the samples.

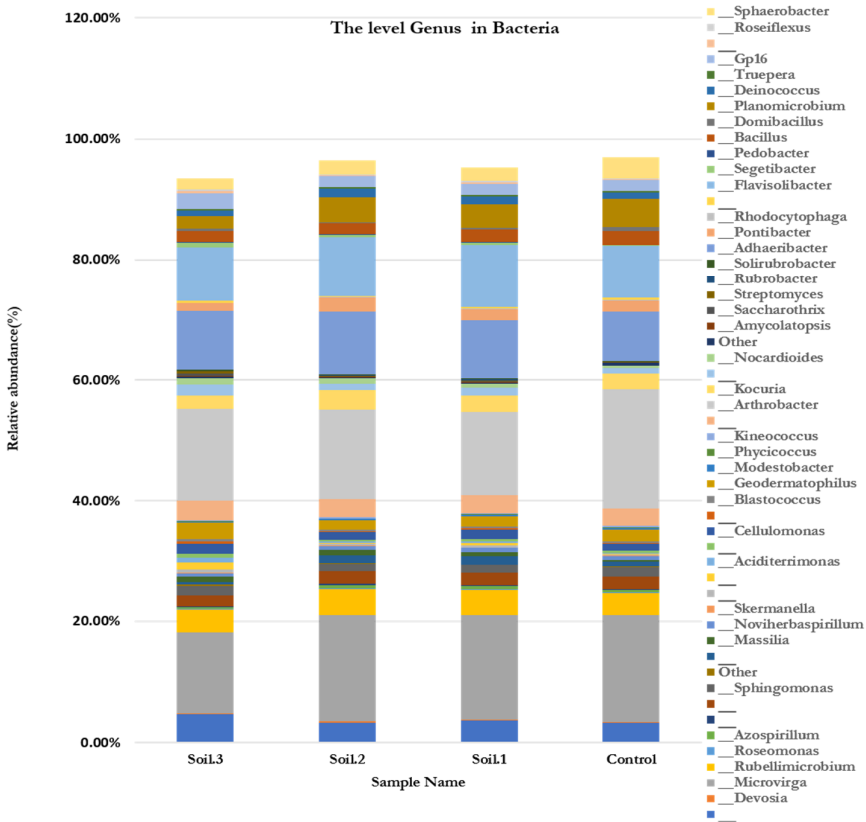

Figure 9. Relative abundance of the taxonomic composition distribution at the Genus level in all samples (Soil. 1, soil. 2, soil. 3, and control samples) associated with *Senna italica* plant) based on the V3-V4 regions of the 16S rRNA. The most abundant genera include, a Genus of unclassified phyla, Acidobacteria, Actinobacteria, Bacteroidetes, Chlolrofleix, Deinococcus Thermus, Proteobacteria, and Firmicutes

Discussion

This study aimed to identify and classify bacterial communities of the drought ecosystems in the soil associated with the *S. italica* plant located in the northeast of Jeddah in the Asfan region, Saudi Arabia. To approach our goals, we applied metagenomic techniques, discovering a new bacterial strain beneficial for biotechnological applications. Our findings agreed with our goals and previous studies (Alsaedi *et al*., 2022; Baeshen *et al*., 2020; Soussi *et al*., 2016) demonstrating that rhizospheric bacteria could be used as indicators of plant growth rate and survival ability in hostile environments. Additionally, previous observations (Adeleke et al., 2022; Kuźniar *et al*., 2019) supported our results indicating the importance of rhizospheric bacterial diversity in the production of many applications, including agriculture, industry, medicine, biofertilization, and bioremediation.

