METAGENOMIC PROFILING OF MICROBIAL COMMUNITY IN SALINE SOIL OF MADINAH, SAUDI ARABIA

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Abstract. For optimal agricultural and other soil utilization, microbial structure analysis is essential. Sabkhat Madinah, Saudi Arabia, boasts high-salinity soil and thriving vegetation. The microbial makeup of the soil in this area is novel and has not been extensively studied. This study aimed to analyze the microbial structure of two locations in Sabkhat Madinah's soil and examine the correlation with soil properties. Soils microbiota were investigated using Illumina sequencing of bacterial 16S rRNA genes. The soil chemical properties were determined. The microbial composition of both sites was investigated at different taxonomic levels. There was a significant difference in salt concentration between the two sites derived from the same sabkha. The second sample had higher sodium content, leading to increased electrical conductivity (EC) measures. Moreover, the samples had different microbial compositions. The first sample was dominated by Bacteroidetes (18.37%), Firmicutes (13.57%), Proteobacteria (13.57%), and Actinobacteria (9.30%), while the second one contained Bacteroidetes (8.96%), Proteobacteria (25.01%), Actinobacteria (12.03%), and Firmicutes (11.36%). Firmicutes were newly recorded and found only in saline habitats in Saudi Arabia. Acidobacteria, Thermodesulfobacteria and Streptophyta were only in the first sample, while Verrucomicrobia sequences were identified only in the second sample which had higher salt content. On the genus level, 16 genera were found across both samples, with *Bacillus* being the most prevalent at 5.17% followed by *Marinoscillum* (4%), *Fibrobacter* (3.57%) and *Rubrobacter* (3.45%) in the first sample. The second soil sample had a dominant genus, *Halomonas*, making up 10.64% of the total sequences. Other genera present included *Fibrobacter* (3.96%), *Nitrospira* (3.92%), *Rubrobacter* (3.36%), and *Methylophaga* (3%). To conclude, the analysis of bacteria in the two sites revealed notable differences in soil properties and bacterial diversity. This study provides insight into the complex microbial compositions of saline soil and a foundation for future studies. Understanding these bacteria' genetics can improve soils or help crops thrive in saline environments.
Keywords: *16S rRNA gene, Illumina MiSeq, saline soil, diversity*

Introduction

The structure of soil is open, and it is always interacting with all the different parts of the ecosystem, such as the atmosphere, biosphere, hydrosphere, and lithosphere (Lin, 2010; Huggett, 2021). Soil composition is constantly changing due to natural geological processes like rock weathering, and winds, as well as human activities such as poor agricultural practices. These factors affect the physical and chemical properties of the soil (Sun et al., 2018; Costantini and Mocali, 2022).

The top layer of soil contains a microbiome that provides nourishment for all forms of life that grow on the soil. This microbiota, along with mineral components, creates living communities of bacteria, lichens, fungi, and algae known as biological soil crusts or biocrusts (Rodriguez-Caballero et al., 2018). The intricacy of soil surroundings can differ significantly based on external factors (Bebber and Richards, 2022). However, it is approximated that every gram of soil is home to six million bacteria, which belong to roughly 20,000 distinct species (Vitorino et al., 2018).

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Soil can be negatively affected by changes in its chemical composition which can cause an imbalance of ions, leading to reduced productivity and compatibility with living organisms. Salinity, which is the concentration of dissolved salts in the soil, is one such property that can be affected. Natural processes can cause primary salinization, while human activities are responsible for secondary salinization (Shrivastava and Kumar, 2015). Soils that have a high concentration of sodium ions, with NaCl levels above 40 mM and exchangeable sodium levels over 15%, are referred to as saline or salt-affected soils. Saline soils are also characterized by their electrical conductivity, which is above 4 dsm^{-1} (Shahid et al., 2018a). There are four categories of soil salinity based on the amount of salt accumulation: slight, moderate, severe, and very severe. In the Gulf region, particularly along the waterways, there are highly saline soils that have been historically referred to as sabkhas. These soils are characterized by excessive salt buildup that forms calcified land covers (Shahid, 2018b).

It has been believed that the sabkhas in the Middle East were created during the Late Pleistocene era, a time when the Gulf waters had submerged these areas. The following ice age caused the waters of the gulf to withdraw, which exposed the lands and created flatlands and depressions with salt deposits. Later on, the Arabian Gulf waters increased, leading to the submergence of the lands once again. At this particular phase, the combination of seawater and land silts was believed to have resulted in the creation of marine sediments, which is currently evident in the distinctive calcium sediments and clays found in sabkhas. Apart from these ancient processes, the movement of wind had caused the secondary accumulation of minerals and sediment concentration in sabkhas. Due to environmental factors such as high temperatures and low rainfall, modern-day sabkhas have formed in arid climates (Al-Amoudi and Abduljauwad, 1995). Throughout Saudi Arabia, sabkhas are prevalent in various areas, including the east and west coasts and the middle region (Parimalarenganayaki, 2021). The west Red Sea coastal strip spans from 16 to 65 kilometers (FAO, 2021) contains several saline areas such as Jizan, Al-Layth, Jeddah, Abhur, and Yunbu (Al-Mhaidib, 2010).

The impact of salinity on microbial communities in saline soils is not yet fully understood, possibly because there are not enough studies on saline soil systems (Xie et al., 2017). Further research is required to fully comprehend the microbial variety and process in saline soils (Rath et al., 2019). Discovering the variety of microbial communities in saline soils is important for assessing their potential for agricultural or other economic purposes. The use of saline-resistant microbiota to assist the growth of other flora in these challenging environments has gained increasing attention in recent years. For example, within dry, salty environments, clusters of microbes in the soil rhizospheres produce valuable fertilizers and metabolites that aid in the plant's ability to withstand both natural and environmental challenges (Alsharif et al., 2020). In turn, plant growth-promoting bacteria can be introduced into soil to stimulate the growth of plants (Mus et al., 2016). A recent study found that introducing deep-sea actinobacteria, which can tolerate high levels of salt, into hypersaline soils helped to stimulate the growth of tomato seedlings that would not have grown otherwise. The same bacteria were also found to prevent the buildup of harmful compounds like hydrogen peroxide in the tomato seedlings' leaves (Rangseekaew et al., 2021).

