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**Microbial Diversity and Unique Soil-
Microbe Interactions Across Western
Saudi Arabian Arid Environments**

Microbial Diversity and Unique Soil-Microbe Interactions Across Western Saudi Arabian Arid Environments

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Abstract

This study investigates microbial diversity in arid soils across Western Saudi Arabia using 16S rRNA analysis. Soil samples were collected from various locations in the Makkah region, with pH levels ranging from 7.00 in Kamil to 7.81 in Khulais. Moisture content varied significantly, from 0.5% in Fair Capital to 12.0% in Jeddah. The sand was identified as the dominant soil component, with varying proportions of silt and clay, classifying all samples as sandy soil. Bacterial incidence showed notable variation, peaking at 20% in Fair Capital and reaching a minimum of 6% in Al-Qunfudhah. Soil composition influenced particle aggregation, with higher clay and moisture content promoting clumping in areas like Jeddah, while lower percentages resulted in loose, scattered particles elsewhere. 16S rRNA sequences from bacterial isolates were matched with similar isolates from the gene bank database. Results reveal diverse microbial communities across the studied region, correlating with varying soil physicochemical properties. This research contributes to our understanding of soil-microbe interactions in arid environments and may inform future soil management strategies in similar ecosystems.

Keywords: Soil microbes, Soil-microbe interactions, Soil biodiversity, SNPs, SEM

Introduction

Since the relationship between the biotic and abiotic content in soil is reciprocal, bio-scanning of a region or area can better understand this area's nature (Berg et al., 2020). Old techniques studying the bacterial content of soil gave estimations of species numbers much lower than the actual number. Metagenomics and molecular techniques approved that one gram of soil may have from one thousand to a million different species (Cullen et al., 2020). This and soil characteristics change with time, leading to changes in biotic content, so new bio scans using molecular techniques must be taken (Ma et al., 2019). Development in molecular and genetic sciences improved the ecological scientists' classification of soil's bio flora by examining the microbial DNA and RNA and recording these strains in the global gene bank databases (Berg et al., 2020; Ma et al., 2019).

Thermotolerant microorganisms have the mesophilic range (30-37°C), therefore gaining the ability to grow in high-temperature environments. However, thermophilic bacteria in

extremely high-temperature environments such as volcanic sites can thrive at up to 60°C (Roumpeka et al., 2017). In response to the foregoing, we can conclude that thermophilic bacteria can prosper in hot environments like deserts where temperatures can reach up to 50°C and more (Escobar-Zepeda et al., 2018; Hasanean et al., 2015). Bacteria living in the deserts have to deal with other extreme factors besides high temperatures, such as dryness, low nutrient levels, robust thermal contrasts, high levels of ultra-violet radiation, and strong wind (Al-Obaid et al., 2017; Herzallah et al., 2019; Al-Hanawi et al., 2021).

Previous studies on desert microflora of Saudi Arabia reported that *Bacillus* species were the most abundant. In particular, the genus *Bacillus subtilis*. Other bacterial species like *Enterobacter*, *Paenibacillus*, *Pseudomonas*, and *Lactobacillus* were also found in Saudi Arabia's different regions (Gaulke et al., 2018; Premaraj et al., 2020).



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This study identifies the bacteria in the isolates gathered from different sites in the West region. Two isolates were examined: soil and olive oil pressers' isolates.

1. Material and method

1.1. Study areas



Figure 1. The map of Saudi Arabia shows the Mecca Region highlighted in red. Its coordinates are 21°30'N 41°0'E.

1.2. Samples collection, preparation, and bacteriological examination

Isolates were collected from 50 soil samples, and different types of dry soil, including rocky, clay, and sand presses in the western region of Saudi Arabia, were collected for microbial investigations. Each sample was collected from a 5-20 cm depth from the land surface. Each sample was stored in a plastic bag and labelled, then immediately transferred to the bacteriological laboratory for analysis according to the technique recommended (Jackson et al., 1967; O'Kelly et al., 2004; APHA 2002).

