



Original article

Microbial Diversity of Some Sabkha and Desert Sites in Saudi Arabia

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ABSTRACT

Several studies isolated fungal and bacterial species from extreme environments, such as Sabkha and hot deserts, as their natural habitat, some of which are of medicinal importance. Current research aimed investigating the microbial (fungi and bacteria) diversity and abundance in Sabkha and desert areas in Saudi Arabia. Soil samples from nine different geographical areas (Al-Aushazia lake, AlQasab, AlKasar, Tabuk, Al-Kharj, Al-Madina, Jubail, Taif and Abqaiq) were collected and cultured for microbial isolation. Isolated fungi and bacteria were identified by molecular techniques (PCR and sequencing). Based on 18S rDNA sequencing, 203 fungal species belonging to 33 genera were identified. The most common fungal genera were *Fusarium*, *Alternaria*, *Chaetomium*, *Aspergillus Cochliobolus* and *Penicillium*, while the most common species were *Chaetomium globosum* and *Fusarium oxysporum*. By 16S rDNA sequencing 22 bacterial species belonging to only two genera, *Bacillus* and *Lactobacillus*, were identified. The most commonly isolated bacterial species were *Bacillus subtilis* and *Lactobacillus murinus*. Some fungal species were confined to specific locations, such as *Actinomyces elegans*, *Fusarium proliferatum*, *Gymnoascus reesii* and *Myzostoma* spp. that were only isolated from Al-Aushazia soil. AlQasab soil had the highest microbial diversity among other areas with abundances of 23.5% and 4.4% of total fungi, and bacteria, respectively. Findings of this study show a higher degree of fungal diversity than that of bacteria in all studied areas. Further studies needed to investigate the connection between some isolated species and their habitat ecology, as well as to identify those of medicinal importance.

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1. Introduction

It has long been believed that extremes of temperature, particularly in cold and dry areas, of the world are not hostile for any forms of biological life. However, many studies have reported that deserts are full of life (Cary et al., 2010). Such reports have even described the hot desert soil as a habitat of some fungal species.

The Sabkha environment is an extremely flat, saline, evaporative area along the coasts that forms widely in the Arabian Gulf and many parts of Saudi Arabia (Al-Amoudi, 1992). It has been

reported that the soil type in this environment is unusual for its neutral pH with lots of calcium carbonate deposition and calcium sulfate, which is very much prone to water (Arifuzzaman et al., 2016). Fungal growth was reported under conditions of low salt concentrations, varying degrees of temperature between 30 and 45 °C and low to moderate NaCl concentrations between 0.5 and 10% (Al Tamie, 2014). Salinity, pH, moisture and available nitrogen were found to be the key determinants for different growth patterns of vegetation in coastal plains (Li et al., 2008).

However, human-induced contamination ultimately caused the growth of *Penicillium* spp. and other ubiquitous fungi in the desert soil (Sterflinger et al., 2012). A recent study conducted in Jordan showed that *Aspergillus niger*, *Aspergillus tubingensis*, *Alternaria tenuissima*, *Alternaria alternate*, *Alternaria gaisen*, *Rhizopus stolonifer*, *Penicillium citrinum*, and *Fusarium oxysporum* were the commonly isolated fungi from the desert (Alsohaili and Bani-Hasan, 2018).

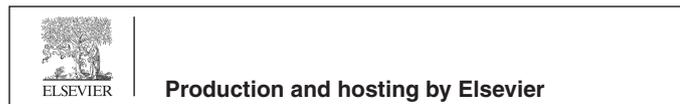
Earlier studies in Saudi Arabia showed that halophilic fungi including *Aspergillus*, *Penicillium*, *Drechslera*, and *Ulocladium* were the common isolated fungal genera (Abdel-Hafez, 1981). Further studies reported the isolation of osmophilic fungi including

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Aspergillus, *Alternaria*, *Penicillium*, *Cephaosporium*, *Acremonium* and *Botryotrichum* from desert soils (Abdel-Hafez, 1982a). Among these genera, *A. fumigatus*, *A. terreus*, *A. niger*, *A. flavus*, *P. citrinum*, *P. coryphilum*, *B. atrogriseum*, *U. botrytis*, *M. phaseoli*, *R. stolnifer*, *F. moniliforme*, *A. alternate* and *C. herbarum* were the most commonly isolated species (Abdel-Hafez, 1982b). Thermophilic and thermo-tolerant fungal species including *Aspergillus fumigatus*, *A. terreus*, *Humicola grisea* var. *thermoidae* and *Chaetomiurn thermophile* var. *copropile*, *A. fumigatus*, *C. thermophile* var. *copropile*, *A. terreus*, *A. nidulans* and *C. thermophile* var. *dissitum* were also reported (Abdel-Hafez, 1982c). Recent studies reported the isolation of hyaline fungi, like *Myrothecium* and *Fusarium*, from the soil in the northern region of Saudi Arabia together with the common *Aspergillus* and *Penicillium* (Hemida and Abdel-Sater, 2016). A large fungal biodiversity including *Ascomycota*, basal fungi, and *Basidiomycota*, *Dothideomycetes*, *Pezizomycetes*, and *Sordariomycetes* were isolated from Jordanian and Saudi Arabian deserts (Murgia et al., 2019).

Recent studies in Saudi Arabia also investigated the biological activity of soil microorganisms. Antimicrobial activity of the extra-cellular extract of *Alternaria alternata* isolated from soil against *Proteus vulgaris* and *Salmonella typhi* has been reported (El hamd et al., 2014). In another investigation, *Penicillium melinii*, *Petriella setifera*, *Aspergillus pseudo-niger*, *Alternaria chlamydospora*, *Pythium nayloroense*, *Phoma glomerata*, *Mucor ramosissimus*, *Mucor racemosus*, *Fusarium chlamydosporum* and *Rhizopus azygosporus* were isolated from soil and showed anticandidal activity (Al-Enazi et al., 2018). However, the previous studies identified microbial abundance from sabkha in Saudi Arabia, but soil microbial diversity in desert of Saudi Arabia was not sufficiently covered. Therefore, the current work has been undertaken with a principal aim of identifying the diversity of fungal and bacterial species in various Sabkha and desert soils in Saudi Arabia. Additionally, to identify some microbes that might be of medical importance. To this end, nine different locations including Sabkha and desert soils differing in their ecologies were assigned for investigation in this study.