Traditional methods for cultivating microorganisms are restricted, resulting in less than 1% of them being able to grow in laboratory settings. However, in recent years, metagenomics has revolutionized the analysis of microbial communities in various environments (Nam et al., 2023). Approaches to metagenomics are divided into two

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categories: targeted and shotgun genomics, depending on the segment of the amplicon or gene used to determine the organisms' phylogenetic classification (Kamble et al., 2020). Unlike the analysis of single types of bacteria, metagenomics allows for the examination of microbiota straight from the soil, which helps in comprehending bacterial networks, their interactions with one another and the surroundings. Furthermore, metagenomics allows for the analysis of microbiota communities and how they change over time and space. This information can be used to predict how human activities may impact these communities in the future (Berg et al., 2020).

The knowledge gained from metagenomic research on soil microbiomes is being applied to enhance agricultural methods and preserve plant and animal life in different habitats (Cullen et al., 2020). However, there is a lack of metagenomic information available from environments in Saudi Arabia according to Alzahrani (2021). Based on this, this research was proposed to explore the microbial communities present in two saline soil sites in Madinah using a metagenomic approach. In addition, it was aimed to compare the diversity of microbial populations in these two locations and identify potential causes for any variations observed.

Materials and methods

Soil sample collection

In July 2020, samples were collected from two different locations (Sabkhat) in the northern part of Madinah, Saudi Arabia in accordance with the methodology of Li et al. (2016). To avoid the influence of grass roots, the locations had no grass cover (*Fig. 1*). Prior to sampling, stones, salt crusts, and roots were removed completely. Using a metallic auger, subsurface soil samples were collected at a depth between 15 and 20 cm. Soil samples from each site were mixed thoroughly and placed in sterile plastic bags. Following collection and preparation, the samples were stored at -80°C until used.

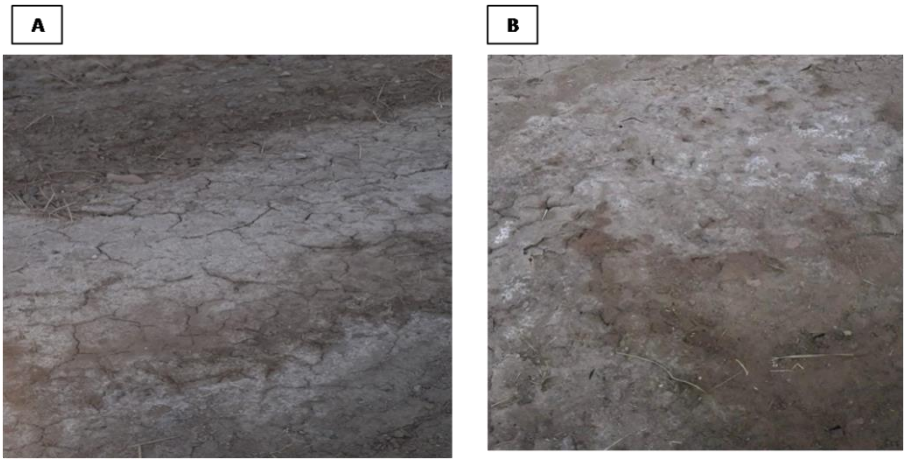

Figure 1. *Site of sample1 (A) and sample 2 (B) at Madinah, Saudi Arabia*

Chemical analysis of soil

The properties of the soil samples were analysed using several analytical procedures. The pH of a 1:2.5 (w/w) aqueous solution was measured using a pH meter (PHS-3C;

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INESA, Shanghai, China) (Pansu, 2006), while EC of a 1:5 aqueous solution was measured using a conductivity meter (FE-30; Mettler Toledo, Switzerland) (Jackson, 1973). Calcium and magnesium were determined volumetrically using the versant method with ammonium perpetuate as an indicator and soil added to Calcium Eriochrome black T. Sodium and potassium were detected photometrically, and carbonates and bicarbonates were analysed volumetrically (Jackson, 1973). Total carbonate was determined volumetrically using the Collins Calcimeter and calculated as calcium carbonate percentage (Piper, 1950). Soluble chlorides were determined by titration with 0.005 N silver and potassium chromate as an indicator. Sulphate ion in soil and water extract (1:1) was determined using an apparatus outlined by Jackson (1973). Total organic carbon (TOC) was estimated using a modified method of Allison (1965), while available phosphorus was extracted using 0.5 N $NaHCO_3$ procedures according to Olsen et al. (1954) and colorimetrically measured using an ascorbic acid-molybdenum blue method at wave length of 406 nm as described by Murphy and Riley (1962). The Nessler method was used to determine available nitrogen (Pratt and Chapman, 1961), and the available potassium was measured by the Flame Photometer in accordance with Jackson (1973).

DNA extraction

Total DNA was extracted from selected samples using PowerSoil® DNA Isolation Kit according to the company instructions. After DNA extraction, the quantity and quality of the purified DNA was measured using a Nanodrop spectrophotometer (Thermo Scientific) until subsequent analysis.

Amplification and sequencing of the bacterial 16S rRNA gene

The composition and diversity of bacterial communities in soil were determined by amplifying the bacterial hypervariable V3–V4 region of the 16S rRNA genes. A set of universal primers 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (CGG TTA CCT TGT TAC GAC TT) was used (Weisburg et al., 1991). The PCR process was carried out using a total volume of 25 μL, with 2.5 μL (25 ng) of DNA placed in a PCR tube and 22.5 μL of a master mixture containing Taq polymerase, dNTPs, reaction buffer and $MgCl_2$. The entire mixture was subjected to an amplification reaction in a thermal cycler (GeneAmp 9700 thermocycler, ABI-USA). PCR conditions were: initial denaturation for 5 min at 95°C, 30 cycles with 1 min at 94°C, 50 s at 55°C, 1 min at 72°C, and final extension for 5 min at 72°C. The PCR products were visualized on agarose gel electrophoresis (2% in TAE buffer) containing ethidium bromide according to the standard method. The Illumina MiSeq 2×250 pb at Macrogen (Seoul, Korea) was used to sequence the purified products. Raw 16S rRNA reads were received in FASTQ (FASTA with quality information) format.

Metagenomic analysis

MG-RAST

The FASTQ files containing metagenomic data were processed and analysed using MG-RAST (v.4.0.3) as described by Meyer et al. (2008). Amplicon metagenomic reads were filtered and trimmed. Each sample was pre-processed to remove sequences with length less than 100 bp with minimum average quality score < 30. Reads with ambiguities and barcode mismatch were discarded. Reads were assigned to operational taxonomic

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units (OTUs) using *de novo* assembly. Consequently, BLAST search was run to find out closest matches and sequence classifier tool was used to determine the taxonomic distribution of soil microbes.

