Microbial diversity and abundance in the hot springs on the west coast of Saudi Arabia as a potential source of novel industrial products

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ABSTRACT

A knowledge gap exists regarding the potential of thermophilic bacteria in the hot springs in Saudi Arabia as a resource for medically useful compounds. This study aimed to investigate the microbial diversity in the soils of hot springs at three locations on the west coast of Saudi Arabia, including Jizan, Al Lith, and Tabuk. Soil samples were extracted for rDNA 16S, and the results were analysed for microbial diversity and abundance. The results showed the dominance of Acidobacteriota, Actinobacteriota, Desulfobacterota, Firmicutes, Halanaerobiaeota, Nitrospirota, and Thermotogota. Actinobacteriota dominated the microbial population in Al Lith soil, while Deinococcota were dominant in Jizan soil and Chloroflexi in Tabuk soil. Firmicutes and Proteobacteria had high microbial populations in all three soils. Based on principal coordinate analysis, the normalized relative microbial diversity of all soils showed differences as well as similarities in the distribution of taxonomic classifications between soil samples. In conclusion, the microbial diversity in Jizan, Al Lith, and Tabuk included a variety of microorganism species that can potentially be used in biotechnology as a novel source for industrial production of medically useful compounds.

Introduction

The chemical and physical features of hot springs, which are consistently among the hottest sites in the world, allow for the growth of thermophilic bacteria (Li et al. 2021; Simmons et al. 2021). A thermophile is an organism that has evolved to thrive in temperatures ranging from 45°C to 80°C. Such challenging environments make thermophilic bacteria highly diverse, as shown by their molecular phylogeny, and a rich source of secondary metabolites (Dobretsov et al. 2011; Gurunathan et al. 2021). Some of these metabolites could serve as new chemotherapeutics, which are needed to treat emerging diseases and other threats to public health. In particular, microbial diseases are constantly evolving and can acquire resistance to existing antibiotics; thus, new and efficient antimicrobials are needed to combat these emerging

threats (Al-Daghistani et al. 2021; Nas et al. 2021). Thermophiles have recently drawn much attention owing to their secondary metabolism, but the biological activity of Saudi Arabia's hot springs has received relatively little research attention. A knowledge gap exists regarding the potential of these hot springs as a resource for medically useful secondary metabolites.

Microbial communities that inhabit the soil-water-sediment of hot springs contribute to a continuum in these coastal environments and providing ecosystem services. Microbial biomonitoring methods are still in their infancy, but their adaptation to this environment stressors, lead the organic compounds and the elements to involve to their cycle and enable the organisms to survival in such environment. Researchers in Saudi Arabia have used chemical and molecular techniques such as 16S rRNA

gene metabarcoding to examine how contaminants affect microbial communities in the aquatic network of lakes, which is heavily affected by human activity (Aabed et al. 2021; Al-Thobaiti et al. 2021; Yasir et al. 2021).

Thermal spring bacteria from Saudi Arabia's southern region have been studied for their bioactivity. During the dry (summer) and wet (winter) seasons, samples were collected from three locations. Fifty microorganisms displayed antimicrobial activity against pathogenic microorganisms, and four isolates demonstrated maximum antagonistic ability against all pathogens. Based on molecular identification and phylogenetic analysis, these isolates were confirmed to be *Bacillus sonorensis, B. thermocopriae, Brevibacillus borstelensis and B. parabrevis* (Alrumman et al. 2019). Analysis of the cell-free extracts by GC-MS revealed the presence of 40 chemical compounds, with some having industrial and medicinal value, as well as secondary metabolites of these 40 strains. Cyclohexyl acrylate and imiloxan were the most common secondary metabolites, and cyclohexasiloxane, dodecamethyl, tabtoxinine-(beta)-lactam, and defensin, as well as etomidate and nicotine alcohol, mephenesin, and nicotine lactate, were naturally occurring in these hot springs (Alrumman et al., 2019). These findings suggest that Saudi Arabia's hot springs could serve as a source of new bioactive chemicals of industrial and medical interest.

