Comparative Metagenomics to Study the Impact of Soil Salinity on Microbial Diversity in Al-Madinah, KSA

Moayad S Waznah (mwaznah@taibahu.edu.sa)
Taibah University
Hibah M Albasri
Taibah University
Hassan A Albahri
Taibah University

Research Article

Keywords: 16S rRNA gene, metagenomic, Illumina HiSeq, soil microbiota, saline soil, MG-RAST, QIIME2 pipelines

Posted Date: November 30th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3480005/v1

Abstract

The chemical properties and fertility of soil are greatly influenced by soil microbes, which are essential to the biogeochemical cycle. Analyzing the microbial structure of soil is important for efficient use of the soil, whether it be for agricultural purposes or other uses. Sabkhat Al-Madinah in Saudi Arabia has soil with high salinity and plants that thrive in that environment. The microbial makeup of the soil in this area has not been extensively researched. This study aimed to analyze the microbial structure of two locations in Sabkhat Al-Madinah's soil and examine the correlation with soil properties. The 16S rRNA gene's V3-V4 region was targeted for metagenomic analysis using Illumina MiSeq. The soil chemical properties including EC, pH as well the concentration of some soil elements were determined. The microbial composition of both sites were investigated at different taxonomic levels using MG-RAST and QIIME2 pipelines. There was a significant difference in salt concentration between the two sites that were derived from the same sabkha. The second sample had higher sodium content, leading to increased E.C measures. Moreover, The two samples had different microbial compositions. The first sample was dominated by Bacteroidetes (18.37%), Firmicutes (13.57%) and Proteobacteria (13.57%), while the second one contained Proteobacteria (25.01%), Actinobacteria (12.03%) and Firmicutes (11.36%). Firmicutes were newly recorded and found only in saline habitats in KSA. Acidobacteria, Thermodesulfobacteria and Streptophyta were present 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%). In conclusion, the analysis of bacteria in the two sites revealed notable differences in soil properties and bacterial diversity.

1. 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). 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).

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 et al., 2018). The intricacy of soil surroundings can differ significantly based on external factors. 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).

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 deci Siemens (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 KSA, sabkhas are prevalent in various areas, including the east and west coasts and the middle region. The west Red Sea coastal strip spans from 16 to 65 kilometers (FAO, 2021) and contains several saline areas such as Jizan, Al-Layth, Jeddah, Abhur, and Yunbu (Al-Mhaidib, 2002).

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. Approaches to metagenomics are divided into two categories: targeted and shotgun genomics, depending on the segment of the amplicon or gene used to determine the organisms' phylogenetic classification (Clarridge, 2004). 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 KSA according to Alzahrani (2021). Based on this, this research was proposed to explore the microbial communities present in two saline soil sites in Al-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.

2. Materials and methods

2.1 Soil sample collection

In July 2020, samples were collected from two different locations (Sabkhat) in the northern part of Al-Madinah, KSA 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.

2.2 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; INESA, Shanghai, China) (Pansu, 2006), while the electrical conductivity (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 (Richard, 1972). 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 Allisson (1965), while available phosphorus was extracted using 0.5 N $NaHCO_3$ procedures and colorimetrically measured using an ascorbic acid-molybdenum blue method at wave length of 406 mm as described by Murphy and Riley (1962). The Nessler method was used to determine available nitrogen. The soil samples were extracted through a 1N ammonium acetate extractant (pH 7.0) (Chapman and Pratt, 1978), and the available potassium was measured by the Flame Photometer in accordance with Jackson (1973).

2.3 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.

2.4 Amplification and sequencing of the bacterial 16S rRNA gene

The composition and diversity of bacterial communities in soil were determined by amplifying the V3–V4 regions of the bacterial 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 (25ng) 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 2×250pb was used by Genoscreen in France to sequence the purified products. Raw 16S rRNA reads were received in FASTQ file format.

2.5 Metagenomic anaylsis using bioinformatics pipelines

2.5.1 MG-RAST

The FASTQ files containing metagenomic data were processed and analysed using Metagenomics Rapid Annotation using Subsystems Technology (MG-RAST) version 4.0.3, which also included OTU clustering, as described by Meyer et al. (2008).

Amplicon metagenomic reads were curated for quality, length and ambiguous bases as a quality filtering step. 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 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.

2.5.2 QIIME2

Quantitative Insights Into Microbial Ecology (QIIME) (v. 2019.1) pipeline was implemented for sequence data analysis (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 Divisive Amplicon Denoising Algorithm package (DADA2). The 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 and beta diversity, evenness and phylogenetic diversity.

2.5.3 PICRUSt

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (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.

3. Results

3.1 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.

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

3.2 Microbial community profiling

3.2.1 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 microbial composition of the soil was determined using 16S rRNA encoding gene (V3-V4 regions) amplicons. 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.

To ensure the accuracy of the sequencing data, several measures were taken. These included examining the K-mer profile, nucleotide histogram, and source hits distribution. The K-mer profile was used as an additional quality control step, revealing a direct relationship between coverage and sequence size. The nucleotide histogram was used to evaluate the proportions of A, T, G, and C nucleotides in the soil

specimens. A biased read in the nucleotide histogram confirmed the varying distribution of species in the two samples. A smaller e-value indicates a more confident sequence alignment of DNA obtained from the soil sample.

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, which suggests that new bacterial species may be present in these areas that could play a crucial role in salt adaptation.

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.

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* (Fig. 3).