2. Materials and methods

2.1. Study areas

Nine different locations representing different ecologies and geographical areas of Saudi Arabia were selected for sampling (Al-Aushazia, AlQasab, AlKasar, Tabuk, Al-Kharj, El- Al-Madina, Jubail, Taif and Abqaiq) as shown in Fig. 1. Studied sites are having various soil types including rocky, clay, and sand. Soil samples (n = 5) were collected from 5 to 20 cm depth in clean plastic bags and transferred to the laboratory for analysis.

2.2. Physicochemical investigations

The soils samples were subjected to examination by a Scanning Electronic Microscope (SEM) (JEOL 7500FA JEOL, Peabody, MA, USA) at 10 kV voltage for identifying the particles features: color, shape and morphology. pH analysis was done using the pH meter, model (HI98107) according to (Jackson, 1958) and the moisture content (MC) was calculated according to (O'Kelly, 2004). Additionally, electrical conductivity (EC) were determined for each soil sample by EC-meter, Matter Toledo-AG).

2.3. Microbiological investigations

2.3.1. Culture for isolation of fungi and bacteria

Direct inoculation method was used for microbial isolation. Culture media for isolation of fungi included potato dextrose agar,

Sabouraud dextrose agar, Czapek-Dox agar, all being supplemented with chloramphenicol. Nutrient agar was used for bacterial isolation. Two culture techniques were employed: (1) The soil dilution plate method as described by Waksman (1922). Briefly, one gram of soil sample was suspended in 9 ml of sterile distilled water to make serial dilutions (10^{-1} to 10^{-3}) and 1 ml from each dilution was placed on the agar medium. The plates were incubated at 28 °C up to one week for fungal growth and at 37 °C for 24 h for bacterial growth. (2) The soil plate method: The plate was prepared by transferring 0.5 g of the soil to sterile Petri dish, 15 ml of the sterilized still liquid (at 45 °C) culture medium was added and the soil particles were dispersed throughout the agar and then the plates were left to solidify. For sandy soils, adequate dispersal was obtained by shaking and rotating the plate before the agar solidifies. Dilution of soil sample was made in three replicates for each media.

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue-stained (LCB) slide mounted with a small portion of the mycelium. Bacterial colonies were also examined macroscopically and then microscopically after Gram's staining.

Further identification of fungal and bacterial isolate was achieved by PCR amplification of the 18S rDNA and 16S rDNA genes, respectively followed by sequencing, as follows:

2.3.2. DNA extraction

Genomic DNA was extracted from fungal and bacterial isolates using the InstaGene™ Matrix Genomic DNA Kit (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions.

2.3.3. PCR amplification and purification

PCR amplification of the 18S rDNA gene from fungal isolates was performed using extracted genomic DNA as the template and the universal primers NS1 F (5' GTAGTCATATGCTTGCTC 3') and NS8 R (5' TCCGCAGGTTCACTACCGA 3'). PCR amplification of the 16S rDNA region of bacterial isolates was performed using the extracted genomic DNA as template and the universal primers 785 F (5' GGATTAGATACCCTGGTA 3') and 907 R (5'CCGCAATTCMTTTRAGTTT3'). The PCR reaction mixture was prepared as follows: 10x Taq PCR Buffer, 2 µl; 2.5 mM dNTP mixture, 1.6 µl, F and R primers (10 pmole/µl), 1.0 µl, KOMA Taq (2.5 U/ µl), 0.2 µl, DNA template (20 ng/µl), 2 µl, and HPLC-grade distilled water to adjust the reaction volume to 20 µl. Amplification was performed in a thermal cycler using the following conditions: initial denaturation for 5 min at 95 °C, 30 cycles each composed 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. PCR amplification was verified by 1% agarose gel electrophoresis. The PCR products were purified using the Montage PCR Cleanup Kit (Millipore Sigma, Burlington, MA, USA).

2.4. DNA sequencing

The purified fungal and bacterial PCR products were sequenced using the amplification primers and the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA USA). PCR product templates were sequenced using 3730xl DNA Analyzer automated DNA sequencing system (Applied Biosystems) at Macrogen, Inc. (Seoul, South Korea).

Sequence analysis: The obtained sequences were edited using Geneious prime software version 2020.1.2 (available online at: <https://www.geneious.com>; Kearsse et al., 2012). Consensus sequences were created from forward and reverse sequences. The sequences were aligned using the Basic Local Alignment Search



Fig. 1. The locations investigated.

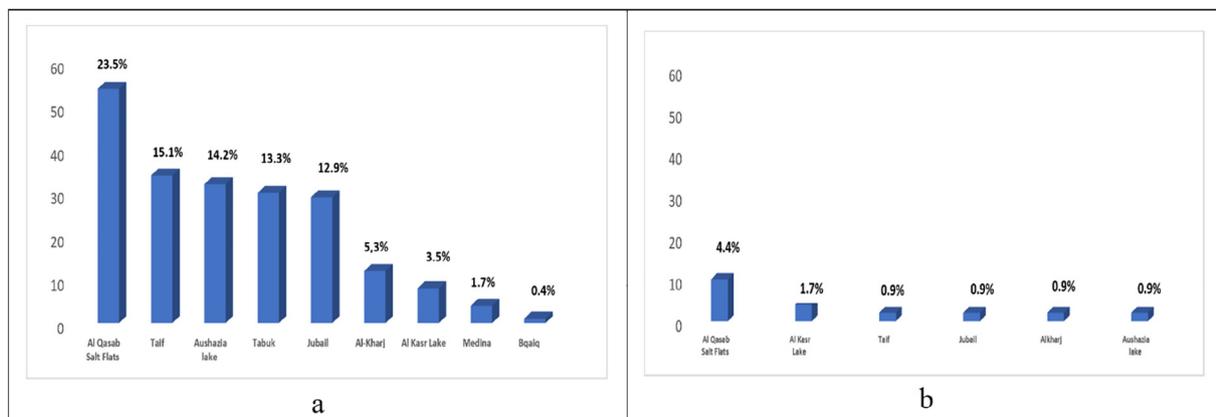


Fig. 2. Microbial abundance in all study sites, (a) is the fungi and (b) bacteria.

Tool (BLAST) of the National Center of Biotechnology Information (NCBI) with available nucleotide sequences database in the GenBank.