Researching bacterial communities is challenging because of their complexity and the possible difficulties in cultivating many of their members. Identifying and characterizing microorganisms at the species and the subspecies levels are possible owing to developments in molecular biology techniques, rep-PCR profiling, and 16S rRNA sequencing. A relatively quick and effective detection method, such as a PCR-based approach for the direct collection and analysis of 16S rRNA gene sequences from the surroundings, does not yet exist (Krych et al. 2018; Kushkevych et al. 2021). Still, the existing molecular biology methods improve identification beyond what is possible with conventional technology.

Sequence analysis of the 16S rRNA gene has helped to identify isolates from hot springs. In a study of sediments from six hot springs in Saudi Arabia, the bacterial diversity of 536 isolates was assessed by 16S rRNA gene analysis. About six novel species were detected, and *Bacillus* species were found to be common in the six hot springs. Moreover, 16 of the isolated thermophilic bacteria had the ability to grow at a temperature of 60°C or greater, and 21 of the isolates had hydrolytic enzyme activity (Yasir, et al. 2019).

Another study in Saudi Arabia investigated hot springs in Jazan and Bani Malik, and two thermophilic bacteria were isolated and identified through enzymatic analysis as *Brevibacterium linens* and *Bacillus subtilis* (El-Gayar et al. 2017). In the current study, we aimed to isolate 16S rDNA from Jizan, Al Lith, and Tabuk hot springs soils and to analyse the microbial diversity in these locations. We also compared the abundance of microorganisms in the soils from the three locations for further investigation of novel microorganisms or microbial products that could be used in microbial industry applications or biotechnology.

Materials and Methods

Sample collection
Hot springs in Jizan, Al Lith, and Tabuk along the west coast of Saudi Arabia were chosen as study sites for the isolation of thermophilic microorganisms from sediment. Soil samples were collected in sterilized screw-lid glass containers, transported to the laboratory, and kept at 4°C in a refrigerator till further processing. The samples were collected from various locations at each site, with the most common samples being soil particles from the surface and at a depth of 1–2 cm. 10g from each soil sample was stored in sterilized tube and transferred to the SGS Laboratories Division, Water and Environment Laboratory, Chemical and Physical Analysis Unit, Jeddah, Saudi Arabia (available on Laboratories (sgs.org.sa)) and then analysed them physical and chemical.

Molecular identification of microbial community of hot springs
Soil DNA was extracted using the Qiagen DNeasy PowerSoil (50) kit (QIAGEN Company) following the manufacturer's protocol (Adjeroud *et al*., 2020). For genomic DNA isolation, 0.25 g from each soil sample was placed in a PowerBead tube provided in the kit and then mixed by vortex. Sixty microliters of solution C1 was added to the sample and briefly vortexed. Twenty-four 1.5- to 2.0-ml PowerBead tubes were secured in a vortex adapter. Each sample was mixed by vortex at maximum speed for 10 min, followed by centrifugation at $10,000 \times g$ for 30 s. Next, 400–500 µl of supernatant was transferred to a clean 2-ml collection tube, 250 µl of Solution C2 was added, and the mixture was vortexed for 5 s. The tubes were placed at 2–8°C for 5 min and then centrifuged at $10,000 \times g$ for 1 min. Up to 600 µl of supernatant was transferred to a clean 2-ml collection tube and 1200 µl of solution C4 was added to the supernatant, followed by vortexing for 5 s. Next, 675 µl of the mixture was loaded on the MB spin column from the PowerSoil kit, which was then centrifuged at $10,000 \times g$ for 1 min twice. Five hundred microliters of solution C5 was added to the MB

spin column, centrifugation was done for 30 s at 10,000 × g, the flow was discarded, and the column was centrifuged for 1 min at 10,000 × g. The MB spin column was then placed in a clean 2-ml collection tube, 100 μl of solution C6 was added to the centre of the white filter membrane, and the tube was centrifuged at room temperature for 30 s at 10,000 × g. The MB spin column was discarded, and the DNA in the tube was ready for downstream applications (Qiagen 2017).