QIIME2

Following 16S rRNA gene amplicon sequencing, taxonomic assignment and downstream statistical analyses were performed on raw reads using QIIME (v.2019.1) (Caporaso et al., 2011), in order to compare OTUs with amplicon sequence variants (ASVs) in marker gene-based amplicon data analysis. Reads were denoised, assembled to one single read and clustered into representative sequences using DADA2. QIIME2 pipeline continues with determining a taxonomy based on the SILVA reference database (Bolyen et al., 2019). Subsequently, QIIME2 calculated various diversity criteria such as alpha diversity, evenness, phylogenetic diversity, and beta diversity.

PICRUSt

PICRUSt was used for functional prediction of the microbiome 16S rRNA gene sequences (Parks et al., 2014). MetaCyc pathway abundances are calculated in PICRUSt2 through structured mappings of EC gene families to pathways.

Results

Properties of soil samples

Table 1 presents the results of the analysis of soil samples to identify their chemical characteristics, such as the minerals present and the percentage of organic matter. The pH values of the soil samples were slightly alkaline and ranged from 7.8 to 8. EC analysis revealed that it was varied between sample collection sites. In one site, the EC was 7.6 ds/m, whereas it was 13.36 ds/m in another site. The two samples were typically sandy, with low concentrations of organic matter. Upon analysing the mineral and ion composition of the soils, it was found that there was an accumulation of minerals including nitrogen, phosphorous, and potassium. Additionally, there were cations present such as calcium, magnesium, sodium, and potassium, as well as anions including hydrocarbonate, chloride, and sulfoxide. These results confirmed that the soils have a saline nature.

Microbial community profiling

Metagenome analysis of the two soil samples using MG-RAST

The DNA samples collected from the soil were analysed using the MG-RAST platform for processing and OTU clustering. The first sample contained 113,699 sequences with an average length of 301 bps, totaling 34,223,399 base pairs. About 94% of the sequences were predicted, while 6% were unknown. Only 25 sequences (0.02%) did not pass the QC pipeline. Similar results were obtained for sample 2, as shown in *Fig. 2*.

Analysis of the MG-RAST results in the following quality outputs showing a higher average quality after trimming of the reads for sample 1 compared to sample 2. Most of the reads for sample 1 can be assigned to a feature (93.6%), while 6.4% of the reads do not seem to contain features. A similar result is obtained for sample 2 expressing feature and unfeatured reads by 93.8% and 6.2%, respectively.

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Table 1. *The properties of soil samples collected for the study*

Parameter	Soil sample 1	Soil sample 2
pH (1:2)	8	7.8
EC (1:2 WATER EXATRACT) (ds/m)	13.36	7.6
TOC (%)	0.37	0.29
$CaCo_3$ (%)	2.6	3.1
Ca^{2+} (mEq/L)	5.06	3.04
Mg^{2+} (mEq/L)	25.4	22.8
Na^{+} (mEq/L)	101.7	49.4
K^{+} (mEq/L)	1.8	1.3
HCO_3 (mEq/L)	4	4
Cl^{-} (mEq/L)	58	32
SO_4^{2-} (mEq/L)	66	38
N (mEq/L)	26.5	30
P (mEq/L)	21.1	20.9
K (mEq/L)	550	950

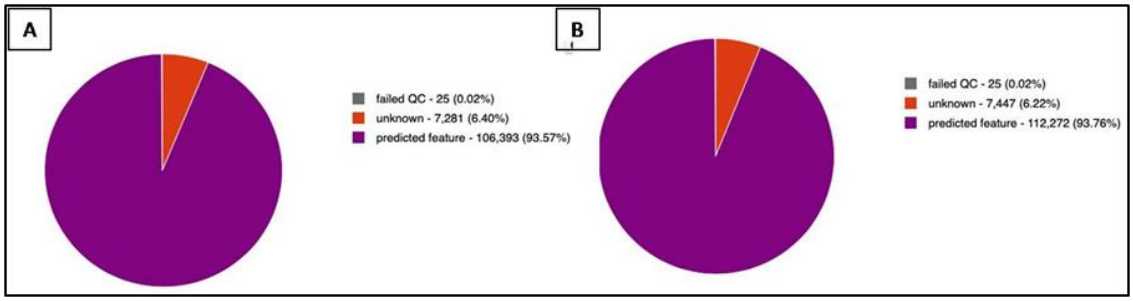

Figure 2. *Sequence breakdown for sample 1 (A) and sample 2 (B)*

Analysis of the MG-RAST results in the following quality outputs showing a higher average quality after trimming of the reads for sample 1 compared to sample 2. Most of the reads for sample 1 can be assigned to a feature (93.6%), while 6.4% of the reads do not seem to contain features. A similar result is obtained for sample 2 expressing feature and unfeatured reads by 93.8% and 6.2%, respectively.

The sequences analysis indicated that bacteria was the prominent species in the two soil samples, making up approximately 96% and 98% of the total species, respectively. The remaining percentages were made up of archaea and eukaryota, with sample 1 containing 1.8% and 1.2% of each, respectively, and sample 2 containing 1.1% and 0.54% of each, respectively. A significant portion of the unclassified sequences derived from bacteria in sample 1 and 2 were estimated to be 23.5% and 33% of the total sequences.

The soil sample1 was mostly composed of Bacteroidetes (18.37%), Firmicutes (13.57%), Proteobacteria (13.57%), and Actinobacteria (9.30%). In sample 2, Proteobacteria (25.01%), Actinobacteria (12.03%), Firmicutes (11.36%), and Bacteroidetes (8.96%) were the dominant phyla.

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Sample 1 was mainly composed of *Bacillus*, *Pseudomonas*, *Clostridium*, *Saccharopolyspora*, *Gemmatimonas* and *Salinibacter* bacterial taxa. Some of these genera, such as *Sphingobium* and *Halomonas* from Proteobacteria, and *Salinbacter* and *Rhodothermus* from Bacteroidetes, were halotolerant. On the other hand, the dominant genera in sample 2 were mainly *Halomonas*, *Fibrobacter*, *Nitrospira*, *Rubrobacter*, and *Methylophaga*. Sample 1 had four genera, including *Coprothermobacter*, *Candidatus*, *Koribacter*, *Verticillium*, and *Halomonas*, while sample 2 had *Prevotella*, *Slackia*, and *Salinibacter* (*Figs. 3* and *4*).