The procedure for preparing the samples was as follows: A gram of soil sample was mixed with 9 ml of sterile distilled water to create serial dilutions ranging from 10^0 to 10^3 . One ml of each dilution was then placed on nutrient agar, mannitol salt agar, and MacConkey agar media to identify various microorganisms present in the soil. Two different methods were used for culturing: (1) The soil dilution plate method (Waksman et al., 1922), where the plates were incubated at 37°C for 24 hours to allow bacterial growth. (2) The soil plate method involved placing 0.5 g of soil in a sterile Petri dish and adding 15 ml of sterilized liquid culture media at 45°C. The soil particles were dispersed throughout the agar, and the plates were left to set. For sandy soils, shaking and turning the plate was done before the agar solidified to ensure proper dispersal. Three replicates of soil sample dilutions were prepared for each media. The results were expressed as CFU/g. The isolated microbes were further identified through gram staining under a microscope and through biochemical methods. The bacterial isolates were also identified through PCR amplification of the 16S rDNA genes and subsequent sequencing.

Sampling was conducted in various regions of Saudi Arabia, specifically in 10 provinces in the Makkah region, covering a total area of 153,128 km² (59,123 sq mi). These provinces include Al-Qunfudhah Governorate, Fair Capital Governorate (City of Mecca), Jamoum Governorate, Jeddah Governorate, Kamil Governorate, Khulais Governorate, Laith Governorate, Rabigh Governorate, Taif Governorate, and Torba Governorate (Figure 1).

1.3. DNA extraction

Extracting genomic DNA from bacterial isolates was carried out using the InstaGene™ Matrix Genomic DNA Kit (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions.

1.4. PCR amplification and purification

The bacterial isolates were subjected to 16S rDNA region analysis using the genomic DNA extracted as a template, and the universal primers 785-F (5'-GGATTAGATACCCTGGTA-3') and 907-R (5'-CCGTCGAATTCMTTTRAGTTT-3'). The PCR reaction mixture was prepared by combining 10x Taq PCR buffer (2 µl), 2.5 mM dNTP mixture (1.6 µl), F and R primers (10 pmol/ml) (1.0 ml), KOMA Taq (2.5 U/ml) (0.2 µl), DNA template (20 ng/ml) (2 µl), and HPLC grade distilled water to reach a total reaction volume of 20 µl. The thermal cycler was used to amplify the samples, with an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 0.5 min, annealing at 55°C for 2 min, and extension at 68°C for 1.5 min, and a final polymerization extension at 68°C for 10 min. The success of the PCR amplification was confirmed through 1% agarose gel electrophoresis. The PCR products were then purified using the Montage PCR Cleanup Kit (Millipore Sigma, Burlington, MA, USA).

1.5. DNA sequencing

The amplified primers and the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) were used to sequence the purified bacterial PCR products. The

sequencing was carried out at Macrogen, Inc. (Seoul, South Korea) using the 3730xl DNA Analyzer automated DNA sequencing system (Applied Biosystems).

1.6. Sequence analysis

The acquired sequences were analyzed using Geneious Prime software version 2020.1.2 (<https://www.geneious.com>) (Kearse et al., 2012). Combined sequences were generated by merging the forward and reverse sequences. To match the sequences with the existing nucleotide sequence database in GenBank, the Basic Local Alignment Search Tool (BLAST) from the National Centre of Biotechnology Information (NCBI) was utilized.

1.7. Physicochemical investigations

The soil samples were examined with a Scanning Electronic Microscope (SEM) (JEOL 7500FA JEOL, Peabody, MA, USA) at 10 kV voltage to identify the particle characteristics: colour, shape, and morphology. The pH was determined with a pH meter model (HI98107) (Jackson et al., 1967), and the moisture content (MC) (Waksman et al., 1922). In addition, electrical conductivity (EC) was determined for each soil sample using an EC-meter (Matter Toledo-AG).

1.8. Statistical analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM, Armonk, NY, USA). The findings were reported in numerical and percentage format (Norussis et al., 2006).