The prepared libraries were quantified using qPCR according to the Illumina qPCR Quantification Protocol Guide as previous (Guo et al. 2017). The Illumina NGS workflow was performed by Macrogen. After quality control (QC) is performed, qualified samples proceed to library construction. The preparation of sequencing library is done by random of DNA or cDNA sample fragments, then followed by 5′ and 3′ adapter ligation. Adapter-ligated fragments are then PCR amplified and gel purified. Cluster generation was sequenced, the loaded of library into flow cell that fragments, then fragments amplified to be clear, and generating clusters through bridge amplification. After completion of cluster generation, templates are ready for sequencing. Illumina SBS technology utilizes reversible terminator-based method by detecting single bases as they are incorporated into DNA template strands. This process yields highly accurate base-by-base sequencing and virtually eliminates sequence-context-specific errors, even in repetitive sequence regions and homopolymers. Sequencing data were converted into raw data for the analysis (Stone et al. 2018; Fariq et al. 2021).

The 16S gene was amplified by PCR using the following forward and reverse primers:
518F 5′- CCAGCAGCCGCGGTAATACG-3′; 800R 5′-TACCAGGGTATCTAATCC-3′;
27F 5′-AGAGTTTGATCMTGGCTCAG-3′; and 1492R 5′-GGTTACCTTGTTACGACTT-3′.

The primers for the amplification of the 16S gene were designed based on the conserved regions in the 518F–800R and 27F–1492R genome sequences (Guillen et al. 2016). Library size was determined to verify the size of the PCR-enriched fragments, and the template size was checked for distribution by running on an Agilent Technologies 2100 bioanalyzer using a DNA 1000 chip. Further, library quantity was checked by illumine library to achieve the highest quality of data; on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. Macrogen sequenced the extracted DNA (Available online: https://www.macrogenusa.com/(accessed on 15 July 2021). The sequences were analysed by BLAST-NCBI (Available online: http://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 7

December 2021), and the diagram of the 16S DNA of the soil samples from the three locations was obtained by JGI GOLD | Home (doe.gov) (accessed on 12 February 2022).

Statistical Analysis and Diversity Indices

Phylogenetic indices serve as a quantitative index that shows how many different species are found in a dataset (a community), as well as the phylogenetic relationships (co-distribution, species affinity, species richness) between distributed individuals. After sequencing, FastQC program was used for quality control steps. According to the quality control results, data amounts, read qualities, GC distributions, kmer distributions, possible adapter contamination of each sample were determined and evaluated (Andrews 2010). After quality control, reads with poor read quality (Phred Score <Q20, 30 bp window range) were removed from all data. Further, low-quality base read at terminal regions, chimeric sequences with possible adapter contaminants were removed by using Trimmomatic tool and Genomes OnLine Database (GOLD) (Bolger et al. 2014). Taxonomic profiling was performed using the Kraken2 (Wood et al. 2019; Silva 2020) database was used as a reference dataset (Quast et al. 2013). OTU groups in each sample were determined after alignment. R scripts were used in data reporting, statistical analysis, and data visualization (https://www.R-project.org/).

Results

The temperature and pH were recorded at the time of sampling. The temperature and pH at Jizan were 60°C and pH 4; Tabuk, 50°C and pH 3; and Al Lith, 80°C and pH 5. In addition, Cl, HCO_3, NH_4, were detected with the values (mg/kg): Cl 858.10, 685.40, and 16.39, HCO_3 231.40, 9110.0, and 9.0, NH_4 ≤0.013, ≤0.013, and 14.40 for Jizan, Al Lith and Tabuk respectively. The microbial diversity of the three hot springs was analysed for the maximum reads of bacterial communities, and the results are presented in Figure 1. Tabuk soil had the largest number of reads, while Jizan showed the lowest number of reads. Further, Tabuk and Al Lith showed reads for unknown bacteria.

The results in Table 1 show that Tabuk had the highest numbers of reads in each taxonomic unit, while Jizan had the lowest numbers of reads in each taxonomic unit.