Metagenome analysis of the two soil samples using QIIME2

The two soil samples were further analysed using the QIIME2 pipeline, using DADA2 as denoising algorithm. The two samples were found to contain high quality reads as in the heat map. After merging, 54,433 reads were non-chimeric for sample 1 and 50,660 reads were non-chimeric for sample 2, which represent 100% of the total reads. These reads will be the basis for further taxonomic and functional inference.

Amplicon sequence variants (ASVs) using QIIME2 revealed noteworthy differences with OTU performed by MGRAST platform. Due to enormous amount of data, the interactive results are available at the following link https://nawat-md.com/Hasanalbahri/qiime2/barplot/.

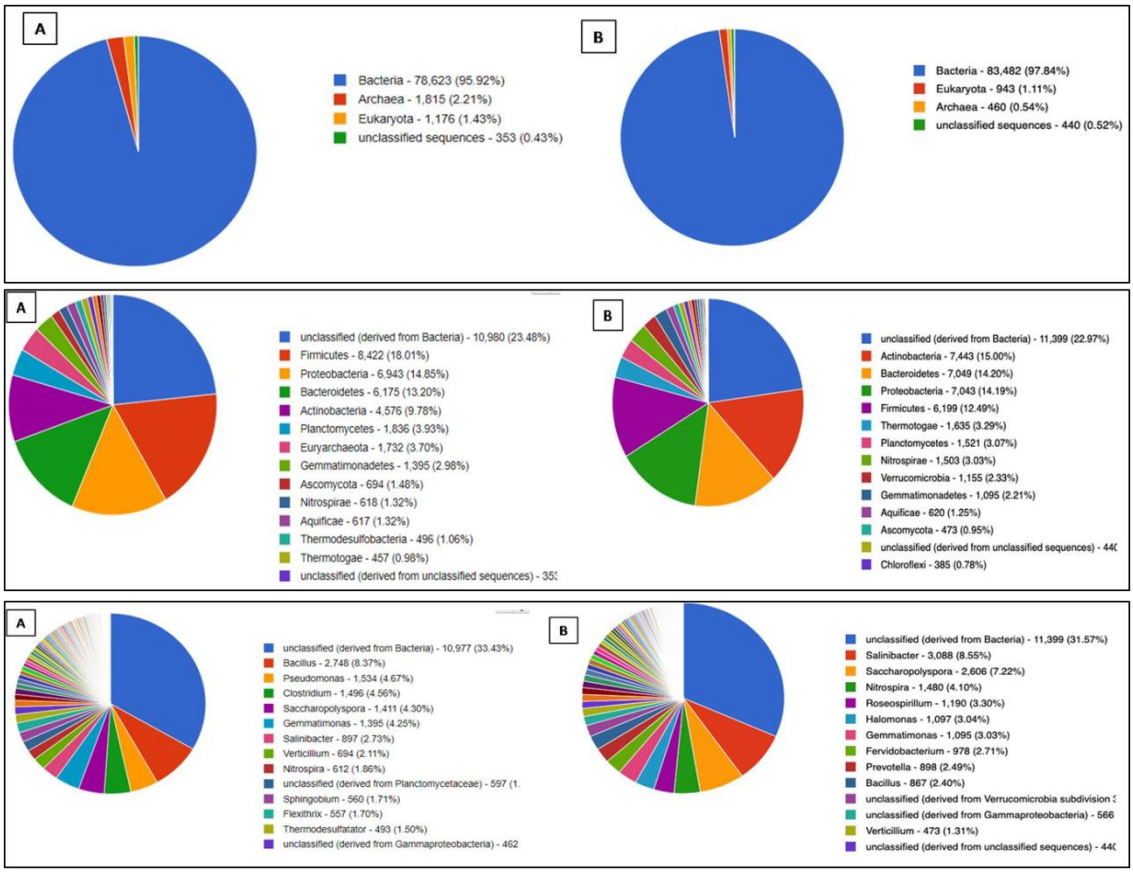

Figure 3. *Relative abundances of bacteria at the domain, phylum, and genus level in sample 1 (A) and sample 2 (B)*

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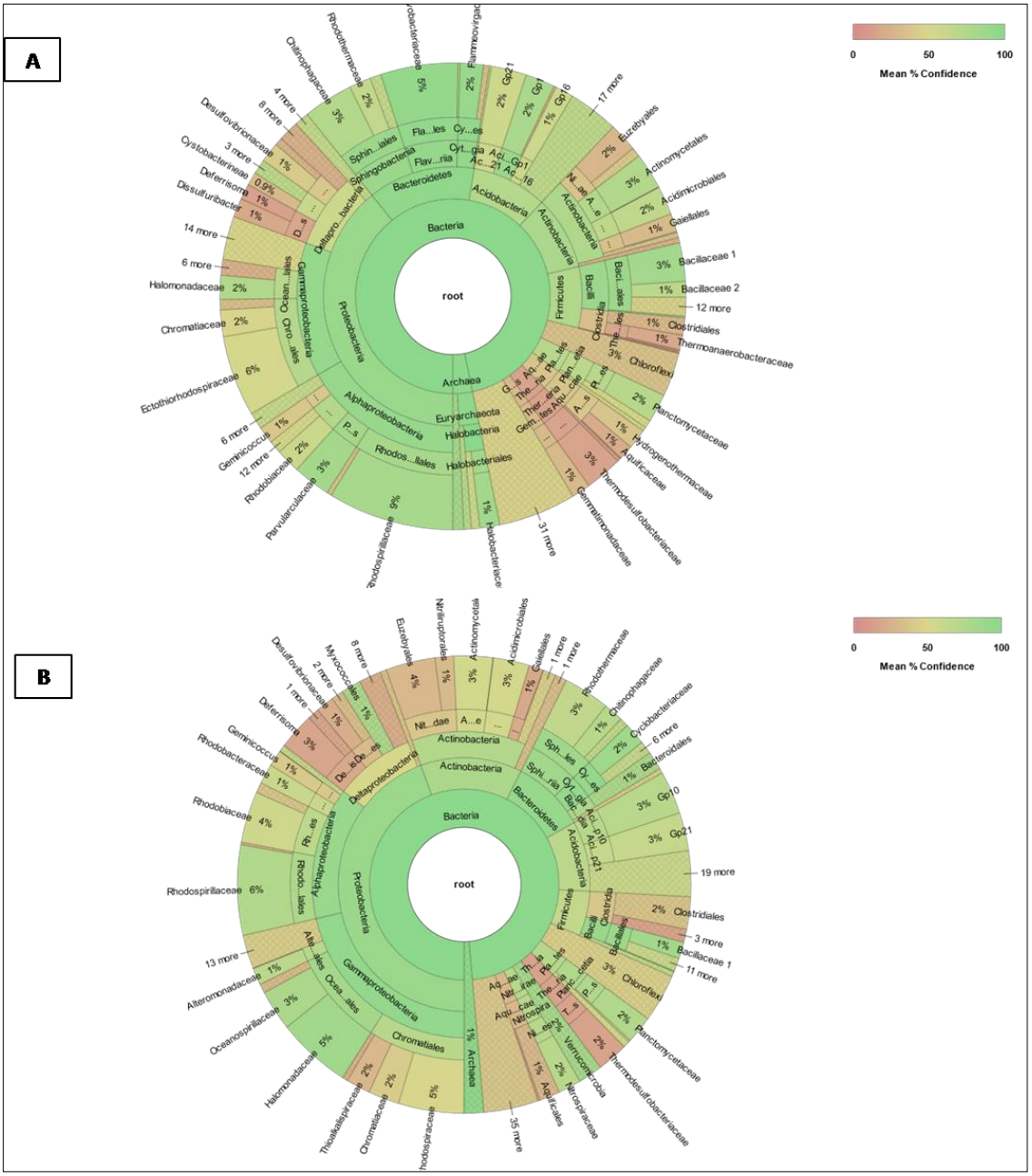