2. Results

It is well known that soil provides a rich home for several microbial populations. This led to differences in microbial kinds and densities between the Makkah and desert locations under investigation, with a few species constituting the core microbiome and others being site-specific. The pH, moisture content, and soil texture from the nine study sites were examined since variances in microbial communities may be explained by changes in soil characteristics among the study sites (Table 1). All soil samples were collected around neutrality; the highest was at Khulais (7.81), and the lowest was at Kamil (7.00). The highest moisture percent was at Jeddah (12.0%), and the lowest moisture percent was at Fair Capital (0.5%). Sand is the dominant component of all soils. Then, the silt was the second, while the lowest percentage was clay; this considered all soil samples to be sandy soil (Table 1).

Table 1: Soil characteristics of the different governorates in the Makkah region.

<i>Governorate</i>	<i>pH</i>	<i>Moisture (%)</i>	<i>Clay (%)</i>	<i>Sand (%)</i>	<i>Silt (%)</i>
<i>Al-Qunfudah</i>	7.10	1.20	13.0	69.0	38.0
<i>Fair Capital</i>	7.20	0.50	7.00	72.0	13.5
<i>Jamoum</i>	7.19	1.20	5.00	64.0	19.5
<i>Jeddah</i>	7.35	12.0	9.00	69.0	21.5
<i>Kamil</i>	7.00	7.00	5.40	70.0	15.5
<i>Khulais</i>	7.81	6.00	4.00	73.0	20.0
<i>Laith</i>	7.15	5.50	4.00	70.0	24.0
<i>Rabigh</i>	7.33	4.50	4.80	90.0	18.0
<i>Taif</i>	7.55	7.55	5.40	85.0	15.0
<i>Torba</i>	7.40	7.33	10.0	88.0	21.0

Bacterial diversity in the Makkah region was earlier investigated and well-documented (Alzahrani et al., 2021; Alharbi et al., 2021; Amasha et al., 2018). The bacterial incidence was declared in Figure 2. It was informed that the highest bacterial incidence was in Fair Capital (20%), followed by Jeddah (15%), Taif (13%), Laith (9%), Jamoum, and Khulais (8% each), and Kamil, Rabigh, and Torba (7% each). The lowest bacterial incidence (6%) was in Al-Qunfudhah.

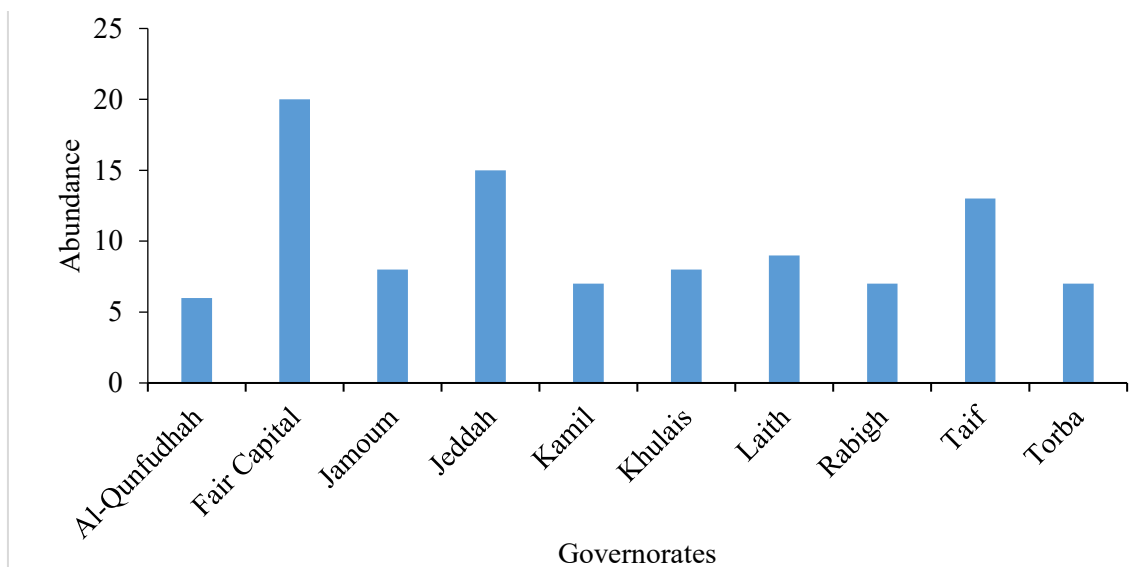


Figure 2. Bacterial abundance is present in the different governorates in the Makkah region.