Soil microbes are essential in soil fertility, plant health, carbon, nitrogen, and other nutrient cycling. Thousands of bacterial, archaeal, and eukaryotic species live in every gram of soil, and the diversity of their protein-encoded activities reflects this taxonomic richness, including an almost infinite range of physiologies and life history strategies. These properties of soil microbial communities have been discovered for decades. However, the ongoing development of high-throughput molecular methods and the tools required for analyzing the additional data assist microbial ecologists in identifying the taxonomic, phylogenetic, and varied functions of soil microbial populations to a previously unimaginable degree (Mashiane *et al*., 2017; Kuźniar *et al*., 2019).

We can undertake extensive comparative soil investigations across vast spatial gradients rather than just individual soils (Fierer *et al*., 2012). Beneficial root-associated rhizospheric bacteria are essential for maintaining host plant growth and could allow drought-resistant agricultural production in the future. Soil type, plant genotype, and soil moisture all have a role in the intricate interaction of root-associated microorganisms. The draught, on the other hand, is the cause. The most damaging environmental stress significantly impacts soil biota and can limit plant growth and productivity (Aslam *et al*., 2022).

This study investigated the metagenomics of the bacterial communities in the rhizosphere of the desert plant *S. italica* and their role as plant growth-promoting factors. Four soil samples were collected from various locations in the same area and were labeled as soil. 1, soil. 2, and soil. 3. The fourth sample was kept from an area with no plant and considered a control. In these severe settings, bacterial diversification was examined using the PCR amplification of the V1-V3 bacterial 16S rRNA gene regions. Therefore, high-quality sequences of 340,319 were classified at the phylum and the genus levels. The differences among bacterial communities were demonstrated in the four soil samples. The richness and diversity of the bacteria were examined in each sample, and a slight change was found among all the samples from the same region (from 235 to 283 OTUs). According to the sequencing results, the taxonomic distribution of the bacterial communities at the phylum level is classified as 17 phyla. Furthermore, multiple investigations have suggested that these bacteria have several environmental effects. Likewise, several studies have reported the significant environmental and ecological impact of the taxonomic distribution of the bacterial communities at the phylum level, including plant growth-promoting bacteria (PGPB) (Alsaedi *et al*., 2022; Baeshen *et al*., 2020; Shailendra Singh, 2015). It has been reported that plant growth stimulation by rhizobacteria stimulates the evolution of the plant and this growth improvement is related to specific rhizobacteria features. Furthermore, PGPR employs various pathways to promote plant growth and development in different environments. PGPR influences the growth of plants either directly by providing nutrients (nitrogen, phosphorus, potassium, and essential minerals), modifying the levels of the plant hormone, or indirectly by eliminating the inhibitory impacts of different pathogens on plant growth and improvement as biocontrol agents, root colonizers, and environmental protectors (Shailendra Singh, 2015).

When compared between soil and rhizosphere bacterial communities, the rhizosphere is usually dominated by many bacterial lineages, with Actinobacteria and Proteobacteria being the major phyla. This was discovered for a variety of crop species, including maize, barley, rice, and grapevine (Bulgarelli *et al*., 2015; Hernández *et al*., 2015; Zarraonaindia *et al*., 2015; Niu *et al*., 2017). Furthermore, pioneer desert plants have intimate and frequently coevolved interactions with soil bacteria to acquire sufficient survival strategies under desert conditions (Marasco *et al*., 2012). Therefore, it was expected that the rhizospheric area would comprise bacteria to colonize, interact, and assist plants to tolerate abiotic challenges based on our observations and due to their high bacterial diversity. As a result, bacteria from the rhizosphere region of *S. italica* plants were isolated and studied further.

Due to Naylor and Coleman-Derr (2018), drought appears to have little impact on bacterial phylogenetic diversity for soil communities. Several PGP characteristics and abiotic stress tolerance capacities were assessed in the bacterial isolates. Bacteria from various phyla demonstrated several PGP properties and the ability to withstand drought stress (Eida *et al*., 2018). According to the sequencing results, the taxonomic distribution of the bacterial communities at the phylum level revealed 17 phyla. Furthermore, multiple investigations have revealed that these bacteria have various environmental effects. The most dominant phyla among the four different soil samples were Actinobacteria, which dominated soil. 3 associated with *S. italica*, while were less abundant in soil. 1. Similar to our results, Actinobacteria frequently present in the rhizosphere and the crust of the Z. album samples (Baeshen *et al*., 2020) due to their participation in the carbon, nitrogen, and sulfur cycles globally (Barka *et al*., 2016).