Phylogenetic analyses: Phylogenetic trees were constructed by alignment of obtained nucleotide sequences using the neighbor-joining analysis (Saitou and Masatoshi, 1987) in the MEGA software version X (Kumar et al., 2018).

Data availability: All data obtained in this study are stated here. The 18S rDNA and 16S rDNA gene sequences of the isolates have been deposited in the GenBank of NCBI. The assigned accession numbers of the fungal strains are MN995471 to MN995561 and for the bacterial strains are MT126329 to MT126339.

2.5. Statistical analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM, Armonk, NY, USA). Results were presented as numbers and percentages.

3. Results and discussion

3.1. Soil characteristics of the study sites

Microbial diversity in Sabkha and desert was earlier investigated and well documented (Murgia et al., 2019; Jaouani et al.,

2014). Soil is known as a rich habitat with different types of microbial communities. Hereby, variations among the investigated Sabkha and desert sites regarding microbial densities and types were observed having few species as core microbiome while others are location-specific. The soil from the nine studied locations were analyzed for pH, moisture content (MC), electric conductivity (EC) and texture, as variations in microbial communities might be explained by differences in soil characteristics among studied sites (Table 1). The soil samples from all locations had neutral to slight alkaline pH. Al-Aushazia lake displayed the highest pH (8.5) while the lowest pH value among all soil samples was detected at AlKasar area (pH, 7.0) as shown in Table 1. Soil samples from Al-Aushazia lake showed the highest MC (14.2%) among studied sites, whereas those from Tabuk area showed the lowest MC (0.3%).

Regarding the EC, it was the highest for AlQasab, and the lowest for Tabuk soils (Table 1). For soil texture, Al-Aushazia, AlQasab, AlKasar, Tabuk, Al-Kharj and Al-Madina had sandy loam soils, whereas the soil from Jubail was sandy, Taif was loamy soil and the soil from Abqaiq was sandy clay loam. It was noticeable that, Taif, Tabuk and Al-Madina were the most elevated areas among the sites studied. Examination of the soils granules by the SEM showed variation between particle size and shape among the studied sites (Figs. 3–11).

3.2. Microbial communities in relation to soil characteristics

Fungal communities were detected in all study sites and identified by 18S rDNA sequencing. The highest microbial population was isolated from AlQasab salt (53 fungal species), while the lowest population was in Abqaiq (1). Other locations showed numbers of isolates as follows: Taif area (34), Al-Aushazia (32), Tabuk (30), Jubail (29), Al-Kharj (12), Al Kasr Lake (8) and Al-Madina (3). Fungi are one of the most common soil microbes that possess great ability for adaptation under various adverse conditions; therefore, they are considered an important soil inhabitant (Sun et al., 2005). Consequently, as an essential member in soil microbial population, they have gained greater concern than bacteria (Coleman-Derr et al., 2016). By constituting the vast majority of microbial communities compared with bacteria (203 species [90.1%] Vs 22 species [9.9%]) in the present investigation, the importance of fungal species as essential inhabitants in the desert soils is very clear. AlQasab salt also had the highest number of bacterial isolates (10), followed by Al Kasr Lake (4), whereas none was isolated from Abqaiq, Al-Madina and Tabuk. The microbial existence and distribution are presented in Fig. 2 and Table 2 and Table 3.

As a consequence to the variations in soil properties, different microbial diversity patterns were found. Microbial population is highly influenced by some variables, such as soil type, soil structure, pH, salinity and moisture content, and these features are regarded as regulating factors for soil microbial diversity (Girvan et al., 2003; Lauber et al., 2008; Shen et al., 2013; López-Bucio et al., 2015; Roupheal et al., 2015; Kim et al., 2016).

The soil pH was in the normal and medium range in almost all locations, except in Abqaiq and near Al-Aushazia lake shore, where alkaline pH was observed according to Kadam (2016). Fungi may survive at varied temperature and pH (Frąc et al., 2015). In this study, isolation of different fungal and bacterial species from neutral and alkaline soils is in accordance with Arifuzzaman et al. (2016). Rousk et al. (2009) noted that fungi generally dominate in soil of low pH. In the present study, the highest number of fungal isolates was found at AlQasab, where the pH was 7.1, but lower number of fungal isolates was detected at AlKsar (pH 7). Moreover, the pH values in the locations investigated were between 7 and 8.5, which is considered the ideal range that enhances the microbial growth. This indicates that microbial abundance is not only pH-dependent. It has been stated that the microbial development in

the soil is not only affected by the pH (Cho et al., 2016), but also other factors contribute significantly to fungal abundance. Such information might explain why no special trend of correlation was observed for the microbial abundance and the soil pH for all study areas. In a similar supporting evidence to our findings, Faoro et al. (2010) showed no significant effect of pH on bacterial communities isolated from the Brazilian Atlantic Forest.

On the other hand, MC tends to affect the microbial communities at the different studied sites, but no special correlation was noticed. A recent study by Borowik and Wyszowska (2016) indicated that soil type has greater effect on soil microbial activity than the moisture content. The lack of a relation between MC and microbial abundance might be linked to the fact that in the natural habitat the MC is not stable, and the microbial abundance is mostly affected and adapted by the stable soil characteristics.

Furthermore, Kim et al. (2016) and Ma et al. (2016) showed that microbial population was influenced by soil electrical conductivity when they studied greenhouse soils and major fresh produce growing soils, respectively. Interestingly, the higher EC at Al Qasab salt flats might be the reason behind the higher fungal and bacterial isolates detected in this area. A significant correlation between the bacterial presence in each area and its EC was found ($R^2 = 0.79$). Such correlation was not clear for fungal abundance; probably due to their high diversity. However, the status of clay, salt and mineral content are mostly mirrored by changes in soil EC with a great effect on microbial community (Xue et al., 2018). Linked to this, it has been noted that the soil type could be an effective factor in relation to microbial abundance since sandy soil encourages microbial population (Zakaria et al., 2011); however, other authors expected clay to be more related to microbial communities (Carney and Matson, 2005). In addition, it was demonstrated that soil with small particle size contains higher microbial population and diversity than those with larger particles size (Sessitsch et al., 2001).