Figure 4 displays the canvas for sample 1 and sample 2. Sample 1 consisted of a bacterial population of 97% and archaea population of 3%. Among the bacterial population, phylum Proteobacteria accounted for 41%, followed by Bacteroidetes (14%), Acidobacteria (10%), Actinobacteria (10%), Firmicutes (8%), Chloroflexi (3%), Planctomycetes (3%), Aquificae (3%), Thermodesulfobacteria (3%), Gemmatimonadetes (1%), Candidatus Calescamantes (1%), Acetothermia (0.9%), and other phylum in less than 1%. The archaea population, on the other hand, was dominated by phylum Euryarchaeota (77%), followed by Nanohaloarchaeota (17%), Pacearchaeota (3%), Woesearchaeota (2%), Thaumarchaeota (0.8%), Aenigmarchaeota (0.2%), Diapherotrites (0.4%), and Crenarchaeota (0.04%). The canvas for sample 2 showed that bacteria comprise 99% of the population and 1% represents archaea. 45% of the bacteria belongs to the phylum Proteobacteria, Actinobacteria (13%), Bacteroidetes (10%), Acidobacteria (9%), Firmicutes (6%), Chloroflexi and Planctomycetes (both 3%), Thermodesulfobacteria (2%), Verrucomicrobia (2%), Nitrospirae (2%), Aquificae (1%) and Gemmatimonadetes(0.9%) along with other phyla in less than 1%. The majority of archaea, around 71%, belongs to the phylum Euryarchaeota. Other

phyla include Thaumarchaeota (17%), Pacearchaeota (8%), Woesearchaeota (2%), Aenigmarchaeota (0.5%), Diapherotrites (0.2%), Crenarchaeota (0.2%), and Parvarchaeota (0.08%).

3.2.2 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, 54433 reads were non chimeric for sample 1 and 50660 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 a 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/

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 (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 and 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%).

3.2.2.1 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.

3.2.2.2 The diversity and richness of the microbial community

Alpha diversity 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 (148) compared to sample 2 (129). Shannon's index indicates more abundance and evenness of the taxa present in sample 1 (6.81) compared to sample 2 (6.62). The evenness value was confirmed by Heip's evenness measure, it was 0.945 for sample 1 and 0.943 for sample 2. Moreover, Faith's phylogenetic diversity measures of biodiversity that incorporates phylogenetic difference between species as the sum of length of branches. More phylogenetic diversity is also obtained for the first sample (13.21) compared to sample 2 (0.943).

The values of beta diversity indicate that the two samples are completely different from each other in spite of their close locations from the same sabkha. Jaccard distance Bray-and Curtis distance was for sample 1 compared to sample 2 (0.9963) and (0.9958), respectively.

3.3 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))-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.

4. Discussion

The quality of soil is a crucial factor in addressing various global issues in the 21st century, such as climate change, food security, biodiversity conservation, water scarcity, and sustainable development. 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 KSA, 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-Badawi, 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.

Yet, the Saudi population continues to grow. KSA needs more sustainable farming practices to achieve their food security goals by 2030 (Brown et al., 2018). To this end, 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).

At first, the study of the vast array of microorganisms in soil was hindered by insufficient methods of cultivation (Doornbos et al., 2012). Nevertheless, 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 KSA. Hence, this study aimed to identify microbial communities in the sabkhas of Al 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 KSA, 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 Al-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, with one site having an EC of 7.6 ds/m and another site having an EC of 13.36 ds/m. 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 the Al-Madinah sabkha differed significantly from previous studies conducted on non-saline regions of KSA, 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 the Al-Madinah province. However, they did not analyse 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 Al-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. While smaller proportions of Archaea and Eukaryota were also detected, it is worth noting that these findings align with previous studies conducted on soil samples in KSA. However, it is important to note that previous studies have identified over 203 fungal species in sabkha soils throughout KSA, whereas a lower proportion of fungus was found in these Al-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 Al-Madinah province (Alotaibi et al., 2020).

The soil in sabkhat Al-Madinah contains five main groups of bacteria that are abundant: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and halobacteriales. Bacteroidetes are found in many desert soils worldwide. These bacteria groups have also 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). Prior studies in KSA have also found similar bacteria compositions, including Proteobacteria, Actinobacteria, and Acidobacteria (Yasir et al., 2015). Researchers have hypothesized that these bacteria are abundant in KSA due to their ability to fix nitrogen and ammonia. However, the study only identified the presence of Firmicutes in these soils (Khan and Khan, 2020). This finding suggests that the prevalence of Firmicutes may be unique to soils with high levels of salinity.

In the Al-Madinah sabkha, *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). Although the researchers did not provide functional annotations of these bacteria, previous studies have shown that *Bacillus* species are highly resilient in stressful environments due to their spore-forming ability (McKenney et al., 2013). 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 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).

The Al-Madinah Sabkha soil was found to have an abundance of Proteobacteria in addition to Firmicutes. 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., 2021). Additionally, Proteobacteria have been associated with nitrogen fixation and are believed to protect desert flora from high salinity (Rath et al., 2019).

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).

Actinobacteria and Bacteroidetes have been discovered in various extreme environments worldwide, including the Arctic, geothermal springs, and acidic or alkaline conditions (Prathyusha and Bramhachari, 2018). These bacteria are able to form spores, which provides them with protection in harsh environments. Some species of Actinobacteria are used as inoculants and biofertilizers to enhance plant growth in commercial fields (Yadav and Yadav, 2019). 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). It is possible that halobacteria play a similar role in the Al-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 used as a crucial indicator of soil biological activity and quality (Melero et al., 2007 and 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 (Rietz and Haynes, 2003; Wong et al., 2010; Lu et al., 2016). Previous research has shown that increased salinity can inhibit mangrove soil enzyme activity (Tilak et al., 2005 and Chambers et al., 2016). The soil samples were analyzed, specifically those which were related to making salt soluble and accessible for plant growth.

Taken together, The study has successfully identified the soil composition present in Al-Madinah Sabkhas. However, 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

5. Conclusion

The microbial community of Sabkhat Al-Madinah was investigated using metagenome analysis. DNA extraction and cluster analysis for using V3- V4 regions in 16s rRNA resulted in the identification of large number of sequences used for the identification of bacterial structure on various levels including phyla, classes, orders, families, genera and species. The soil sample consisted mostly of bacteria, comprising around 96% of the microbiota present. However, there were also smaller amounts of Archaea and

Eukaryota. The five most abundant groups of bacteria in the Al-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. Further research is necessary to examine the relationship between soil and microbial structure, as well as their potential functions. An initial view on inferred metabolic capabilities based on reference species in this study, shows what can be expected from follow up studies.