Table 1. Number of reads in Jizan, Al Lith, and Tabuk for each taxonomic unit

Soil ID	Kingdom	Phylum	Class	Order	Family	Genus
Jizan	73,734	391,273	308,901	226,604	148,579	73,012
Al Lith	572,089	472,158	372,288	272,517	173,161	82,772
Tabuk	655,086	536,797	418,566	300,396	186,526	84,435

Figure 2 shows the 20 most abundant genera in Jizan, Al Lith, and Tabuk, including *Acinetobacter*, *Bacillus*, and *Sphingomonas*, which had high percentages of relative abundance. These genera were followed by uncultured microorganisms, *Paenibacillus*, *Flavobacterium*, *Pullulanibacillus*, and finally, the genera *Thermus*, *Anoxybacillus*, *Arthrobacter*, *Geobacillus*, *Caldicellulosiruptor*, *Roseovarius*, *Microbacterium*, *Dictyoglomus*, *Fervidobacterium*, *Rhodothermus*, *Mycoplasma*, *Pseudarthrobacter*, and SEEP-SRB1.
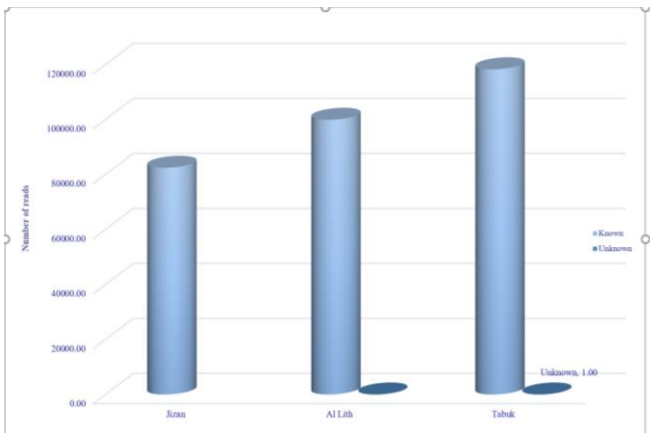

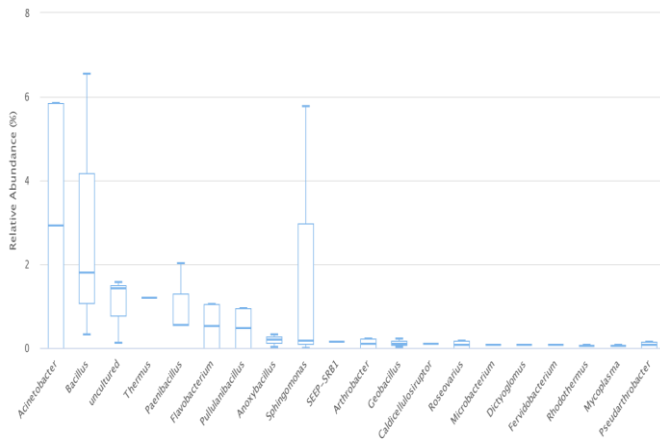**Fig. 1**. Number of reads in Jizan, Al Lith and Tabuk soil samples.

Fig 2. The top 20 genera with the highest relative abundance in Jizan, Al Lith and Tabuk

As a result of metagenomics analysis, a total of 61 phyla were identified from Jizan, Al Lith, and Tabuk soils. The 20 phyla with the highest abundance in all soils are shown in Figure 3. They included the most prominent in the hot springs functions; Acidobacteriota, Actinobacteriota, Aquificota, Armatimonadota, Bacteroidota, Chloroflexi, Cyanobacteria, Deinococcota, Desulfobacterota, Firmicutes, Halanaerobiaeota, Nitrospirota, Patescibacteria, Planctomycetota,