In Al-Qunfudhah governorate, the clay and silt percentages were highest, so the soil particles appeared coagulated and coherent. The decrease in clay percentage caused the soil particles to appear loose and scattered, in addition to the very low moisture in the Jamoum governorate. On the contrary, a high rate of silt and humidity caused the aggregation of soil particles in clumps, as in the Jeddah governorate. The high percentage of sand makes wide pores between the soil particles, which depend on the percentage of sand and moisture as in the rest of the sites under study (Figure 3).

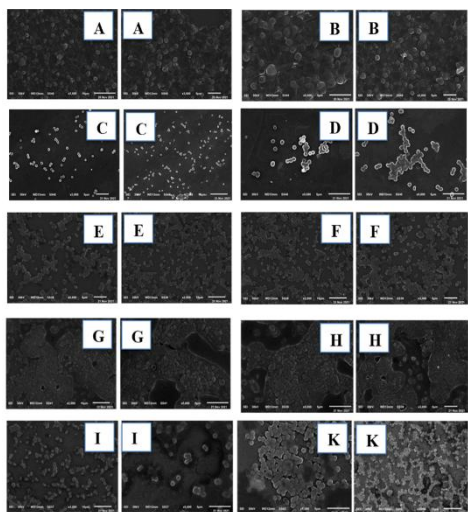
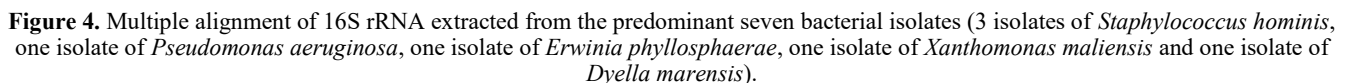


Figure 3. Images of microbial communities concerning soil characteristics. The image represents the microbial and the soil particle size detected in different governorates using SEM at a magnification of 100X, and the scale bar represents 500 μ m as follows: A) Al-Qunfudhah, B) Fair Capital, C) Jamoum, D) Jeddah, E) Kamil, F) Khulais, G) Laith, H) Rabigh, I) Taif, K) Torba.

The 16S rRNA extracted from the predominant seven bacterial isolates (3 isolates of *Staphylococcus hominis*, one isolate of *Pseudomonas aeruginosa*, one isolate of *Erwinia phyllosphaerae*, one isolate of *Xanthomonas maliensis* and one isolate of *Dyella marenensis*) was extracted and sequenced. 16S rRNA gene sequences of several bacterial strains that had substantial similarity with query sequences were obtained from the Gene Bank database, and multiple alignments using ClustalW were performed before performing phylogenetic analysis. With the aid of the MEGA 7 software program and the neighbor-joining technique, the resulting alignment was utilized to create a phylogenetic tree. The robustness of the phylogenetic tree building was assessed using the bootstrap percentage (1000 bootstrap replicon) (Figures 4 and 5).