Declarations

I certify that the information given is true and complete to the best of our knowledge. I understand that if I have deliberately given any false information or have withheld any information regarding any situation, I am liable for prosecution for fraud and/or perjury.

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript and have no relevant financial or non-financial interests to disclose.

Ethical Approval

The manuscript is original work of author(s). All data, tables, figures, etc. used in the manuscript are prepared originally by authors, otherwise the sources are cited, and reprint permission is attached.

Consent to Participate

I voluntarily agree to participate in this study.

Consent to Publish

I agree to publish this study.

Authors Contributions

Moayad S. Waznah contribution to the concept and design of the article.

Hibah M. Albasri contribution to write the article.

Hassan A. Albahri contribution to the lab work and analysis the data of the article.

Funding

No funds have been received in this work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

1. Abbas A, Khan S, Hussain N, Hanjra MA, Akbar S (2013) Characterizing soil salinity in irrigated agriculture using a remote sensing approach. Phys Chem Earth Parts A/B/C 55:43–52
2. Abduljauwad SN (1995) Compressibility and collapse characteristics of arid saline sabkha soils. *Compressibility and collapse characteristics of arid saline sabkha soils*
3. Ahmed V, Verma MK, Gupta S, Mandhan V, Chauhan NS (2018) Metagenomic profiling of soil microbes to mine salt stress tolerance genes. Front Microbiol 9:159
4. Al-Barrak S, Al-Badawi M (1988) Properties of some salt affected soils in Al-Ahsa, Saudi Arabia. Arid Land Research and Management 2(2):85–95
5. Al-Hassoun SA (2007) Monitoring salt-affected soils in a region in Saudi Arabia using remote sensing techniques. In *Proceedings of the Eleventh International Water Technology Conference, Sharm El-Sheikh*
6. Allison L (1965) Organic carbon. *Methods of soil analysis: Part 2 Chemical and microbiological properties*, *9*, 1367–1378
7. Al-Mhaidib AI (2010), June Effects of fiber on swell of expansive soils. In *The Twentieth International Offshore and Polar Engineering Conference*. OnePetro
8. Alotaibi MO, Sonbol HS, Alwakeel SS, Suliman RS, Fodah RA, Jaffal ASA, …, Mohammed AE (2020) Microbial diversity of some sabkha and desert sites in Saudi Arabia. Saudi J Biol Sci 27(10):2778–2789
9. Alsharif W, Saad MM, Hirt H (2020) Desert microbes for boosting sustainable agriculture in extreme environments. Front Microbiol, 1666
10. Alzahrani KJ (2021) Microbiome Studies from Saudi Arabia over the Last 10 Years: Achievements, Gaps, and Future Directions. *Microorganisms*, *9*(10), 2021
11. Bashir Y, Singh P, S. and, Kumar Konwar B (2014) 'Metagenomics: An Application Based Perspective', Chinese Journal of Biology, 2014, p. e146030
12. Berg G, Rybakova D, Fischer D, Cernava T, Vergès MCC, Charles T, …, Sessitsch A, Schloter M (2020) Microbiome definition re-visited: old concepts and new challenges
13. Bhatia S, Batra N, Pathak A, Joshi A, Souza L, Almeida P, Chauhan A (2015) Metagenomic analysis of bacterial and archaeal assemblages in the soil-mousse surrounding a geothermal spring. Genomics data 5:195–200
14. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, …, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37(8):852–857
15. Brown JJ, Das P, Al-Saidi M (2018) Sustainable agriculture in the Arabian/Persian Gulf region utilizing marginal water resources: making the best of a bad situation. Sustainability 10(5):1364
16. Bünemann EK et al (2018) 'Soil quality – A critical review', Soil Biology and Biochemistry, 120, pp. 105–125

17. Canfora L, Bacci G, Pinzari F, Lo Papa G, Dazzi C, Benedetti A (2014) Salinity and bacterial diversity: to what extent does the concentration of salt affect the bacterial community in a saline soil?. PLoS ONE, 9(9), e106662
18. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, …, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the national academy of sciences*, *108*(supplement_1), 4516–4522
19. Chambers LG, Guevara R, Boyer JN, Troxler TG, Davis SE (2016) Effects of salinity and inundation on microbial community structure and function in a mangrove peat soil. Wetlands 36:361–371
20. Chapman HD, Pratt PF (1961) Methods of analysis for soils, plantsand waters. University of California, Division of Agricultural Science, Riverside, (USA
21. Chen K, Neimark H, Rumore P, Steinman CR (1989) Broad range DNA probes for detecting and amplifying eubacterial nucleic acids. FEMS Microbiol Lett 57(1):19–24
22. Cullen CM, Aneja KK, Beyhan S, Cho CE, Woloszynek S, Convertino M, …, Rosen GL (2020) Emerging priorities for microbiome research. Front Microbiol 11:136
23. Doornbos RF, van Loon LC, Bakker PA (2012) Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agron Sustain Dev 32:227–243
24. Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, …, Khan ML (2019) Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28:2405–2429
25. Elhadj E (2004) Camels don't fly, deserts don't bloom: An assessment of Saudi Arabia's experiment in desert agriculture. Occasional paper, *48*(6)
26. FAO (2021) Soil salinization as a global major challenge. Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/global-soil partnership/resources/highlights/detail/en/c/1412475/ (Accessed: 29 August 2021)
27. FAO, GSBI ITPS, SCBD,EC (2020) State of knowledge of soil biodiversity—Status, challenges and potentialities, Report 2020. FAO
28. Frankenberger W Jr, Bingham FT (1982) Influence of salinity on soil enzyme activities. Soil Sci Soc Am J 46(6):1173–1177
29. Gamalero E, Bona E, Todeschini V, Lingua G (2020) Saline and arid soils: Impact on bacteria, plants, and their interaction. Biology 9(6):116
30. Garcia-Garcia S, Cortese MF, Rodríguez-Algarra F, Tabernero D, Rando-Segura A, Quer J, …, Rodríguez-Frías F (2021) Next-generation sequencing for the diagnosis of hepatitis B: Current status and future prospects. Expert Rev Mol Diagn 21(4):381–396
31. Hou Y, Zeng W, Hou M, Wang Z, Luo Y, Lei G, …, Huang J (2021) Responses of the soil microbial community to salinity stress in maize fields. Biology 10(11):1114
32. Ibarra-Villarreal AL, Gándara-Ledezma A, Godoy-Flores AD, Herrera-Sepúlveda A, Díaz-Rodríguez AM, Parra-Cota FI, de los Santos-Villalobos S (2021) Salt-tolerant Bacillus species as a promising