Figure 4. *Bacterial communities among the samples. Sample 1 (A) and sample 2 (B)*

The bacterial population percentage increased to reach 97.7%, which in turn reduced archaea to 2.3 in sample one. In sample 2 the bacterial population reaches 98.4, to raise the percent of archaea to 1.6 compared to 1% for the MG-RAST OTU analysis.

Keeping the relative ratios for different phyla, the percentages were slightly different between OTU and ASV analyses. In sample 1, the bacterial population 33.4% belongs to phylum Proteobacteria, Bacteroidota (Bacteroidetes, 15.5%), Gemmatimonadota (Gemmatimonadetes, 9.7%), Actinobacteriota (Actinobacteria, 6.2%), Acidobacteriota (7%), Chloroflexi (6.8%), Firmicutes (4.3%), and Myxomycoccota (2.5%). 38.2% of the bacterial population for sample 2, belongs to the phylum Proteobacteria, Bacteroidota (Bacteroidetes, 9.7%), Gemmatimonadota (Gemmatimonadetes, 8.4%), Actinobacteriota

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(Actinobacteria, 10.3%), Acidobacteriota (9%), Chloroflexi (6.2%), Firmicutes (1.9%), and Myxomycoccota (2.2%).

On the genus level, *Halomonas* and *Marinobacterium* were the predominant genera in sample 1 represented by 6.5% and 3.8% respectively. In sample 2 the ratio of both genera decreased to 1.8% and 0%, respectively. On the other hand, *Sinomicrobium* and *Pelangbius* were the dominant genera in sample 2 representing 6.7% and 4.4% of the bacterial population. Such genera were completely absent in sample 1. In addition, genera belonging to the order Actinomarinales and family Rhodothermiaceae represented by 4.8% and 4.1% in sample 1, 1.7% and 1.6% in sample 2, respectively. Halobacterota and Nanohaloarcheota represented the dominant Archaea by 1% and 0.5% in sample 1 and completely absent from sample 2. Thermoplasmatota were present in sample 2 with higher percent than sample 1, 1% and 0.5% in the same respect. Chenarcheota was only present in sample 2 (0.3%).

Phylogenetic reconstruction of soil metagenome

The phylogenetic trees in *Figs. 5* and *6* illustrate the genetic distance between different bacterial and archaeal species found in the closest BLAST search. One clade contains the closest relative, while those with greater genetic variation are located in farther clades.

The diversity and richness of the microbial community

Alpha diversity (the variance within one sample) analysis exhibited a notable difference between the two samples which indicate a significant variation in their metagenome structure. The number of taxa present in the first sample was higher as indicated by the greater number of the observed features. Shannon's index indicated more abundance and evenness of the taxa present in sample one compared to sample 2. The evenness value was confirmed by Heip's evenness measure. Moreover, more phylogenetic diversity was also obtained for the first sample. Faith's phylogenetic diversity measures of biodiversity that incorporates phylogenetic difference between species as the sum of length of branches (*Table 2*).

Table 2. *Shannon's index, Heip's evenness, and Faith's phylogenetic diversity of sample 1 and sample 2*

	Obs. features	**Shannon's index**	**Faith's phylogenetic diversity**	**Heip's evenness**
Sample 1	148	6.81	13.21	0.945
Sample 2	129	6.62	12.56	0.943

The values of beta diversity (the variance between multiple samples) indicated that the two samples are completely different from each other in spite of their close locations from the same sabkha (*Tables 3* and *4*).

Table 3. *Bray-Curtis dissimilarity fraction of over abundant counts of sample 1 and sample 2*

	Sample 1	**Sample 2**
Sample 1	0	0.9958
Sample 2	0.9958	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Table 4. *Jaccard distance: Fraction of unique features, regardless of abundance of sample 1 and sample 2*