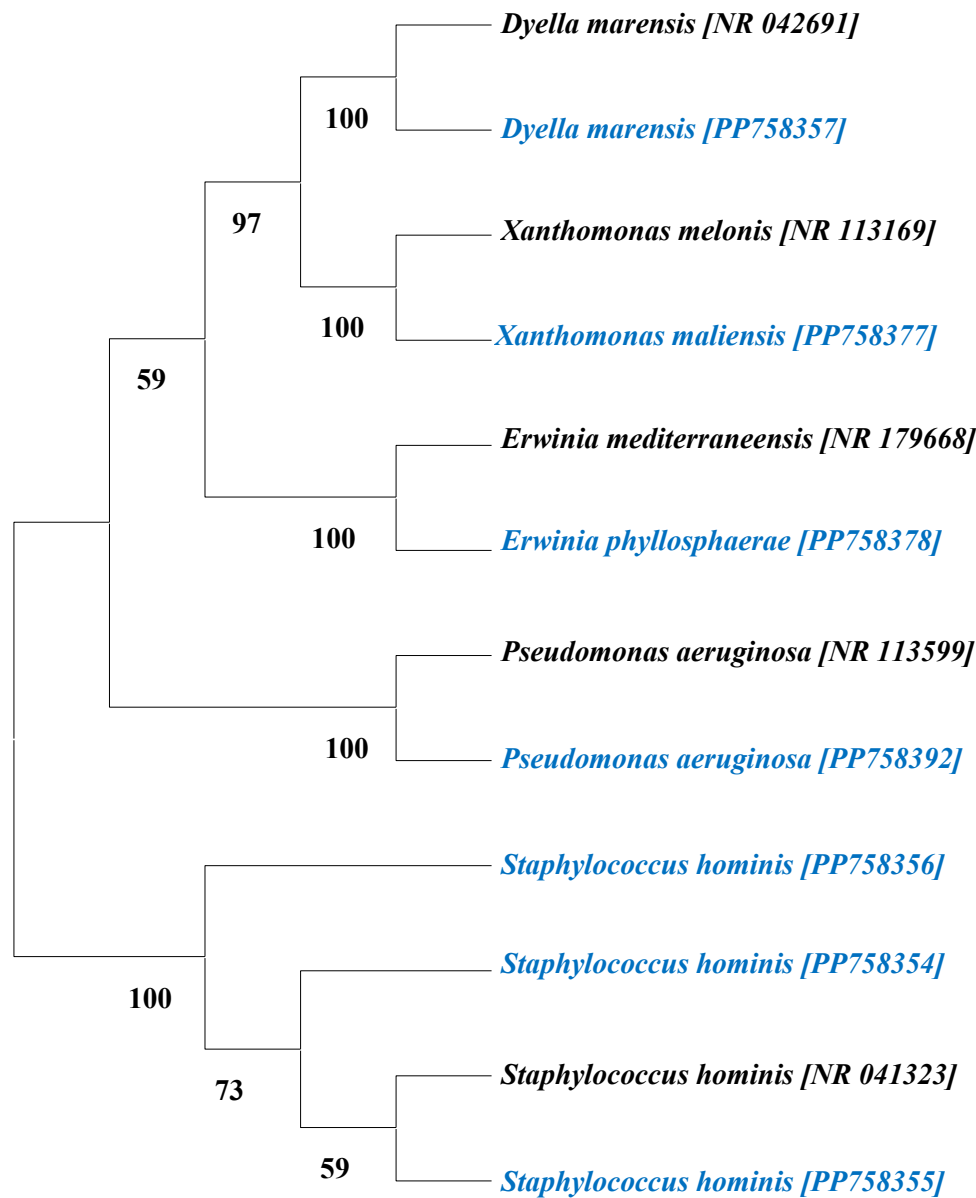


Figure 5. Phylogenetic analysis. Maximum likelihood phylogenetic tree of 16S rRNA extracted from the predominant seven bacterial isolates (3 isolates of *Staphylococcus hominis*, one isolate of *Pseudomonas aeruginosa*, one isolate of *Erwinia phyllosphaerae*, one isolate of *Xanthomonas maliensis* and one isolate of *Dyella marenensis*).

SNPs were detected in the seven bacterial isolates, and declared that the highest number of SNPs were found in *Pseudomonas aeruginosa* while the lowest number was in *Erwinia phyllosphaerae*. The highest type of SNPs was transversion (26) compared with transition (18). The highest transition SNP was

A→G (8), and the lowest transition SNP was G→A and T→C (3 for each). The highest transversion SNP was G→T, C→G, and A→C (5 for each), and the lowest transversion SNP was T→A and G→C (2 for each). The interchange of A→G or G→A was the highest (11) (Table 2).