Altitude can be considered an effective factor influencing the microbial population, because it is highly connected to different factors, such as precipitation, temperature, and richness of vegetation (Singh et al., 2013). Reduction in microbial population is expected at high altitude since environmental factors become harsh as altitude rises (Margesin et al., 2009), which is obvious at Al-Madina, where low percentage of fungal isolates were found and no bacteria have been detected. However, Siles and Margesin (2016) found higher microbial abundance at high altitudes when they studied the diversity of microbial communities in Alpine forest soils. In the current study, no special trend of observations was found between the fungal distribution and single soil properties since altitude of geographical locations, soil pH, moisture content and electrical conductivity showed significant effect on special trend with the specific fungal distribution.

The microbes that exist abundantly in all study areas with 1% are known as core microbiome (Qi et al., 2018). Accordingly, *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Bacillus* spp., and *Lactobacillus* spp. are considered as core microbiome in the current study. Fungi are considered as essential members in soil microbes, but also known to better tolerate dry soils and cold environment compared to bacteria (Coleman-Derr et al., 2016; Rousk and Bååth, 2007; Kirchman, 2012). High abundance of *Fusarium*, *Alternaria* and *Chaetomium* species could be attributed to the fact that *Fusarium* is able to withstand a wide range of soil pH (Zakaria et al., 2011); *Alternaria* spp. from Soda showed variations in their alkali-tolerance ability (Grum-Grzhimaylo et al., 2016); *Chaetomium* spp. also tolerates different pH, MC, temp and organic compounds in the soil (Khan and Bhaduria, 2019). *Bacillus* is linked to *Lactobacillus* spp.; together they have the same class under the Firmicutes phylum (Elshaghabee et al., 2017). Different lactic acid bacteria have been identified from various soils (Chen et al., 2005). The isolated bacteria in the present study are spore-forming

Table 1
Soil characteristics and microbial abundance.

Location	pH	Moisture%	EC %	Sand %	Silt %	Clay %	Texture	Presence of fungal isolates (%)	Presence of bacterial isolates (%)
AlQasab	7.19	5.5	78.6	72.1	20.9	7.0	SL	23.5	4.4
Taif	7.5	1.0	2.0	48	38	14	L	15.1	0.9
Al-Aushazia	8.54	14.2	27.6	77.9	13.1	9.0	SL	14.2	0.9
Tabuk	7.33	0.3	0.6	73.8	19.4	6.8	SL	13.3	0
Jubail	7.28	1.4	4.4	92.6	2.5	4.9	S	12.9	0.9
Al-Kharj	7.5	0.5	1.5	78	18	4	SL	5.3	0.9
AlKasar	7.0	1.3	1.3	73.3	21.3	5.4	SL	3.5	1.7
Al-Madina	7.6	0.6	17.8	70.0	15.5	14.5	SL	1.7	0
Abqaiq	8.4	0.5	1.0	64.0	14.0	22.0	SCL	0.4	0

EC = electrical conductivity, SL = sandy loam soil, L = loamy, SCL = sandy clay loam soil.

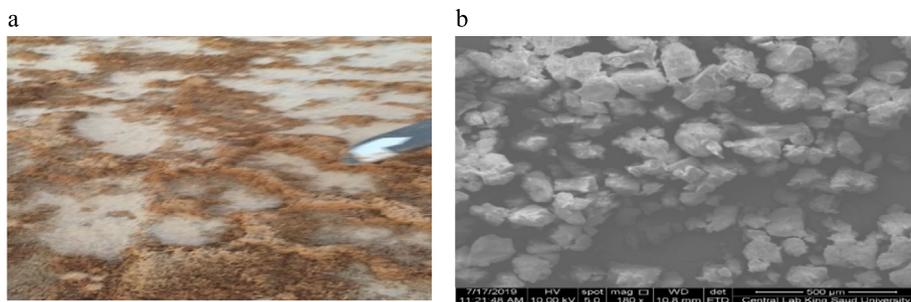


Fig. 3. Images of study area of AlQasab (a) and the soil particle size (b) detected using SEM at a magnification of 10.00. The scale bar represents 500 μ m.

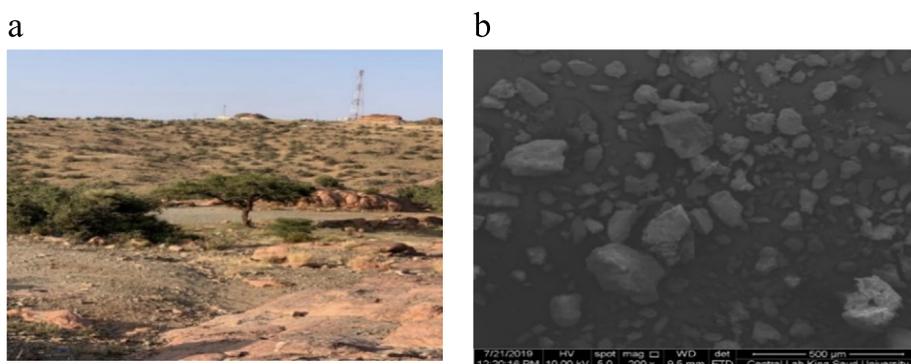


Fig. 4. Images of study site of Taif (a) and the soil particle size (b) detected using SEM at a magnification of 10.00. The scale bar represents 500 μ m.

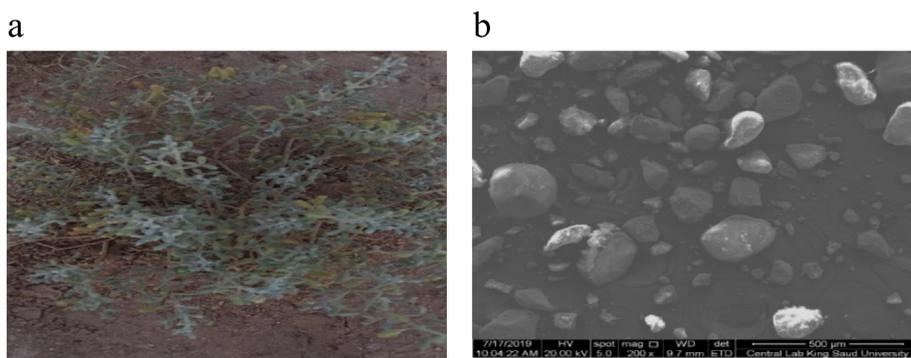


Fig. 5. Images of study site of Al-Aushazia lake (a) and the soil particle size (b) detected using SEM at a magnification of 20.00. The scale bar represents 500 μ m.