strategy to mitigate the salinity stress in wheat (Triticum turgidum subsp. durum). J Arid Environ 186:104399

33. Jackson ML (1973) Soil chemical analysis, pentice hall of India Pvt. Ltd New Delhi India 498:151–154
34. Jansson JK, Hofmockel KS (2018) The soil microbiome—from metagenomics to metaphenomics. Curr Opin Microbiol 43:162–168
35. Kearl J, McNary C, Lowman JS, Mei C, Aanderud ZT, Smith ST, …, Nielsen BL (2019) Salt-tolerant halophyte rhizosphere bacteria stimulate growth of alfalfa in salty soil. Front Microbiol 10:1849
36. Khan MA, Khan ST (2020) Microbial communities and their predictive functional profiles in the arid soil of Saudi Arabia. Soil 6(2):513–521
37. Li YL, Chen JX, Zhou YT, Yang Z, Wang YG, Zhang JY, Yue L et al (2016) Diversity and distribution of culturable microbial communities of semiarid desert steppe and cultivated land in northwestern China. J Agricultural Resour Environ 33:244–252
38. Li YQ, Chai YH, Wang XS, Huang LY, Luo XM, Qiu C, …, Guan XY (2021) Bacterial community in saline farmland soil on the Tibetan plateau: Responding to salinization while resisting extreme environments. BMC Microbiol 21(1):1–14
39. Lin H (2010) Earth's Critical Zone and hydropedology: concepts, characteristics, and advances. Hydrol Earth Syst Sci 14(1):25–45
40. Lu N (2016) Generalized soil water retention equation for adsorption and capillarity. J Geotech GeoEnviron Eng 142(10):04016051
41. McKenney PT, Driks A, Eichenberger P (2013) The Bacillus subtilis endospore: assembly and functions of the multilayered coat. Nat Rev Microbiol 11(1):33–44
42. Melero S, Madejón E, Ruiz JC, Herencia JF (2007) Chemical and biochemical properties of a clay soil under dryland agriculture system as affected by organic fertilization. Eur J Agron 26(3):327–334
43. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, …, Edwards R (2008) The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9(1):1–8
44. Mukhtar S, Mirza BS, Mehnaz S, Mirza MS, Mclean J, Malik KA (2018) Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome. World J Microbiol Biotechnol 34:1–17
45. Murphy JAMES, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36
46. Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, …, Peters JW (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl Environ Microbiol 82(13):3698–3710
47. Olsen SR, Cole CV, Watanabe FS, United States Department of Agriculture Circular 19 (1954) ; Washington DC: Estimation of Available Phosphorus in Soils by Extraction With Sodium Bicarbonate; p. 939

48. Pansu M, Gautheyrou J (2006) Handbook of Soil Analysis. Mineralogical, Organic and Inorganic Methods
49. Parks DH, Tyson GW, Hugenholtz P, Beiko RG (2014) STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30(21):3123–3124
50. Prathyusha AMVN, Bramhachari PV (2018) Novel perspectives of biotic and abiotic stress tolerance mechanism in Actinobacteria. New and future developments in microbial biotechnology and bioengineering, 235–244
51. Rahman SS, Siddique R, Tabassum N (2017) Isolation and identification of halotolerant soil bacteria from coastal Patenga area. BMC Res Notes 10:1–6
52. Rangseekaew P, Barros-Rodríguez A, Pathom-Aree W, Manzanera M (2021) Deep-sea actinobacteria mitigate salinity stress in tomato seedlings and their biosafety testing. Plants 10(8):1687
53. Rath KM, Fierer N, Murphy DV, Rousk J (2019) Linking bacterial community composition to soil salinity along environmental gradients. ISME J 13(3):836–846
54. Rath KM, Maheshwari A, Bengtson P, Rousk J (2016) Comparative toxicities of salts on microbial processes in soil. Appl Environ Microbiol 82(7):2012–2020
55. Rietz DN, Haynes RJ (2003) Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol Biochem 35(6):845–854
56. Shahid SA, Zaman M, Heng L (2018a) Introduction to Soil Salinity, Sodicity and Diagnostics Techniques. In: Zaman M, Shahid SA, Heng L (eds) Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Springer International Publishing, Cham, pp 1–42
57. Shahid SA, Zaman M, Heng L (2018b) Soil Salinity: Historical Perspectives and a World Overview of the Problem. In: Zaman M, Shahid SA, Heng L (eds) Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Springer International Publishing, Cham, pp 43–53
58. Shrivastava P, Kumar R (2015) Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22(2):123–131
59. Shrivastava P, Kumar R (2015) Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22(2):123–131
60. Sun R, Li W, Dong W, Tian Y, Hu C, Liu B (2018) Tillage changes vertical distribution of soil bacterial and fungal communities. Front Microbiol 9:699
61. Thoma F (1999) Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. EMBO J 18(23):6585–6598
62. Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, …, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci, 136–150
63. Vitorino LC, Bessa LA (2018) Microbial diversity: the gap between the estimated and the known. Diversity 10(2):46

64. Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37(1):29–38
65. Wang S, Sun L, Ling N, Zhu C, Chi F, Li W, …, Wei D (2020) Exploring the diversity of bacteria along a soil salinity gradient in Songnen Plain of Northeast China. Front Microbiol 10:1–10
66. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173(2):697–703
67. Wong VN, Greene RSB, Dalal RC, Murphy BW (2010) Soil carbon dynamics in saline and sodic soils: a review. Soil Use Manag 26(1):2–11
68. Xie K, Deng Y, Zhang S, Zhang W, Liu J, Xie Y, …, Huang H (2017) Prokaryotic community distribution along an ecological gradient of salinity in surface and subsurface saline soils. Sci Rep 7(1):13332
69. Xie K, Deng Y, Zhang S, Zhang W, Liu J, Xie Y, …, Huang H (2017) Prokaryotic community distribution along an ecological gradient of salinity in surface and subsurface saline soils. Sci Rep 7(1):13332
70. Yadav N, Yadav AN (2019) Actinobacteria for sustainable agriculture. J Appl Biotechnol Bioeng 6(1):38–41
71. Yasir M, Azhar EI, Khan I, Bibi F, Baabdullah R, Al-Zahrani IA, Al-Ghamdi AK (2015) Composition of soil microbiome along elevation gradients in southwestern highlands of Saudi Arabia. BMC Microbiol 15:1–9
72. Yuan BC, Li ZZ, Liu H, Gao M, Zhang YY (2007) Microbial biomass and activity in salt affected soils under arid conditions. Appl Soil Ecol 35(2):319–328
73. Zakaria L, Yee TL, Zakaria M, Salleh B (2011) Diversity of microfungi in sandy beach soil of Teluk Aling, Pulau Pinang. Trop life Sci Res 22(1):71

Figures

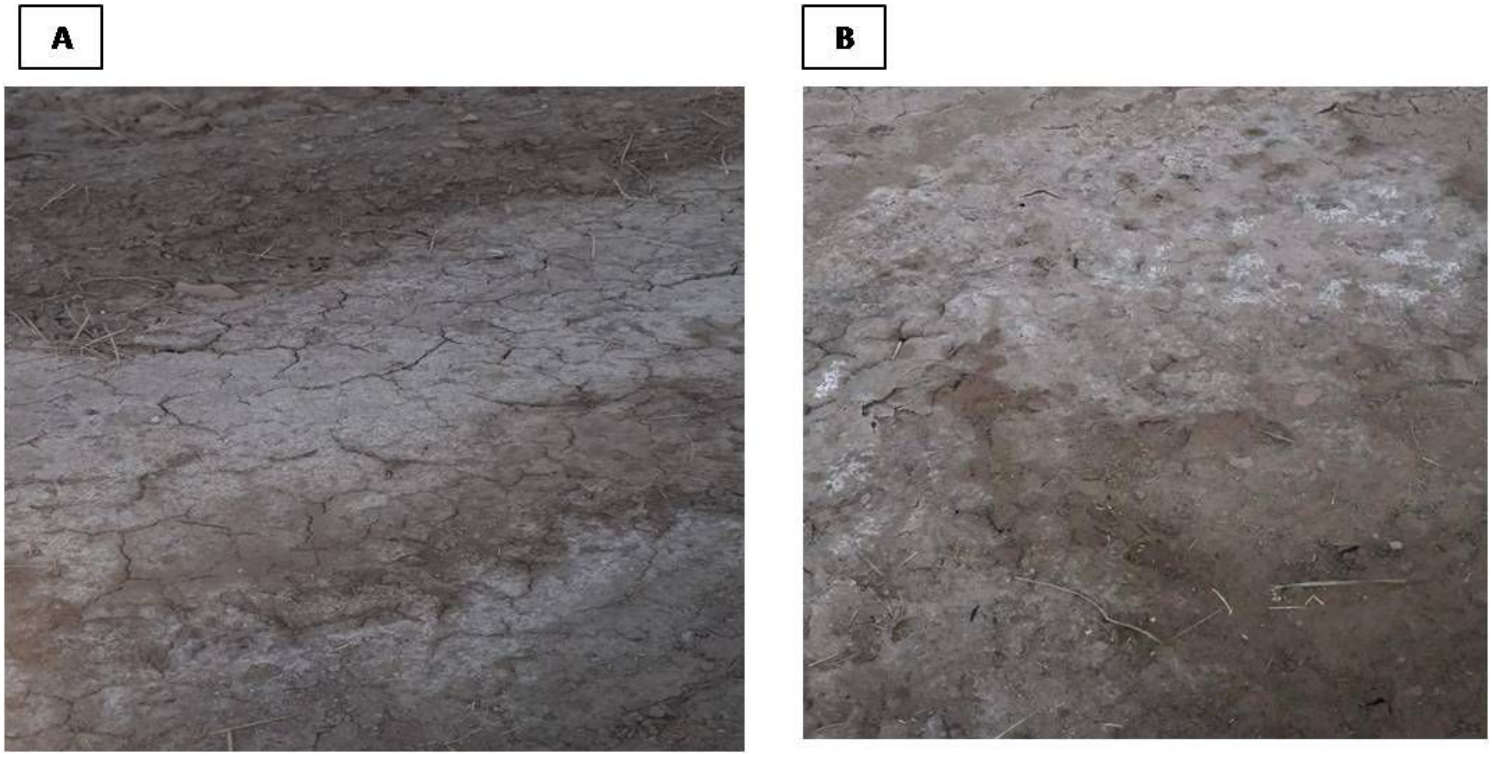

Figure 1

Site of sample 1 (A) and sample 2 (B)