	Sample 1	**Sample 2**
Sample 1	0	0.9963
Sample 2	0.9963	0

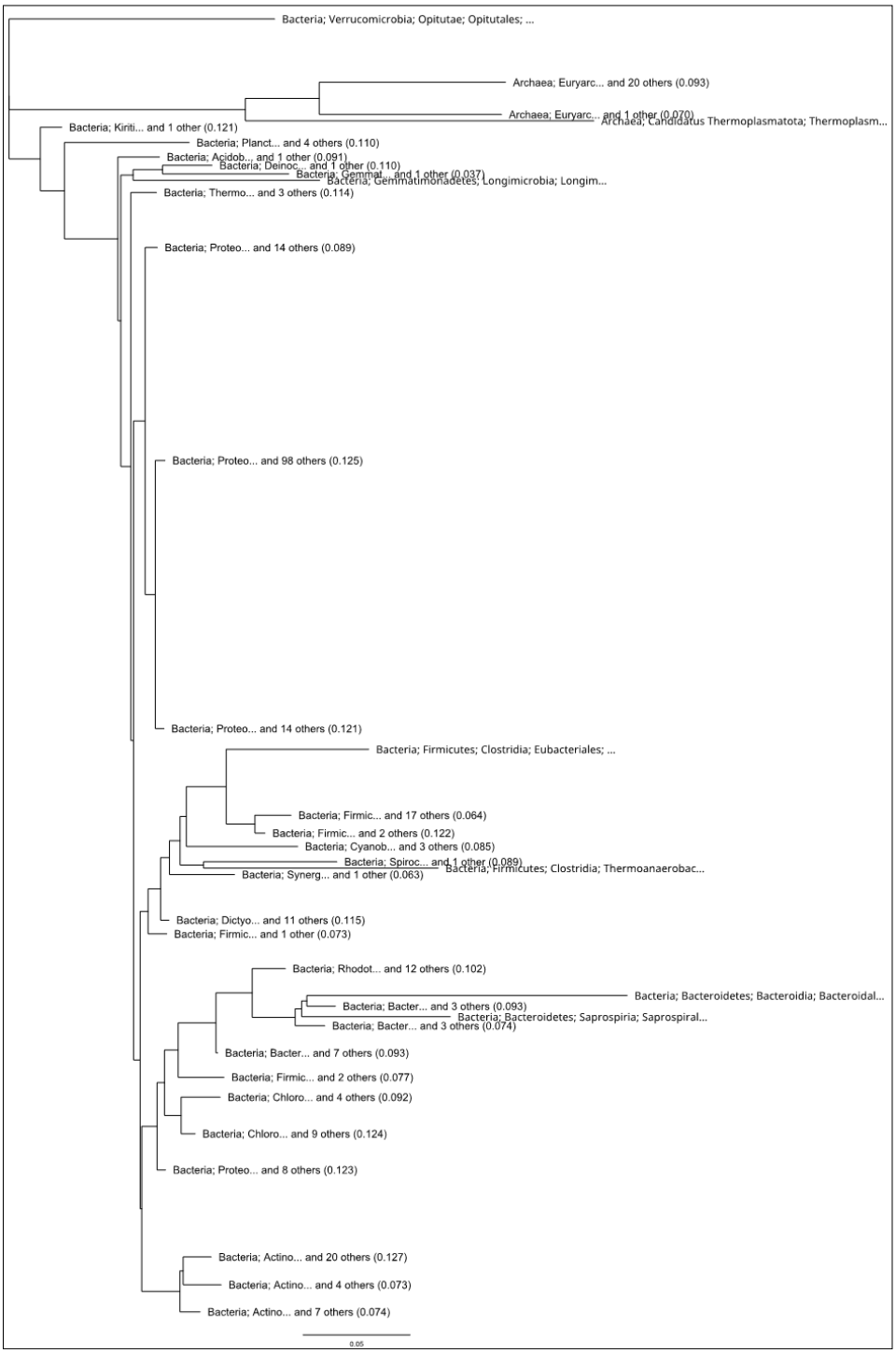

Figure 5. *Phylogenetic tree of the microbial composition of sample 1*

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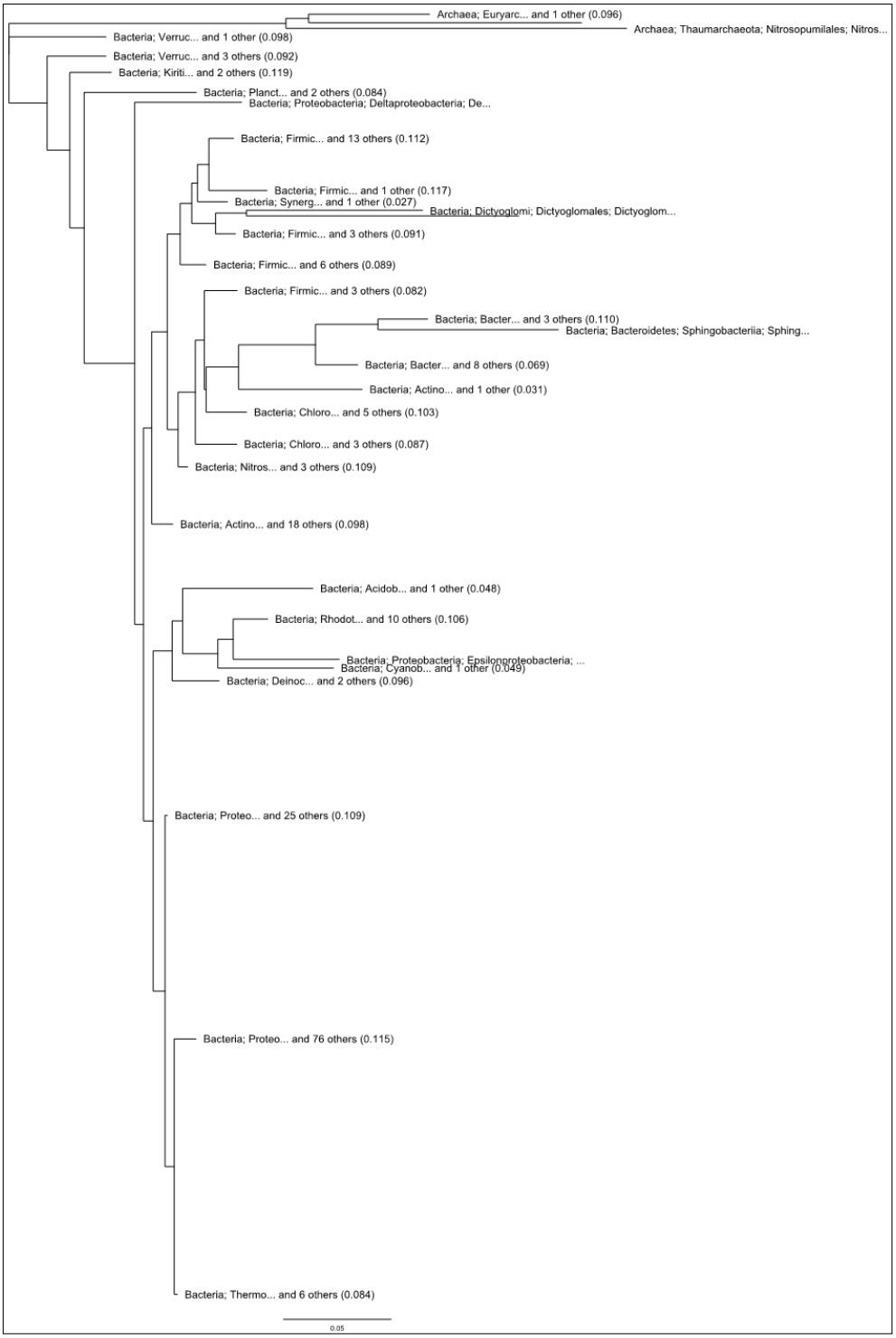