and their ability to withstand the condition in Sabkha and deserts might be related to this. Wang et al. (2018) commented that not only microbial population is affected by soil properties, but also microbes could modify soil characteristics. Such abilities for microbes to modify soil might suggest their distribution in differ-

ent habitats. No bacterial species was isolated from Tabuk, Al-Madina and Abqaiq which might indicate their sensitivity to MC, since such locations suffered low MC compared with other locations; but also it could be related to the high altitude of Tabuk and Al-Madina. Remarkably, the nine study areas varied greatly

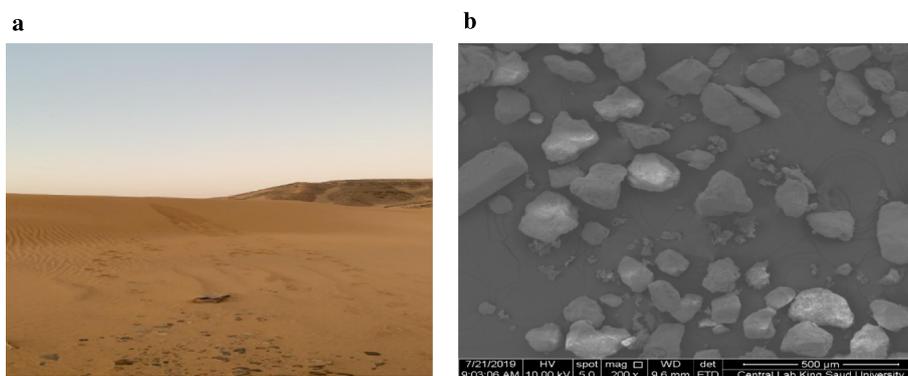


Fig. 6. Images of study site of Tabuk (a) and the soil particle size (b) detected using SEM at a magnification of 20.00. The scale bar represents 500 µm.

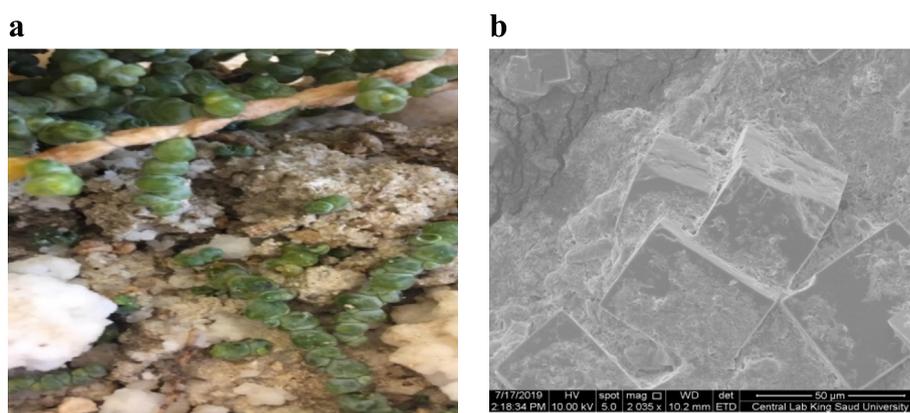


Fig. 7. Images of study site of Jubail (a) and the soil particle size (b) detected using SEM at a magnification of 10.00. The scale bar represents 500 µm.

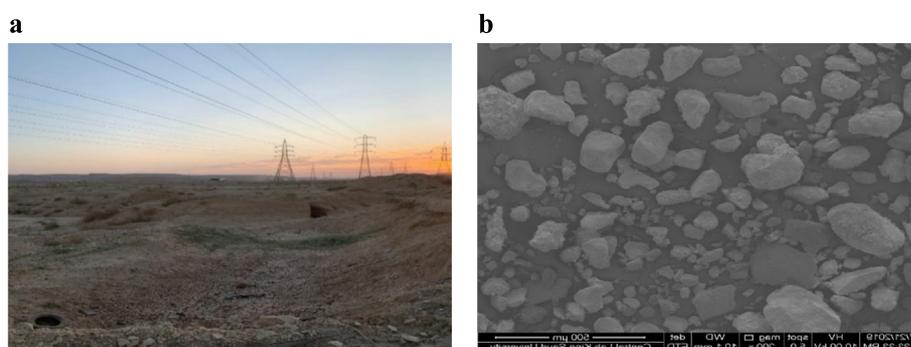


Fig. 8. Images of study site of Al-Kharj (a) and the soil particle size (b) detected using SEM at a magnification of 10.00 and scale bar represents 500 µm.

in their characteristics and microbial abundance, therefore, results regarding each location will be discussed in details.

3.3. Microbial abundance at AlQasab salt flats

AlQasab area contains the highest number of fungi compared to other sites, with 53 fungal species constituting **23.5%** of all identified isolates, as shown in Fig. 2 and Table 1. *Gymnoascus* spp. was the most abundant (11.3%) followed by *Chaetomium globosum* (9.4%), *Alternaria* spp. (7.5%) and *Aspergillus* spp., *Penicillium* spp., *Preussia terricola* and *Lithothelium septemseptatum* (5.7% for each). Various fungal genera, like *Aspergillus*, *Alternaria* and *Penicillium*, were reported from Sebkh El Melah in southern Tunisia as an alkali-halotolerant (Jaouani et al., 2014). *Gymnoascus* spp. is one of the most soil plant pathogenic fungi (Bai et al., 2018). *Chaeto-*

mium spp. also showed capacity to develop in Na and Ca environment (Steiman et al., 2004); therefore, it was abundant in the salty soil types like AlQasab salt flat. Various *Aspergillus* species were identified in Saudi Arabia and Libya deserts (Abdel-Hafez, 1982c; El-Said and Saleem, 2008). AlQasab area has the highest EC (78.6) among all studied sites. The soil EC was suggested to be one of the main factors influencing the soil microbial community compositions in our study. EC is actually correlated with the salinity of the soil; thus, salinity could be reliably evaluated by the EC (Hardie and Doyle, 2012). The most important soluble salts related to the EC of the soil are Na⁺, K⁺, Mg⁺ and Ca⁺ salts (Shi and Wang, 2005); therefore, the higher the EC, the higher the concentration of these salts as evident in the image of the study area (Fig. 3). AlQasab soil also showed the highest number of identified bacterial isolates (n = 10), which were *Bacillus* spp. and *Lactobacillus* spp. A