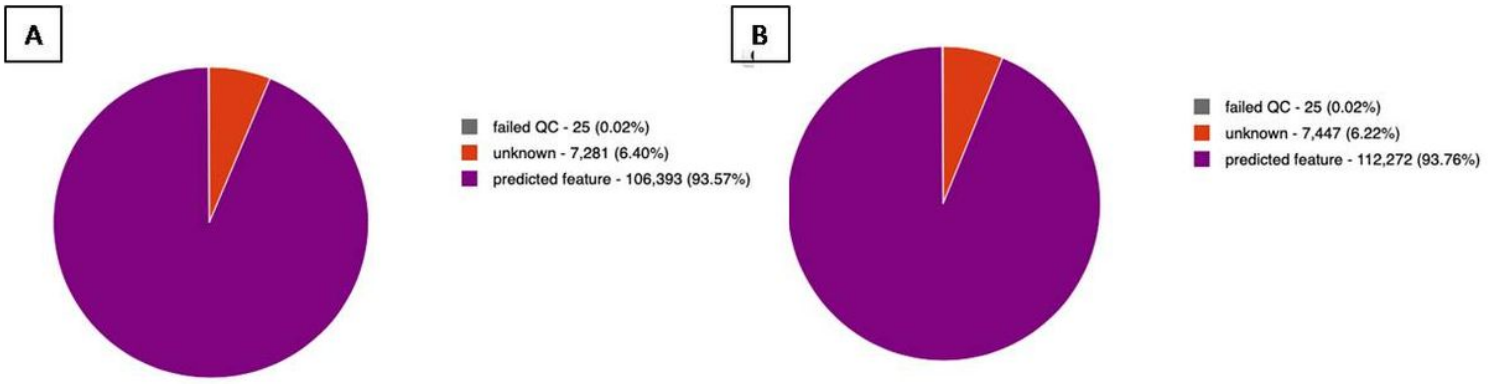

Figure 2

Sequence breakdown for sample 1 (A) and sample 2 (B)