Figure 6. *Phylogenetic tree of the microbial composition of sample 2*

Metabolic function profiling

PICRUSt 2 was used to infer the metabolic pathways present in the two soil samples. According to the data in the top enzyme activities in sample 1 were recorded for Salicylate 5-hydroxylase, Succinyl-CoA--L-malate CoA-transferase, tRNA (guanine (6)-N(2))-

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methyltransferase, 6-hydroxynicotinate 3-monooxygenase, N-acyl-D-glutamate deacylase, and Mandelamide amidase. On the other hand, sample 2 enzyme activities were completely different with the highest levels for Hydroxybutyrate-dimer hydrolase, Phosphonate dehydrogenase, N-acetylphosphatidylethanolamine-hydrolyzing phospholipase D, Nucleoside diphosphate phosphatase, and Glutaconyl-CoA decarboxylase.

Discussion

Soil salinization, especially in desert ecosystems, is a significant global threat that endangers soil viability (Bünemann et al., 2018). While various environmental factors contribute to this phenomenon, human activities have accelerated soil salinization. Unfortunately, this problem is expected to continue, and by 2050, half of the world's agricultural lands are likely to become non-arable (Shrivastava and Kumar, 2015). Soil salinity has been a persistent problem in desert ecosystems like the Middle East, including Saudi Arabia, where varying degrees of soil salinity have severely impacted agriculture and biodiversity (Abbas et al., 2013).

Despite being perceived as unproductive, sabkhas have been found to be viable for agriculture in various projects (Al-Barrak and Al-Badaw, 1988). However, some researchers suggest that irrigation practices aimed at transforming arid ecosystems into farmland may have led to increased salinization (Elhadj, 2004). Indeed, these areas have received less attention from environmental researchers, but their potential for cultivation should not be underestimated. The microbiome of soil offers a valuable opportunity to improve soil fertility. The ecosystem that inhabits the soil greatly influences its fertility, and the microbiome plays an important role in developing and maintaining physical characteristics and chemical composition. These bacteria not only help maintain the soil's content through metabolic repertoires, but also aid in the growth of plants and facilitate nutrient cycling to sustain other life (Dubey et al., 2019). However, our understanding of these microbes is limited and not fully explored (Bashir et al., 2014).

The emergence of metagenomics provided a way to sequence and identify a large quantity of microbial diversity in environmental samples. Consequently, this has resulted in the identification of unique compounds within the soil and plant metabolites that are now being utilized in various fields such as agriculture, industry, and healthcare (Jansson and Hofmockel, 2018). Limited research has been conducted on microbiome communities in salty desert habitats, particularly in Saudi Arabia. Hence, this study aimed to identify microbial communities in the sabkhas of Madinah using a metagenomics approach. Alotaibi et al. (2020) identified some strains of salt-resistant fungi like *Fusarium, Alternaria, Chaetomium, Aspergillus, Cochliobolus* and *Penicillium* in the sabkhas of Saudi Arabia, however, the bacterial communities in these areas remain largely unexplored. Thus, this study was carried out to contribute new insights to the field.

Upon analyzing soil samples collected from the Madinah sabkha in this study, it was confirmed that the soil was saline in nature. The pH of the soil was found to be slightly alkaline, around 8, which is typical of both saline and saline-sodic soils (Shahid et al., 2018a). EC analysis showed that it varied between the different sample collection sites, however, according to FAO categorization, an EC exceeding 4 ds/m confirmed the soil was saline (FAO, 2021). The total soil salinity and concentration of individual elements also varied between the different sites. Interestingly, the chemical composition of the soil in Madinah sabkha differed significantly from previous studies conducted on non-saline

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regions of Saudi Arabia, such as Al-Ahsa (Al-Barrak and Al-Badawi, 1988). EC of the soil obtained from this study was similar to that of soil collected from Skaka city, which had become saline due to human activities (Al-Hassoun, 2007). However, there were significant differences in the EC values and the presence of bacterial isolates compared to the results of a study conducted by Alotaibi et al. (2020). They studied soil from various regions in Saudi Arabia, including Madinah province. However, they did not analyze saline soils, which could explain the differences observed. Sabkha soil, on the other hand, showed evidence of nutrient accumulation, pH changes and EC due to salt buildup.

Upon analyzing the sequences in samples taken from sabkhat Madinah soils, it was found that the soil is home to a diverse array of microorganisms. Bacteria were the most common microorganism present, accounting for approximately 96% of the microbiota in the sample. It is worth noting that these findings align with previous studies conducted on soil samples in Saudi Arabia. However, it is important to note that previous studies have identified over 203 fungal species in sabkha soils throughout Saudi Arabia, whereas a lower proportion of fungus was found in these Madinah sabkha soils. Interestingly, researchers have found that the proportion and abundance of fungal isolates varies with the altitude of the soil, with a lower proportion of fungal communities found in the high-altitude Madinah province (Alotaibi et al., 2020).

The soil in sabkhat Madinah contains five main groups of bacteria that are abundant: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Halobacteriales. These findings were in accordance with Ayangbenro and Babalola (2021). They reported that Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Cyanobacteria are most frequently found in various saline and xeric soils. Moreover, Baeshen et al. (2020) studied the microbial diversity found in soils associated with halophytes located in Jeddah, Saudi Arabia. Results indicated that the most common phyla were Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Deinococcus-Thermus, and Gemmatimonadetes. Prior studies in southwestern highlands of Saudi Arabia have also found similar bacteria compositions, including Proteobacteria, Actinobacteria, and Acidobacteria (Yasir et al., 2015). Khan and Khan (2020) identified the presence these phyla in the arid soil of Saudi Arabia. More significantly, a number of studies have demonstrated the various advantages these bacteria have for the environment and ecology, including the ability to promote plant growth.

Bacteroidetes are found in many desert soils worldwide. These bacteria have been found in deserts across China, Pakistan, Northern America, India, and the Middle-East (Canfora et al., 2014; Bhatia et al., 2015; Xie et al., 2017; Mukhtar et al., 2018; Wang et al., 2020; Hou et al., 2021). The reason for this is that they have all been linked to osmo-tolerance which is necessary for thriving in arid and saline ecosystems (Ahmed et al., 2018). Bacteroidetes are a sensitive biological indicator of agricultural soil usage, and there may be a connection between their antibacterial and antifungal properties (Eida et al., 2018). Environment-found Bacteroidetes species are believed to specialize in the biosphere, namely in the breakdown of complex organic materials, particularly polysaccharides (Church, 2008). Higher electrical conductivity was shown to be linked with Bacteroidetes in a research involving soils from 18 distinct sites (Muhammad et al., 2021).