Table 2. SNPs detected in the seven bacterial isolates and their positions compared to similar ones retrieved from the database

Query Organisms	Reference	Position	SNPs	Type
<i>Staphylococcus hominis</i> A3 (PP758354)	<i>Staphylococcus hominis</i> (NR 041323)	12	G → T	Transversion

		49	A → T	Transversion
		1042	A → G	Transition
<i>S. hominis</i> E2 (PP758355)		14	A → T	Transversion
		39	C → G	Transversion
		53	G → T	Transversion
		57	G → A	Transition
<i>S. hominis</i> E3 (PP758356)		24	C → A	Transversion
		38	G → T	Transversion
		476	T → C	Transition
		867	G → T	Transversion
		972	T → G	Transversion
		1068	A → G	Transition
		1086	A → C	Transversion
		1087	T → A	Transversion
<i>Dyella marensis</i> F5 (PP758357)	<i>Dyella marensis</i> (NR042691)	8	A → T	Transversion
		23	A → C	Transversion
		289	A → G	Transition
		644	T → C	Transition
		682	A → G	Transition
		954	A → G	Transition
		971	T → C	Transition
		1067	A → T	Transversion
		1068	G → A	Transition
		1069	T → G	Transversion
		1090	C → T	Transition
<i>Xanthomonas maliensis</i> F6 (PP758377)	<i>Xanthomonas maliensis</i> (NR113169)	50	A → C	Transversion
		52	A → G	Transition
		58	T → G	Transversion
		96	C → T	Transition
		97	C → G	Transversion
<i>Erwinia phyllosphaerae</i> S13 (PP758378)	<i>Erwinia phyllosphaerae</i> (NR179668)	51	A → C	Transversion
		114	A → G	Transition
<i>Pseudomonas aeruginosa</i> W3 (PP758392)	<i>Pseudomonas aeruginosa</i> (NR113599)	25	C → T	Transition
		34	A → G	Transition
		35	T → A	Transversion

		60	C	→	G	Transversion
		65	G	→	C	Transversion
		77	C	→	T	Transition
		82	A	→	T	Transversion
		88	T	→	G	Transversion
		102	A	→	C	Transversion
		114	G	→	C	Transversion
		126	A	→	T	Transversion
		129	C	→	G	Transversion
		139	C	→	G	Transversion
		153	G	→	T	Transversion

In addition, there were insertion and deletion mutations, the number of insertions was the highest in *Pseudomonas aeruginosa* (6), and the lowest was in *Erwinia phyllosphaerae* (one). There

was no insertion in *S. hominis* E3 and *Xanthomonas maliensis*. The deletion was the highest in *S. hominis* E2 (2), and there was no deletion in *Dyella marenensis* (Table 3).

Table 3. InDels detected in the seven bacterial isolates and their positions compared to similar ones retrieved from the database

Query Organisms	Reference	Position	REF (hit)	ALT (query)	Type
<i>Staphylococcus hominis</i> A3 (PP758354)	<i>Staphylococcus hominis</i> (NR041323)	1061	T	-	Deletion
<i>S. hominis</i> E2 (PP758355)		26	G	-	Deletion
		1083	-	G	Insertion
		1095	-	G	Insertion
		1106	T	-	Deletion
<i>S. hominis</i> E3 (PP758356)		11	G	-	Deletion
<i>Dyella marenensis</i> F5 (PP758357)	<i>Dyella marenensis</i> (NR042691)	1070	-	C	Insertion
		1078	-	G	Insertion
<i>Xanthomonas maliensis</i> F6 (PP758377)	<i>Xanthomonas maliensis</i> (NR113169)	12	T	-	Deletion
<i>Erwinia phyllosphaerae</i> S13 (PP758378)	<i>Erwinia phyllosphaerae</i> (NR179668)	14	G	-	Deletion
		1101	-	T	Insertion
<i>Pseudomonas aeruginosa</i> W3 (PP758392)	<i>Pseudomonas aeruginosa</i> (NR113599)	20	-	G	Insertion
		27	-	A	Insertion
		36	-	T	Insertion
		37	-	A	Insertion
		50	-	T	Insertion
		148	T	-	Deletion

		155	-	T	Insertion
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3. Discussion

Saudi Arabia's diverse habitats make a noteworthy contribution to the world's plant biodiversity in arid environments. This is reasonable given that Saudi Arabia is about two million square kilometers in size and experiences distinct weather variations from north to south. The inland marshes, also referred to as Sabkha or Chott, are among these environments (Al-Amro et al., 2018; Neffar et al., 2016).