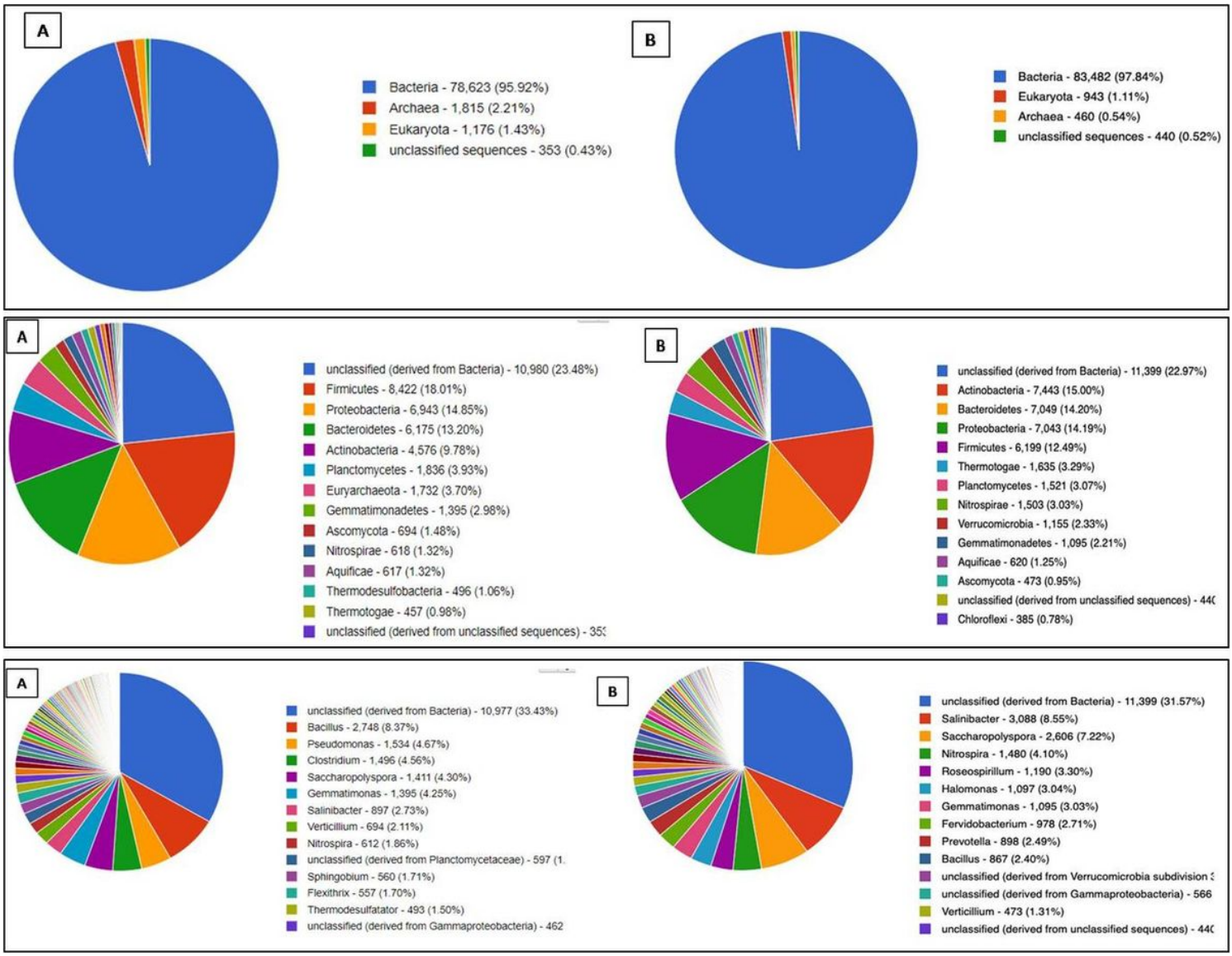

Figure 3

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

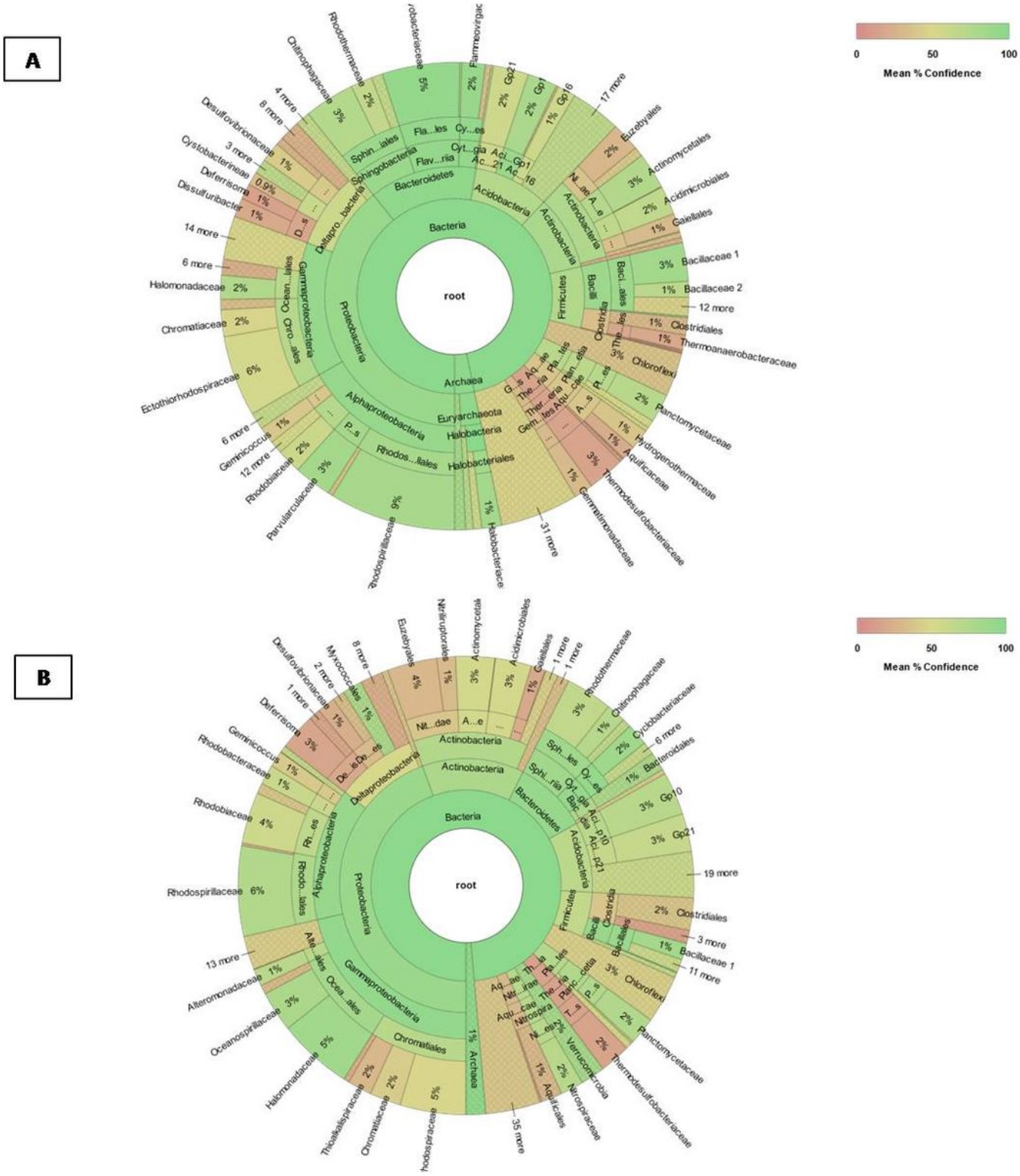

Figure 4

The canvas for sample 1 (A) and sample 2 (B)