Proteobacteria have been linked to environmental stress tolerance, such as resistance to UV radiation, nucleotide excision repair, and photoreactivation pathways (Thoma, 1999). They have also been found in high numbers in extreme environments, such as the high Tibetan mountains, where they are believed to play a role in salt tolerance (Li et al.,

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2021). Proteobacteria have been associated with nitrogen fixation and are believed to protect desert flora from high salinity (Rath et al., 2019). Additionally, they have been employed as a biological treatment for a variety of toxic compounds (Mukhtar et al., 2018).

Firmicutes have also been linked to the advancement of soil bioremediation for sustainable agriculture, according to Hashmi et al. (2020). To counteract osmotic pressures caused by salt, Firmicutes can create salt stress chemicals (Meena et al., 2017). *Bacillus*, which belong to the Firmicutes family, were found to be abundant and naturally resistant to high salt and pH levels (Alotaibi et al., 2020). Previous studies have shown that *Bacillus* species are highly resilient in stressful environments due to their spore-forming ability (McKenney et al., 2013). Halophilic *Bacillus* strains offer several benefits, including the ability to aid in the bioremediation of hazardous substances, stimulate plant growth, and produce industrially significant enzymes (Mukhtar et al., 2018). They promote plant development by preventing phytopathogens and improving nutrient availability in the rhizosphere. In Mexico, certain strains of *Bacillus* have been found to facilitate the growth of wheat in high saline soils, with the diversity of *Bacillus* directly correlating with wheat growth (Ibarra-Villarreal et al., 2021). Other members of the Firmicutes family have also been shown to tolerate a wide range of soil pH levels (Zakaria et al., 2011). Hou et al. (2021) demonstrated a positive relationship between the relative abundances of Firmicutes and Bacteroidetes and the saline gradient.

Actinobacteria and Bacteroidetes have been discovered in various harsh environments worldwide, such as the Arctic, geothermal springs and acidic or alkaline conditions (Prathyusha and Bramhachari, 2018). The bacteria's ability to form spores is a significant factor in their protective capabilities in these environments. This unique trait has led to the use of some Actinobacteria species as biofertilizers and inoculants for plant growth in commercial fields (Yadav and Yadav, 2019). Active antimicrobial biomaterials are frequently derived from actinobacteria (Elbendary et al., 2018). They play a crucial role in the decomposition of deceased animals and plants (Barka et al., 2016).

Halobacteriales, also known as halophiles, were found in the soil sample as well. These bacteria constitute about 4.04% of the sample and have been previously found in the rhizospheres of plants growing in saline soils in Utah deserts (Kearl et al., 2019). Gibberellins, abscisic acid, cytokinins, indole acetic acid, and other growth regulators are among the phytohormones produced by Halobacteriales that support plant development. In situations when plants are exposed to salt, all of these phytohormones improve plant health by prolonging root stimulation by significantly extending the length and surface area of the roots (Jha et al., 2013). Moreover, they may produce aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces the amounts of ethylene in salt-stressed plants by converting ACC to α-ketobutyrate and ammonia (Sagar et al., 2020). Halophilic strains contribute to soil P levels being maintained as well as the conversion of insoluble P to soluble P. It is also known that several Halobacteriales generate antioxidative enzymes (superoxide dismutase, peroxidase, and catalase), which help plants survive in salt stress environments (Kumawat et al., 2022). Furthermore, by producing siderophores, halophiles boost iron availability and help the plant thrive in salt stress environments (Mukhtar and Mehnaz, 2020). It is possible that halobacteria play a similar role in Madinah sabkhas. Inoculation with halotolerant bacteria has been previously utilized to improve crop productivity in saline soils in Bangladesh (Rahman et al., 2017).

This study looked at the microbiome's metabolic abilities. Soil enzyme activity is affected by various factors like soil properties, types, and environmental conditions. It is

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used as a crucial indicator of soil biological activity and quality (Melero et al., 2007; Yuan et al., 2007). These enzymes play a significant role in the soil biochemical cycle, and their activity can impact soil metabolism, nutrient conversion, and fertility. Salinity can alter the environment for microorganisms, which are the primary source of soil enzymes. It can also cause protein denaturation and affect enzyme activity (Frankenberger and Bingham, 1982). High salinity can cause soil particles to clump or disperse and impact the solubility of soil organic matter and element mineralization (Wong et al., 2010; Lu et al., 2016; Rietz and Haynes, 2019). Previous research has shown that increased salinity can inhibit mangrove soil enzyme activity (Tilak et al., 2005; Chambers et al., 2016). The soil samples were analyzed, specifically those which were related to making salt soluble and accessible for plant growth.

Conclusion

The microbial community of Sabkhat Madinah was investigated using metagenome analysis. The soil sample consisted mostly of bacteria, comprising around 96% of the microbiota present. The five most abundant groups of bacteria in Madinah sabkha soil were Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Halobacteriales. Even though the samples were taken from the same sabkha, slight variations in the soil due to human activity or climate could lead to changes in microbial diversity.

Although the findings from this study carry several strengths, some limitations should also be carefully considered. It has been proven by Gamalero et al. (2020) that the bacterial communities and their makeup are not always consistent. In contrast, bacterial communities differ depending on the soil nutrients, plant growth, and other environmental factors. Moreover, in contrast to full 16s rRNA sequencing, targeted sequencing of hypervariable regions has previously been shown to be associated with false reads. This is because high variability in sub-regions has been detected even within the same species. Another limitation of this study is that several novel and unknown species detected through metagenomic screening were not fully characterized. Shot-gun metagenomic approach would help in the characterization of these novel sequences. Moreover, the study did not identify the distinct genes that confer resistance to abiotic stressors. An understanding of the functional genes that allow bacteria to survive in these hypersaline soils can help in several ways, including the development of saline resistant crops. Therefore, going forward, the results from this study can be further analysed using functional annotation metagenomics to putatively identify and characterize salt-resistant genes.

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Data availability. Results are available at the following link https://nawat-md.com/Hasanalbahri/qiime2/barplot/.

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