Proteobacteria, and Thermotogota. The most numerous of bacterial populations were Actinobacteriota in Al Lith soil, Bacteroidota and Deinococcota in Jizan soil, and Chloroflexi in Tabuk soil, while Firmicutes and Proteobacteria had high microbial populations at all three sites. Thermotogota and Halobacterota were common in Jizan and Al Lith soils, while Euryarchaeota was common in Al Lith and Tabuk soils. Further, a total of 130 classes were identified from the three sites, which had 40 classes in a common, and the 20 highest abundances are shown in Figure 4, with the most prominent being Actinobacteria, Bacilli, Bacteroidia, Chloroflexia, Cyanobacteria, Desulfobacteria, Desulfobulbia, Gammaproteobacteria, Rhodothermia, Symbiobacteriia, Thermoanaerobacteria, and Thermotogae. The classes Bdellovibrionia, Deferribacteres, and Rhodothermia were common to Jizan and Tabuk; Thermotogae and Methylomirabilia were common to both Jizan and Al Lith; and Thermoanaerobaculia was common to both Al Lith and Tabuk.
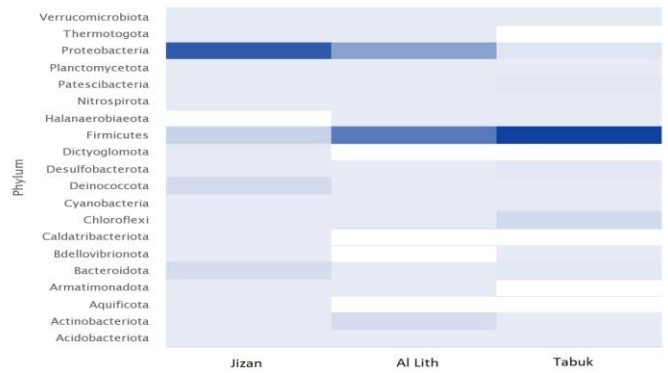

Fig 3. The 20 phyla with the highest abundances in Jizan, Al Lith, and Tabuk.
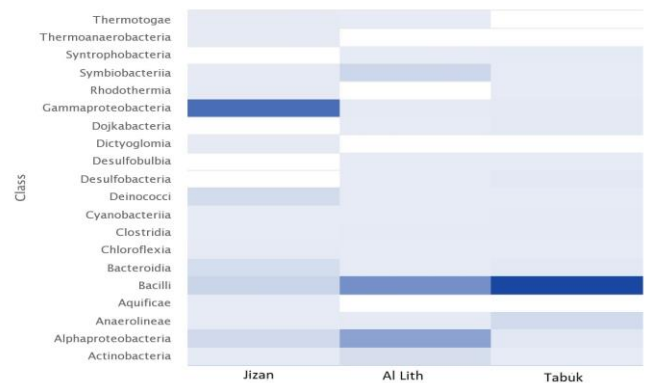

Fig 4. The 20 classes with the highest abundances in Jizan, Al Lith, and Tabuk.

Figure 5 shows the 20 orders with the highest abundances, and a total of 304 orders were identified in Jizan, Al Lith, and Tabuk soils, with 86 orders being common in all three locations. Further, the highest abundances were found for Bacillales, Desulfobacterales, Enterobacterales, Lactobacillales, Pseudomonadales, Rhizobiales, Rhodobacterales, Symbiobacteriales, Thermales, and Thermotogales. The orders Beggiatoales, Caldicoprobacterales, Gammaproteobacteria incertae sedis, and Thiomicrospirales were common in Jizan and Tabuk soils; the orders Thiohalorhabdales, Thermosynechococcales, and Thermomicrobiales were common in Jizan and Al Lith soils; and Acholeplasmatales, Aminicenantales, and Desulfobaccales were common in Al Lith and Tabuk soils.
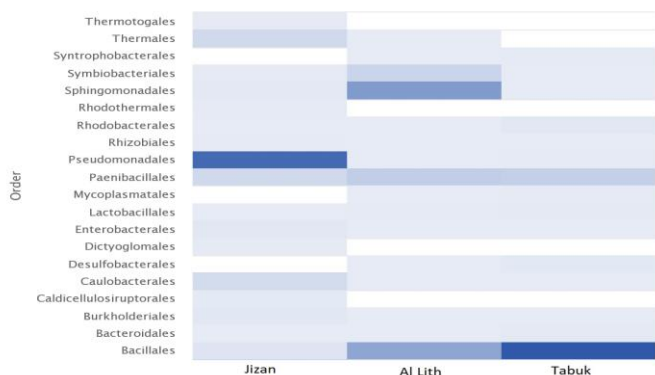