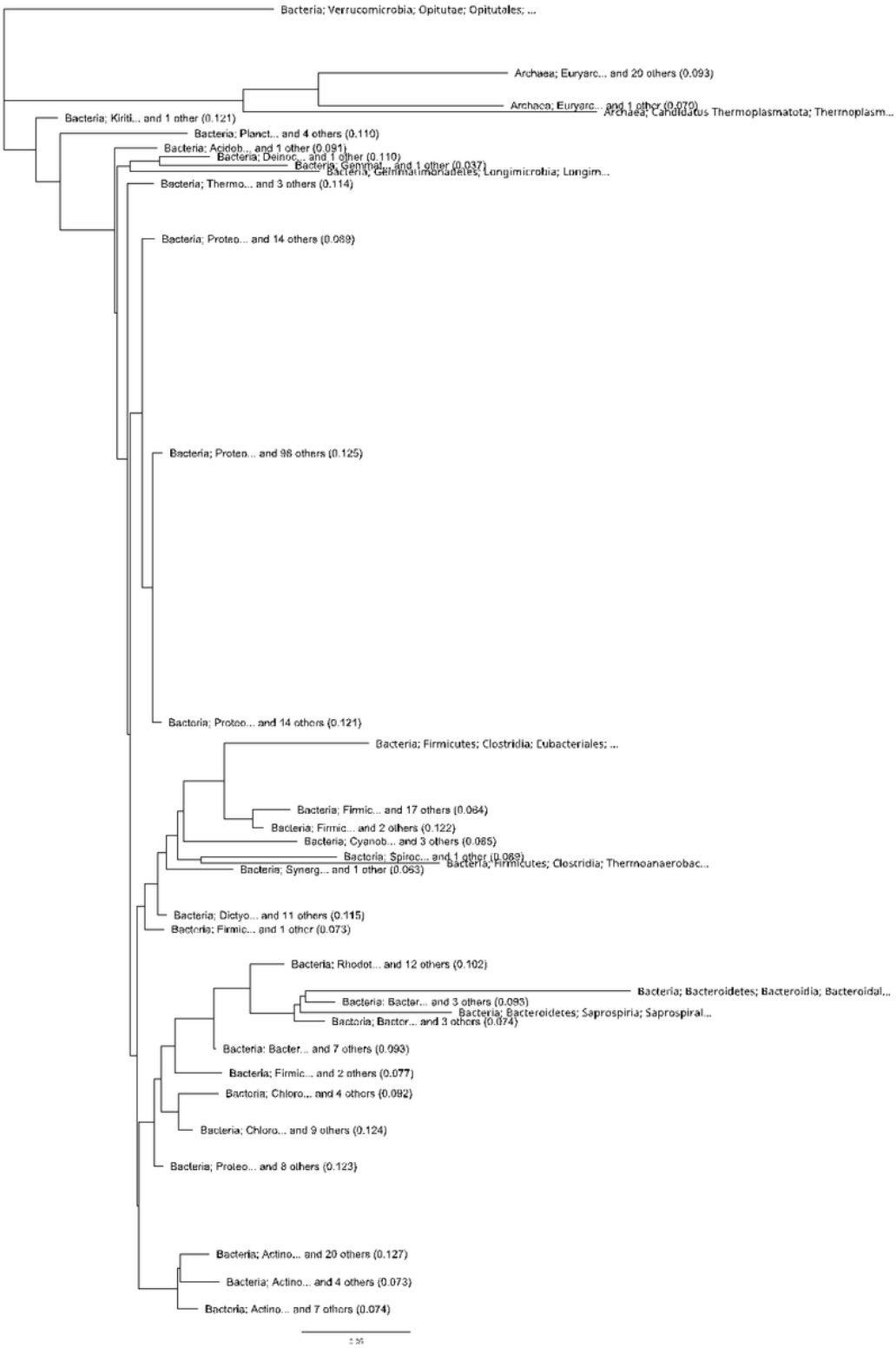

Figure 5

Phylogenetic tree of the microbial composition of the sample 1.

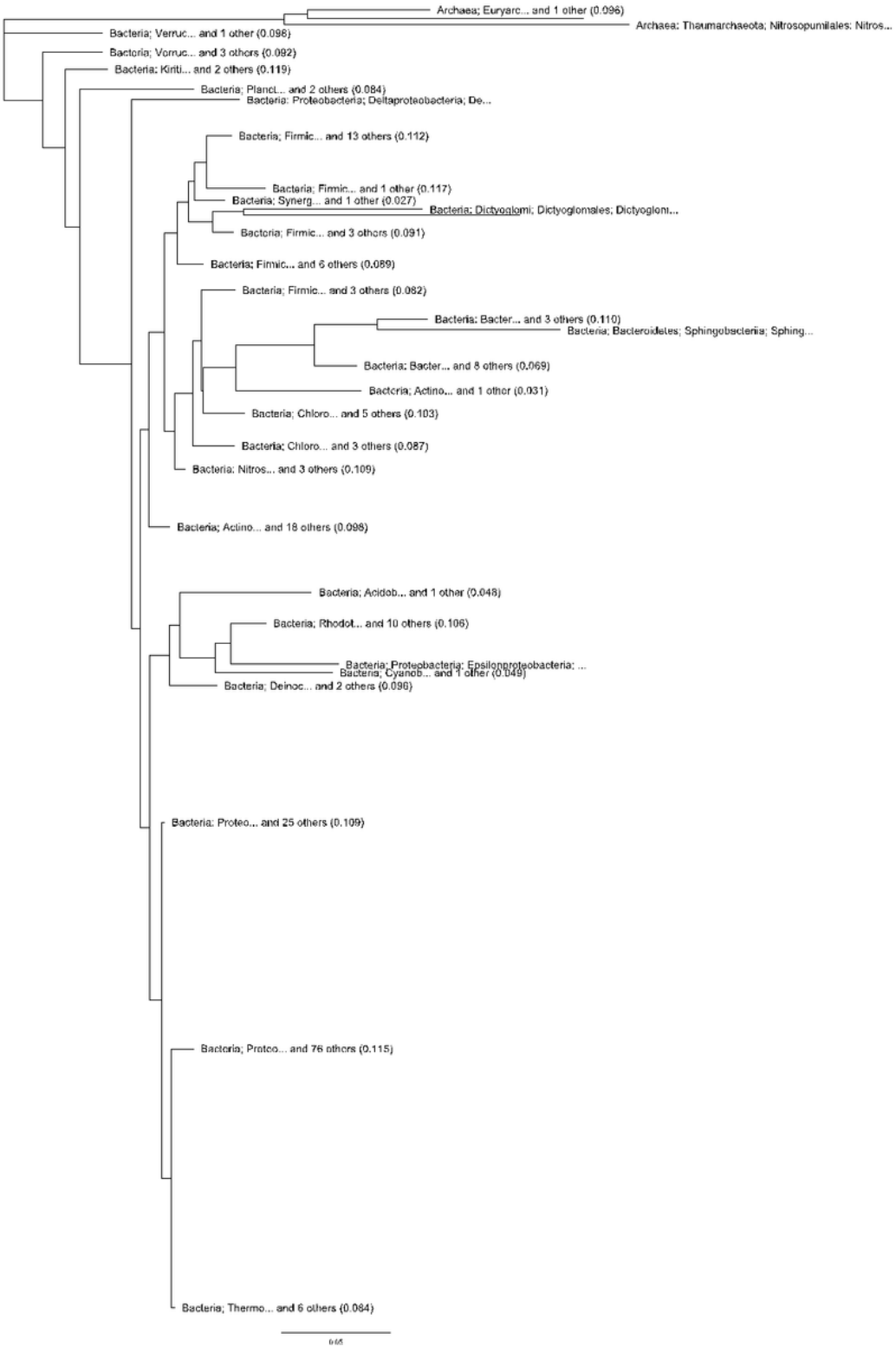

Figure 6

Phylogenetic tree of the microbial composition of the sample 2.