We observed a phylogenetic signal related to changes in community composition due to variations in soil moisture, which seemed to favor Actinobacteria. Actinobacteria are essential phylum for decomposing plants and deceased animals, which restores unregulated biomaterials. They play an important role in manufacturing antibiotics, with a strong resistance to UV radiation and dehydration (Baeshen *et al*., 2020). Actinobacteria are a globally distributed bacterial species with a well-studied ecology. They exhibit adaptive drought resistance and can be found in the highest abundance in arid soils. Actinobacteria have been shown to grow in dry soil at low osmotic potential and relatively increased prevalence (Bouskill *et al*., 2013).

The phylum Proteobacteria were highly abundant in the sample of soil. 1 and soil. 2 associated with *S. italica*, and less abundant in soil. 3. We found that drought conditions drastically reduced the relative abundance of Proteobacteria. Contrary to our findings, the study of (Jang *et al*., 2020) demonstrated that Proteobacteria appear to have the most essential function in rice survival under drought conditions and are able to support plants to overcome drought stress. The decreased soil Easily oxidizable organic carbon (EOC) concentrations in the drought plots could be the reason for this, as there is a strong link between Proteobacteria and soil labile organic carbon availability. Furthermore, in the absence of plants, Proteobacteria are dramatically reduced under drought conditions, a discovery consistent with the prior study indicating that Proteobacteria abundance is relatively low in water-limited desert soil (Bu *et al*., 2018). Due to their role in the global carbon, nitrogen, and sulfur cycles, they are known to be climate change sensitive and impact the soil biosphere (Bodenhausen *et al*., 2013; Zhao *et al*., 2018).

According to previous results, Bacteroidetes are sensitive to biological indicator for agricultural soil use, and they may have an antibacterial and antifungal performance relationship (Eida *et al*., 2018). Firmicutes are more abundant in control sample associated with *S. italica* and less prevalent in soil. 3. Other studies found that Firmicutes were more abundant in both of the Z. album samples (Baeshen *et al*., 2020). In addition, Firmicutes is a drought tolerant and could withstand the harsh conditions (Soussi *et al*., 2016).

Our results found that Chloroflexi had a higher abundance in the control sample associated with *S. italica* than in the soil. 3 sample. The study of (Noor *et al*., 2020) demonstrated that Chloroflexi existed in five medicinal plants. Chloroflexi is an essential and beneficial organism in drought-stricken soils and relies on photosynthesis to thrive in low-fertility soils (Steven *et al*., 2013). We found Acidobacteriait in a small number

compared with other samples. It is found with more abundance in soil. 3 than in the control sample. Acidobacteria in soil linked with the decomposition of soil organic matter (SOM) have been reported implying their importance in the carbon cycle (Kalam *et al*., 2020).

Deinococcus-Thermus was observed in the samples of soil. 1 and soil 2 connected with *S. italica,* with less occurrence in soil. 3 sample. In contrast, Deinococcus-Thermus were significantly prevalent in the control samples in addition to both of the *H. perfoliata* samples (Baeshen *et al*., 2020). Similar to our findings, Deinococcus-Thermus were also known to be highly resistant to harsh environmental stresses and radiation (Theodorakopoulos *et al*., 2013).

The unclassified bacteria at the phylum level were found with 2.42% in the soil. 3 associated with *S. italica*. The emergence of the unclassified bacteria at the phylum level may be due to the lack of a reference sequence in the database, and these bacteria might include a potential candidate that is still unexplored.