Fig 5. The 20 orders with the highest abundances in Jizan, Al Lith, and Tabuk

A total of 447 families were determined in Jizan, Al Lith, and Tabuk soils, and 109 families were common in soils from all three locations. The 20 most abundant families in Jizan, Al Lith, and Tabuk soils are shown in Figure 6, with the most prominent being Bacillaceae, Desulfosarcinaceae, Pseudomonadaceae, Rhodothermaceae, Sedimenticolaceae, Symbiobacteraceae, Thermaceae, and Thermomicrobiaceae. The families Acidaminococcaceae, Aeromonadaceae, and Desulfovibrionaceae were common in Jizan and Tabuk, while the families Hydrogenophilaceae, Marinifilaceae, and Oscillatoriaceae were common in Jizan and Al Lith soils. The families Desulfococcaceae and Magnetospiraceae were common in Al Lith and Tabuk soils. Halobacteroidaceae was only detected in Al Lith soil. At the genus level, a total of 1014 genera were identified in Jizan, Al Lith, and Tabuk soils, and 120 genera were common to all three locations. The 20 genera with the highest abundances are shown in Figure 7, with the most prominent being *Arthrobacter*,

Bacillus, *Dictyoglomus*, *Fervidobacterium*, *Flavobacterium*, *Geobacillus*, *Pseudarthrobacter*, *Rhodothermus*, and *Thermus* uncultured. Moreover, *Actinobacillus*, *Arthrobacter*, and *Cellulomonas* were common in Jizan and Tabuk soils; *Desulfovibrio* and *Halochromatium* were common in Jizan and Al Lith; and [*Eubacterium*] *eligens* group, *Lachnoclostridium*, and *Nitrospira* were common in Al Lith and Tabuk soils.
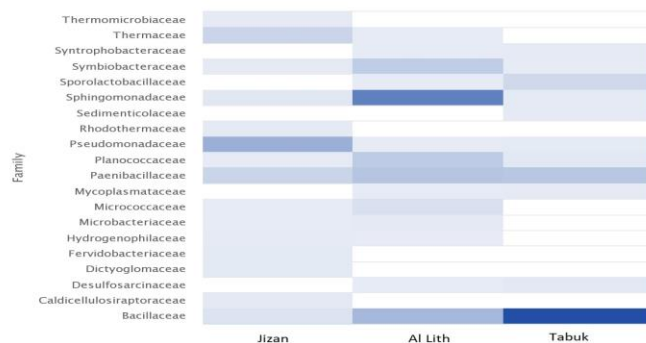

Fig 6. The 20 families with the highest abundances in Jizan, Al Lith, and Tabuk.

Fig 7. The 20 genera with the highest abundances in Jizan, Al Lith, and Tabuk

The correlation of the relative abundance of the dominant phylotype with soil location and the relationship between the reads and species numbers, the principal coordinate analysis, and the normalization of the relative microbial diversity of Jizan, Al Lith, and Tabuk soils are shown in Figure 8 (A, B, C). Figure 8A shows a positive relationship between the reads and species numbers; as the number of reads increases, the number of species that can be identified increases as well. Moreover, the variety of these species are being rapidly investigated as evidenced by the graph's steep slope on the left. When the curve begins to flatten toward the right, it is a sign that a sufficient number of bacteria have been sampled. New species are less likely to be discovered when the sample