It is well known that soil provides a rich home for several microbial populations. This led to differences in microbial kinds and densities between the Makkah and desert locations under investigation, with a few species constituting the core microbiome and others being site-specific. All soil samples were collected around neutrality; the highest was at Khulais (7.81), and the highest moisture percent was at Jeddah (12.0%). Sand is the dominant component of all soils. The soil ecosystem is complex and ever-changing, consisting of both living and non-living components. Typically, soil is composed of 45% minerals, 5% organic matter, and 20-30% each of water and air. There is a wide range of soil compositions due to geographical and climatic differences, which can vary daily due to factors such as water infiltration, climate, and human activities (Li et al., 2021; Voltr et al., 2021). In a recent study, different soil properties and depths were observed in isolated bacterial strains (Al-Yemeni et al., 2006). The presence of specific host plants may also play a role in the diversity of bacteria found in the soil, as they may select beneficial microbes to aid in their growth in harsh environments such as the deserts of Saudi Arabia (Eida et al., 2018). The combination of high temperatures and little moisture resources in hot deserts creates special adaptations that enable bacteria to survive in these harsh environments (Takacs-Vesbach et al., 2013; Makhallanyane et al., 2015). Essential ecosystem services that soil offers include biomass production, biodiversity preservation, drinking water filtration, and insect control. However, the general public is not well aware of the ecosystem services that soils provide (Brevik et al., 2019).

Bacterial diversity in the Makkah region was earlier investigated and well-documented (Alzahrani et al., 2021; Alharbi et al., 2021; Amasha et al., 2018). The highest bacterial incidence was in Fair Capital (20%), and the lowest (6%) was in Al-Qunfudhah. In Al-Qunfudhah governorate, the clay and silt percentages were highest, so the soil particles appeared coagulated and coherent. The decrease in clay percentage caused the soil particles to appear loose and scattered, in addition to the very low moisture in the Jamoum governorate. On the contrary, high silt and humidity caused the aggregation of soil particles in clumps, as in the Jeddah governorate.

The proportionate amount of sand, silt, and clay (mineral particles smaller than 2 mm) in the soil is known as its texture. This is established following the aggregates' physical disruption and chemical dispersion (Faúndez Urbina et al., 2023). Therefore, the amount and size of these mineral particles determine the particle size distribution (PSD) of soil, which is frequently determined by sieving and sedimentation studies (Carter et al., 2007). The primary elements influencing a soil's maximum capacity to store carbon and nitrogen are its silt and clay contents, as well as the mineralogy of the clay (Matus et al., 2021).

The findings of our study are backed by the evidence that alterations in moisture levels significantly impact microbial

diversity. This is especially prominent in soils with lower moisture levels, where it was observed that bacterial diversity is negatively affected by a higher percentage of sand and lower water-holding capacities. On the other hand, soils with a higher proportion of silt and clay, which typically have better water-holding abilities, were found to have a positive association with bacterial diversity (Lee et al., 2018; Skariah et al., 2023).

The 16S rRNA extracted from the predominant seven bacterial isolates (3 isolates of *Staphylococcus hominis*, one isolate of *Pseudomonas aeruginosa*, one isolate of *Erwinia phyllosphaerae*, one isolate of *Xanthomonas maliensis* and one isolate of *Dyella marensis*) was extracted and sequenced. 16S rRNA gene sequences of several bacterial strains that had substantial similarity with query sequences were obtained from the Gene Bank database.