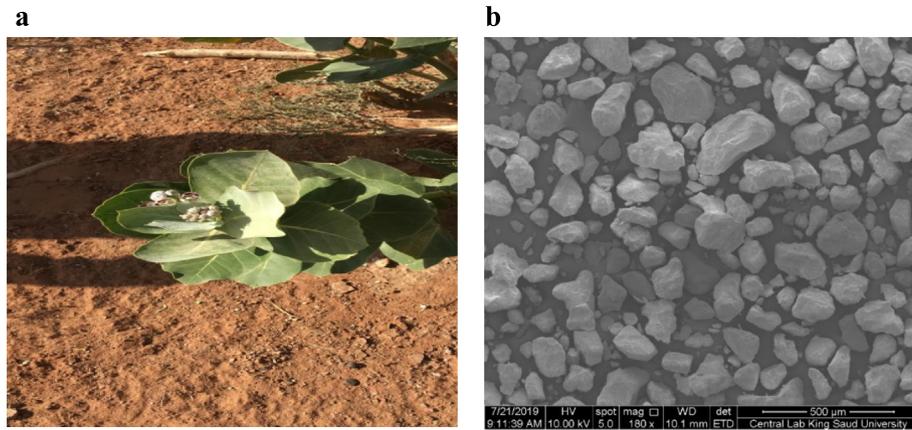


Fig. 9. Images of study site of Al-Kasr (a) and the soil particle size (b) detected using SEM at a magnification of 10.00 and scale bar represents 500 μm .

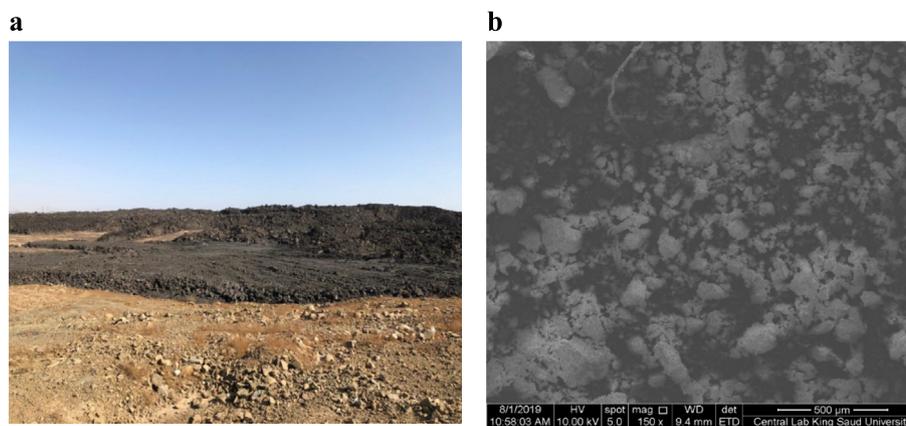


Fig. 10. Images of study site of Al-Madina (a) and the soil particle size (b) detected using SEM at a magnification of 10.00 and scale bar represents 500 μm .

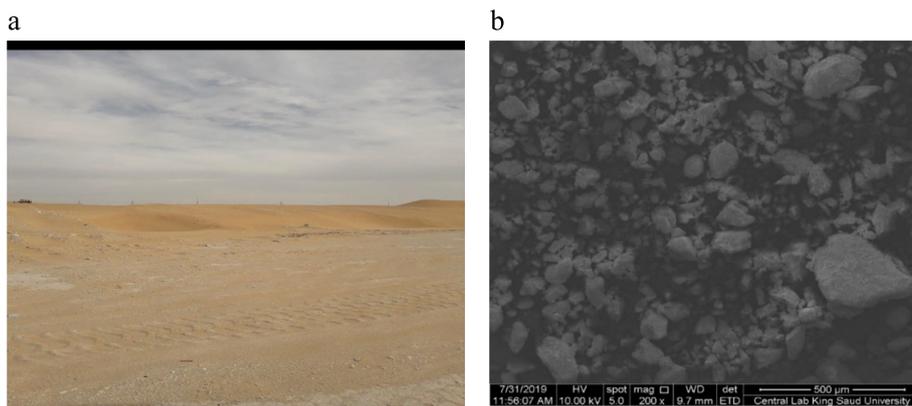


Fig. 11. Images of study site of Abqaiq (a) and the soil particle size (b) detected using SEM at a magnification of 10.00 and scale bar represents 500 μm .

recent study found that the bacterial communities were strongly linked to EC (Siles and Margesin, 2016).

On the other hand, the soil pH influences the chemical solubility by affecting the level of ionization for microbial growth, the required pH is between 6 and 8 (Maier and Pepper, 2009) which is the case at AlQasab and together with the high sand content, both factors might be the reasons for high microbial communities. Therefore, high EC, pH and the sandy soil may contribute to high microbial diversity detected at AlQasab.

Some fungal species were only limited to this area compared with other sites; these were *Ascobolus carbonarius*, *Ascobolus crenu-*

latus, *Aspergillus ustus*, *Gymnoascus* spp., *Hypocreales* spp., *Lithothelium septemseptatum*, *Madurella* spp., *Myceliophthora thermophila*, *Paraphaeosphaeria* spp., *Penicillium* spp. *Phoma herbarum*, *Preussia terricola*, *Sirodesmium olivaceum*, and *Sporomia lignicola*. In addition, *Bacillus amyloliquefaciens* and *Bacillus siamensis* were also only isolated from AlQasab.

However, isolates confined to this location might be linked mainly to EC since other soil properties were not significantly different among studied areas. Such isolates might also be confined to the combination of neutral pH with 5.5% moisture on sandy loam soils of AlQasab.

Table 2
Detailed distribution of isolated fungal genera and species.