density flattens. After an initial spike, rarefaction curves often level off as the number of new species per read decreases. Based on the principal coordinate analysis (PCoA), the normalized relative microbial diversity of all soils shows differences and similarities in the distribution of taxonomic classifications between soil samples; within the plot, cluster-forming soil samples show a similar microbial distribution. The microbial profile at the genus level was taken as a basis for the PCoA calculation (Figure 8B). Further, soil samples showing a similar microbial profile distribution in hierarchical clustering are shown in branches close to each other (Figure 8C).
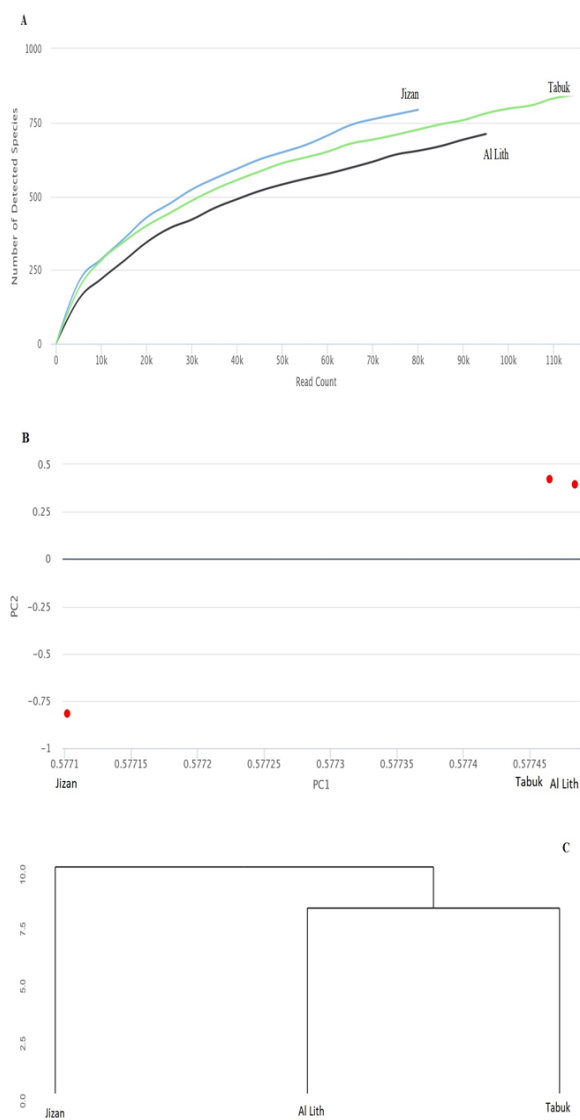

Fig 8. (A)The correlation of reads number and the identification of species, (B) the principal coordinate analysis (PCoA), and (C) the hierarchical clustering.

Discussion

There are only a few places worldwide where thermophilic organisms may thrive in the presence of geothermal energy. Three locations with hot springs on the west coast of Saudi Arabia were investigated to detect microbial diversity in the soil. To understand to diversity of the microorganisms in each location physical and chemical analysis of some soil parameters were detected and the results agreed with several studies (Hamilton & Having 2022; Mathan Kumar et al. 2022; Oestreicher et al. 2022; Yuan et al. 2022). Microorganism populations in hot springs may be rarely identified because of the harsh conditions where they occur or because culture-dependent identification methods can only identify a small fraction of their whole communities (Rashad & Abdel-Azeem 2017; Medhavi et al. 2018; Fachrial et al., 2020).

Microbial diversity can differ between locations due to physical and chemical conditions. Several studies have shown that microorganisms adapt to their environment based on the temperature and water activity. In a comparison of 1114 soil samples derived from the Antarctic, Arctic, Tibetan Plateau, and nonpolar regions, microbial diversity was found to increase from the Antarctic to non-polar regions and from the winter to the summer (Ji et al. 2022). Thus, temperature could act as a controlling key of microbial diversity in soils, which also reflects other environmental factors, such as pH, water availability, and vegetation biomass (Robinson et al. 2018), as well as the concentrations of carbon, nitrogen and soluble anions (Fernández-Martínez et al. 2021). The microbial diversity in one habitat ranges from aerobic to anaerobic and from autotroph and heterotroph in the soil cycle. This variation influences the degradation of organic compounds and polymers. The decomposition of organic compounds and the availability of inorganic compounds increase the percentage of autotrophic bacteria, as well as the difference in the percentages of dissolved gases in the soil water (Peng et al. 2022). In the presence of such bacteria the bioactivity in thermos-ecosystem allows the complete and continuity of the elements cycle in the soil, which reflect on microbial diversity in these environments. as an example of extreme environment, a study on oil refining waste degradation and under harsh conditions include high temperature this study examined the degradation of oil refining waste were the bacterial communities acclimated in temperature reach 60°C, and results showed *Bacillus* and *Geobacillus* sustainable degradation ability in the presence and absence of oxygen (Peng et al. 2022). Several studies have investigated the bioactivity of *Bacillus* isolated from hot springs in Saudi Arabia, these bacteria showed antimicrobial activity against pathogenic bacteria include *Bacillus cereus*,