The 16S rRNA sequences of our bacterial isolates were matched with the similar bacterial isolates retrieved from the gene bank database where *Staphylococcus hominis* strains showed 99.81%, 99.37 and 99.36% similarities, *Dyella marensis* isolate showed 99.0% similarity, *Pseudomonas aeruginosa* showed 80.45% similarity, *Xanthomonas maliensis* showed 99.55% similarity, *Erwinia phyllosphaerae* showed 98.39% similarity with that retrieved from database. Bacteria are considered the most vital component of the soil microbial community (Coleman-Derr et al., 2016). Nearly similar results were observed when *Bacillus subtilis* and *B. amyloliquefaciens* were isolated from soil (Nanamiya et al., 2008; Zhang et al., 2003). A different study, which conducted a comprehensive examination of *B. subtilis* transcriptional reactions (Krishnan et al., 2005), revealed that the *B. axarquiensis* and *B. malacitensis* strains cannot be differentiated from the *B. mojavensis* strain (Wang et al., 2007).

According to research, when bacteria are exposed to environmental pressures such as a lack of amino acids, their metabolism shifts towards survival, and ribosomal protein synthesis decreases (Yus et al., 2009). This is a way for the cells to conserve energy and avoid investing too many resources in ribosome synthesis, which can be costly (Krásný et al., 2004). As a result, if bacterial cells are experiencing a shortage of amino acids during transient growth, it is expected that genes involved in protein synthesis will be further repressed rather than induced (Bais et al., 2006). Previous studies have shown that strains of *B. axarquiensis* and *B. malacitensis* have been found to have a genetic similarity lower than 46.9% with strains of other closely related species (Ruiz-Garcia et al., 2005), which is significantly lower than the values (83-99%) observed in our research.

Bacillus species are round-ended rods that are Gram-positive, aerobic, and can be found either singly or in pairs. They may also appear in short chains or filaments. These bacteria possess peritrichous flagella, which enables them to move. The endospores are mainly elliptical and are in subterminal positions within sporangia that are not swollen. There are no parasporal crystals or accumulation of poly-hydroxybutyrate (PHB). These bacteria can grow within a temperature range of 15 to 45°C and a pH range of 5 to 10. They are also able to tolerate high salt concentrations (mixture of sea salts) of up to 12% w/v. The optimal temperature for their growth is 32°C (Logan et al., 2015).

The highest number of SNPs were found in *Pseudomonas aeruginosa* while the lowest number was in *Erwinia phyllosphaerae*. The highest type of SNPs was transversion, the

highest transition SNP was A→G, and the highest transversion SNP was G→T, C→G, and A→C. The number of insertions was the highest in *Pseudomonas aeruginosa*, and there was no insertion in *S. hominis* E3 and *Xanthomonas maliensis*, while the deletion was the highest in *S. hominis* E2 (2) and there was no deletion in *Dyella marensis*.

The identification of species may be facilitated by SNP detection and analysis of mutation heterogeneity within or across species, mainly when this information is critical to the development of primers and probes that are specific to a given species (Abdella et al., 2023). In the cases of *B. cereus* and *B. subtilis*, the SNP analysis yielded more intra-specific information than the phylogenetic analysis (Fernández-No et al., 2015).

2. Conclusion

The current study's findings showed that different parts of the Makkah region in Saudi Arabia have very diverse microbial communities with varying soil physiochemical properties. The greater diversity of bacteria may reflect the soil's capacity to endure harsh environments. The most recent research showed that some microorganisms are unique to a particular place. The trend of changes in soil factors in connection to bacterial dispersion patterns is often inconsistent. The importance of such information will be of significant benefit for comprehending the ecology of bacterial habitats, and it will then be simple to develop a unique procedure to deal with such bacteria. Additionally, research on rare fungus species that have been separated individually may be

significant in supplying important biological components for therapeutic use.

Acknowledgments

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Data Availability

All the relevant data have been provided in the manuscript.

Compliance with Ethical Standards

- **Conflict of Interest:** The author declares that I have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- **Ethical approval:** Not applicable.
- **Consent to Publish:** The author has consent to publish the paper.
- **Consent to participate:** The author has consent to participate in the study.

Statement of informed consent: The research does not involve human participants and animal experiments.

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