Fungal genera and species	n (%)	n (%)	Fungal genera and species	n (%)	n (%)	Fungal genera and species	n (%)	n (%)
<i>Fusarium</i> sp.	28 (12.5%)		<i>Microascus cirrosus</i>	4 (1.7%)		<i>Sporomia lignicola</i>	2 (0.8%)	
<i>F. equiseti</i>		3 (1.5%)						
<i>F. graminearum</i>		7 (3.1%)						
<i>F. oxysporum</i>		17 (8.2%)						
<i>F. proliferatum</i>		1 (0.5%)						
<i>Alternaria</i> sp.	25 (11.1%)		<i>Lithothelium</i>	3 (1.5%)		<i>Thielavia</i> sp.	3 (1.3%)	
<i>A. Spp</i>								
<i>A. Alternata</i>		12 (5.3%)						
<i>A. maritima</i>		10 (4.4%)						
		3 (1.5%)						
<i>Chaetomium globosum</i>		19 (8.4%)	<i>Phoma</i>	3 (1.5%)		<i>Westerdykella cylindrica</i>	2 (0.8%)	
			<i>P. herbarum</i>		1 (0.4%)			
			<i>P. spp</i>		2 (0.8%)			
<i>Aspergillus</i>	19 (8.4%)		<i>Actinomyces elegans</i>	2 (0.8%)		<i>Embellisia</i> spp.	1 (0.4%)	
<i>A. spp</i>		9 (4%)						
<i>A. terreus</i>		4 (1.7%)						
<i>A. flavus</i>		3 (1.5%)						
<i>A. ochraceus</i>		2 (0.8%)						
<i>A. ustus</i>		1 (0.4%)						
<i>Cochliobolus</i>	13 (5.8%)		<i>Ascobolus</i>	2 (0.8%)		<i>Eupenicillium</i> sp.	1 (0.4%)	
<i>C. eragrostidis</i>		8 (3.5%)	<i>A. carbonarius</i>		1 (0.4%)			
<i>C. spp</i>		5 (2.2%)	<i>A. crenulatus</i>		1 (0.4%)			
<i>Penicillium</i>	11 (4.9%)		<i>Eremothothis angulata</i>	2 (0.8%)		<i>Hypocreales</i> spp.	1 (0.4%)	
<i>P. chrysogenum</i>		4 (1.7%)						
<i>P. spp</i>		4 (1.7%)						
<i>P. camemberti</i>		2 (0.8%)						
<i>Cladosporium spp</i>	6 (2.6%)		<i>Mucor</i> spp.	4 (1.7%)		<i>Madurella</i> spp.	1 (0.4%)	
<i>Gymnoascus</i>	6 (2.6%)		<i>Pleosporales</i> spp	1 (0.4%)		<i>Myceliophthora</i> sp.	1 (0.4%)	
<i>G. reesii</i>		3 (1.5%)						
<i>G. spp</i>		3 (1.5%)						
<i>Ulocladium botrytis</i>	6 (2.6%)		<i>Pleospora herbarum</i>	3 (1.3%)		<i>Myzostoma</i> sp.	1 (0.4%)	
<i>Aureobasidium</i>	4 (1.7%)		<i>Preussia terricola</i>	3 (1.3%)		<i>Paraphaeosphaeria</i> sp.	1 (0.4%)	
<i>A. pullulans</i>		3 (1.5%)						
<i>A. namibiae</i>		1 (0.4%)						
<i>Emericella nidulans</i>	4 (1.7%)		<i>Pseudochaetosphaerone</i>	2 (0.8%)		<i>Sirodesmium olivaceum</i>	1 (0.4%)	

3.4. Microbial distribution at Al-Taif

Al-Taif (Fig. 4) is the second highest location for microbial abundance that contains 34 fungal isolates with the core microbiome *Aspergillus* spp. (34.5%) and *Fusarium* spp. (24.1%), beside *Chaetomium globosum*, *Mucor* spp. and *Microascus cirrosus* (6.9% for each). *Bacillus* spp. and *Lactobacillus* spp. were the abundant bacterial isolates. *Aspergillus* spp. are weak alkalitolerants and *Fusarium* spp. are moderate alkalitolerants (Grum-Grzhimaylo et al., 2016) that tolerate to some extent the soil pH at Al-Taif location (7.2). Such fungal isolates were also reported in different studies by Kladwang et al. (2003) and Gunde-Cimerman et al. (2009) as halotolerant and alkalitolerant fungi. The EC 1.96 is an acceptable range for low salinity at Al-Taif. Soil type and particle size may contribute significantly to microbial diversity, since it affects the adsorption affinity (Maier and Pepper, 2009). The loamy soil at Taif contain high percentage of silt and sand in relation to clay, which beside the small particle size, may enhance the microbial diversity (Cho et al., 2016). *Aspergillus ochraceus*, *Aspergillus terreus*, *Eupenicillium crustaceum* and *Mucor* spp. as well as the bacterial species *Bacillus cereus* were only isolated from Taif area. Such occurrence might be related to loamy soil or other environmental factors, since no special soil characteristics were observed. Higher microbial occurrence at Taif compared to other elevated locations (Tabuk and Al-Madina) might be linked to the loam soil with a relatively high MC.

3.5. Microbial distribution at Al-Aushazia

Al-Al-Aushazia area (Fig. 5) showed 14.2% fungal isolates and 0.9% bacterial species. *Fusarium* spp. (21.9%), *Cochliobolus* spp. (15.6%) *Aspergillus* spp. (12.5%), *Alternaria* spp. (9.4%); *Bacillus*

spp. and *Lactobacillus* spp. were the most abundant fungal and bacterial species. Some characteristics here are comparable to those at AlQasab area, which could be the reason for same species abundance, like *Aspergillus* and *Alternaria* species. Such characteristics as high EC and sandy loamy soil suggest ability of these species to withstand salty soils, since salinity is more correlated to EC (Hardie and Doyle, 2012). The highest MC among all study areas was from Aushazia indicating higher microbial diversity; however, sandy loamy soil type also might have affected the diversity of microbes. *Aspergillus*, *Penicillium* and *Fusarium* were commonly isolated in the Northern region of Saudi Arabia (Hemida and Abdel-Sater, 2016). *Actinomyces elegans*, *Fusarium proliferatum* and *Gymnoascus reesii* and *Myzostoma* spp. were only isolated from the sandy loam Al-Aushazia Lake soil. However, pH is more alkaline at Alushazia (8.5) suggesting that identified isolates were halotolerants and alkalitolerant. Combination of alkalinity, salinity, high MC in sandy loam soil might contribute altogether to such species limitation to Al-Aushazia.