Staphylococcus aureus, Enterococcus faecalis, *Streptococcus agalactiae*, *Klebsiella pneumonia*, *Shigella flexneri, Proteus mirabilis, Cryptococcus neoformans*, *Trichophyton mentagrophytes, Candida albicans* and *Aspergillus niger* (Al-Dhabi et al. 2019; Alrumman et al. 2019). Further, the high ratio of Firmicutes population in the soil from the three sites in the current study could be due to their high resistance to environmental stresses through forming spores and resting cells under abnormal environmental conditions. These abilities allow the bacteria to survive harsh conditions, and our results agree with the finding of previous studies on this topic (Parkes & Sass 2009; Bottos et al. 2014; Schmidt, 2019). *Bacillus* was common in all types of soil and was detected in Jizan, Al Lith, and Tabuk; these findings accorded with the results of studies focused on extreme soils (Alrumman et al. 2018). Further, prokaryotes, including bacteria and archaea, were the main source of microbial diversity. The detection of unknown microorganisms that were associated with these extreme environments was a novel finding, and our finding of unknown microorganisms in Al Lith and Tabuk offers the possibility new microorganisms with potential biotechnology applications. Previous studies have shown applications for such novel microorganisms (El-Gayar et al. 2017; Alrumman et al. 2018, 2019; Yasir et al. 2019). Further, thermophilic bacteria were effective in wastewater treatment especially in the treatment with high temperature (Al-rasheedi et al. 2022). In Egypt, 6 thermophilic enzymes were isolated from thermophilic bacteria from thermophilic hot springs include Pharaoh Baths, Ras Sedr, Kharga Oasis and Dakhla Oasis, protease-,lipase- and amylase-producing thermophiles utilized diverse of products that modified several bio-industrial applications (Kahled et al. 2022).

Microorganisms were able to colonize and demonstrate rapid growth in the extreme environments in our study. Based on the high temperature in Jizan and Al Lith compared with the temperature in Tabuk, our results agreed with those from a previous study (Yasir et al. 2017). Cyanobacteria and Actinomycetes were isolated in studies on hot springs (Hug et al. 2018; Abdelwahab & Amin 2019). Halobacteria, which belong to the family Halobacteriaceae, were detected in Al Lith. The presence of this archaea in Al Lith may be due to the temperature of this hot spring and the pH, which are close to the optimum conditions for archaeal growth. This family has been detected in several locations, including the eastern lowlands of Eritrea, Lake and Little Magadi hot springs in Kenya, and Yunnan and Tibetan hot springs in China (Song et al., 2013; Kambura et al. 2016; Ghilamicael et al. 2017). Given the microbial functions in soil, certain microbial species under particular environmental conditions could improve soil fertility by fixing nitrogen; thus, the presence of nitrogen-fixing cyanobacteria and archaea in Jizan, Al Lith, and Tabuk agree with the results of (Al-Sodany et al. 2018; Al-Quwaie 2020). The high abundance of Oscillatoriaceae in Jizan and Al Lith and *Nitrospira* in Al Lith and Tabuk impact on soil content and texture due to the complete cycles of carbon, nitrogen and hydrogen (Li et al. 2019; Hayden et al. 2021). The presence of Desulfococcaceae in Al Lith and Tabuk explain the increase of the pH value compared to Jizan pH, since the optimal pH for growth is 7.1 (Reischl et al. 2018). Previous studies detected the presence of Desulfococcaceae in similar environments (Bonch-Osmolovskaya et al. 2018; Iacono et al. 2020). The smallest known living organisms are the mycoplasmas, classified as Mollicutes, and belong to the order Mycoplasmatales. They do not have a cell wall like bacteria do, but are instead surrounded by a membrane. This explains why they are so diverse in appearance. Sterol is required for the growth of the majority of mycoplasmas. In this case, they have the lowest relative abundance (Borroni et al. 2019; Green et al. 2021). In conclusion, data can demonstrate that nutrient-depleted soil environments are extensively colonized by microorganisms capable of actively participating in various biogeochemical cycles, particularly the nitrogen cycle. Moreover, microorganisms isolated from these hot springs give a promising on finding new hydrolytic enzymes, antimicrobial, anticancer and antioxidants products, further studies on isolated microorganisms are needed.

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