Several studies have proved the bacteria's benefits in agricultural, environmental, medical, and industrial uses at the genus level. For example, Microvirga, the most abundant genus in soil samples, belongs to the Proteobacteria, which can improve soil nutrients, promote plant growth, and control soil-borne diseases (Wang *et al*., 2017). In addition, Microvirga may fix nitrogen; however, biochemical information about this genus is currently limited (Li *et al*., 2020).

Arthrobacter (Phylum: Actinobacteria) was detected in a soil sample and frequently used in many applications. Its unique functions have been widely recognized by reputable research institutions in fields, such as agriculture, medicine, industry, and the environment. It should also be noted that some types of arthropod bacteria accelerate the functions of insecticides, the production of beneficial enzymes and other processes (Fu *et al*., 2014). Additionally, Arthrobacter plays a crucial function in the nitrogen cycle in rhizospheric burnt soils, therefore it could help the transition from oligotrophic to copiotrophic conditions. We can expect to learn more about the environmental functions, applications, and plans for this type of ecological environment in the future. Biological phosphorous removal has become increasingly popular in recent years, bypassing other methods. Among these bacteria, Arthrobacter sp. had the greatest ability to accumulate phosphorous (Fu *et al*., 2014). Adhaeribacter and Flavisolibacter are two genera found in the soil sample under the Bacteridets.

Adhaeribacter are Gram-negative, non-motile, chemo-organotrophic bacteria that produce many extracellular fibrillar material (Zhang *et al*., 2009). Flavisolibacter are also Gram-negative, non-motile, deep yellow, rod-shaped bacteria and radiation resistant (Lee *et al*., 2016).

Conclusions

This study shows the diversity of the microbial communities in the soil associated with stressed desert plants in Asfan, northern Jeddah, Saudi Arabia. Our findings demonstrated that rhizospheric bacteria could be used as indicators of plant growth rate and survival ability in hostile environments. This distinctive feature was found among all members of the phylum presented in the study. Two species of the genus Adhaeribacter and Flavisolibacter were found to be elevated, but we had no previous reports of their role in mitigation. Abiotic factors deserve attention in future studies, which will help researchers better comprehend new candidates for biological agents employed to improve agricultural and industrial processes. In addition to using high-throughput molecular tools to identify soil bacterial communities for taxonomic and phylogenetic characterization, future detailed representation and comparative functional and biochemical studies for the diversity of the soil microbiome are needed to highlight different metabolic pathways. Furthermore, future correlations between taxonomic composition and functional aspects of the soil microbiome will aid in discovering novel prospective candidates for improving humanity's and its resources' fates.

Authors' Contributions

Conceptualization (A.M.A ,R.S.J, Z.S.A, M.N.B, RA.A); Data curation(A.M.A, R.S.J, A.Y.S, M.A.M, Z.S., AL.B, R.A.A); Formal analysis (A.M.A, R.S.J, A.Y.S, M.A.M, Z.S., AL.B, R.A.A); Funding acquisition(A.M.A, R.S.J, A.Y.S, M.A.M, Z.S.A, L.B, M.Y.R, M.N.B); Investigation; (A.M.A, R.S.J, A.Y.S, M.A.M, Z.S.A, L.B, M.Y.R, M.N.B); Methodology(A.M.A ,R.S.J, Z.S.A, M.N.B,N.M.B, A.A.H, RA.A); Project administration; Resources(A.M.A ,R.S.J, Z.S.A, M.N.B,N.M.B, A.A.H, RA.A; Software (A.M.A,M.A.M,Z.S.A); Supervision(R.S.J, M.N.B, R.A.A); (A.M.A, R.S.J, A.Y.S, M.A.M, Z.S.A, L.B, R.A.A) Validation; Visualization; Writing - original draft(A.M.A ,R.S.J, Z.S.A, RA.A);; Writing - review and editing (A.M.A ,R.S.J, Z.S.A, RA.A).

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R31), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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