3.6. Microbial distribution at Tabuk

From all fungal isolates, 13.3% (about 30) were isolated from Tabuk (Fig. 6) including *Alternaria* spp: (30%), and *Chaetomium globosum* (26.7%) as well as *Cochliobolus eragrostidis*, *Ulocladium botrytis* and *Fusarium* spp. (13.3% each); however, no bacterial species were isolated. Bacteria reported to grow in hostile and sandy desert areas include *Bacillus*, *Corynebacterium*, *Listeria* and *Staphylococcus* (Al-Yemeni and Hashem, 2006). In contrast to those findings, we were not able to isolate bacterial species from Tabuk, which might be related to the combination of different factors, such as soil pH, EC and MC as well as the depth of collected soil sample. EC and MC might have significant effect, since the lowest

EC (0.59) and MC (0.26) were detected at Tabuk. Furthermore, in the above mentioned study soil was collected from nearly the soil surface (1.6 cm depth), however we collected the soil from 5 to 20 cm depth. Although microbial abundance is not expected at Tabuk because of their altitude compared to other areas, it is not typically the case regarding fungal abundance suggesting that some other soil properties might enhance their growth.

3.7. Microbial distribution at Jubail

A percentage of 12.9% (n = 29) from all fungal isolates were obtained from Jubail (Fig. 7) including *Alternaria* spp. (17.2%), *Penicillium* spp. (17.2%), *Emericella nidulans* (13.8%), *Cladosporium* spp. (13.8%) with few species of *Aspergillus* spp. and *Fusarium oxysporum*. *Bacillus* spp. and *Lactobacillus* spp. were also isolated. Jubail contains sandy soil with pH 7.2 and EC 4.4. Such EC supported salinity and enhanced microbial abundance. However, nidulantes of *Aspergillus* optimally grows at 5.5–6 pH (Grum-Grzhimaylo et al., 2016); their clear presence in 7.2 pH might suggest that some other factors enhanced their growth and could likely be the sandy soil, since it was isolated from sand of Casa Caiada and "Bairro Novo" beaches (Gomes et al., 2008). *Embellisia* spp. and *Emericella nidulans* and *Penicillium camemberti* were only isolated from Jubail location. *Penicillium* spp. and *Cladosporium* spp. are halotolerant species that identified in different hypersaline environments (Zalar et al., 2007). However, their presence in Jubail is an indication to their ability to sustain neutral pH.

3.8. Microbial distribution at Al-Kharj

Results showed that 5.3% was the ratio of fungal isolates from Al-Kharj (Fig. 8) including *Fusarium oxysporum* (50%) as the core fungal strain and bacterial species *Bacillus* spp. and *Lactobacillus* spp. *Fusarium* spp. are moderate alkalitolerants (Grum-Grzhimaylo et al., 2016) that tolerate to some extent the soil pH 7.5 at Al-Kharj. Generally, the low microbial abundance here in comparison with the above mentioned locations could be related to other not detected factors, since no significant variation among soil physiochemical characteristics for others areas was found. *Pleosporeales* spp. was only isolated from Al-Kharj.

3.9. Microbial distribution at Al-Kasr

Eight fungal isolates were obtained from Alkasr (Fig. 9) having *Aureobasidium* spp. (50%) as the core microbiome beside *Chaetomium globosum* and *Cladosporium* spp. as the only fungal species isolated in addition to *Bacillus* spp. and *Lactobacillus* spp. Bacteria were found in relatively high distribution, which might be related to the MC (1.28) as well as the soil type (sandy loamy), which in turn might enhance bacterial abundance. It was also observed that *Bacillus axarquiensis* and *Bacillus velezensis* were only isolated from Alkasr.

Aureobasidium spp. and *Cladosporium* spp. were isolated from the hypersaline Inland Sea in Qatar with a pH ranging from 7.99 – 8.7 and salinity between 57.3 and 75 indicating their halotolerant ability (Fotedar et al., 2018), but our study suggests their existence at neutral pH and low saline environment.

3.10. Microbial distribution at Al-Madina and Abqaiq

Alternaria spp. are the only four fungal isolates isolated from Al-Madina (1.7%) and those only isolated from Abqaiq were the alkaliphiles, *Thielavia* spp. (0.4%) with no bacterial species found at these locations (Figs. 10 and 11). The relatively high microbial abundance at Al-Madina compared with Abqaiq might be explained by soil EC (17.8) that was low at Abqaiq (0.9). Another

factor that might limit the microbial abundance is the extremely low or high pH (Luo and Zhou, 2006). However, such factor didn't limit the microbial presence indicating that other limiting factors might play a significant role in the abundance of soil microbiome at Al-Madina and Abqaiq. *Alternaria maritima* grew on an alkaline sandy loam soil with low MC in Al-Madina compared to the growth of *Alternaria alternata* and *Alternaria* spp., which grew on the sandy to sandy loam soil of various areas in Saudi Arabia that had neutral to alkaline pH of 7.33 to 8.54.

3.11. Molecular identification of fungal and bacterial isolates

Based on the 18S rDNA gene sequences, molecular identification showed that the 92 fungal isolates from the nine geographical areas had 89% to 99% identities to fungal sequences available in the GenBank database. However, four isolates were excluded from the current analysis due to redundancy of identification on the genus/species level or due to low sequence identity in the database. The six most commonly isolated fungal genera were *Fusarium*, *Alternaria*, *Chaetomium*, *Aspergillus*, *Cochliobolus* and *Penicillium*. Whereas *Chaetomium globosum*, and *Fusarium oxysporum* were the most abundant species. Several of uncommon/new fungi were isolated including *Eremothia angulata*, *Pleosporeales* spp., *Pleospora herbarum*, *Preussia terricola*, *Pseudochaetosphaerone*, *Sporomia lignicola*, *Embellisia* spp., *Hypocreales* spp., *Madurella* spp., *Myceliophthora*, *Myzostoma*, *Paraphaosphera* and *Sirodesmium olivaceum*.

For the bacterial isolates, molecular identification by 16S rDNA sequencing showed that the 22 isolates were assigned to two bacterial genera, *Bacillus* and *Lactobacillus*, with 99.0% identity to the nucleotides sequences in the GenBank database. However, 11 isolates were excluded due to redundancy of identification on the genus/species level or low sequence identity in the database. The most commonly isolated bacteria were *Bacillus subtilis* (n = 7, 3.1%) and *Lactobacillus murinus* (n = 6, 2.6%), as mentioned in Table 3. A phylogenetic tree using the Neighbor-joining method in MEGAX displayed clear clustering and a high degree of homology for *Bacillus* isolates with related available species data in NCBI database (Fig. 12). *Escherichia coli* (GenBank accession number NR 024570) was used as outgroup.

To study the taxonomical relationships among the isolated fungi, the 18S rDNA gene sequences of the 92 fungal isolates were compared with nucleotide sequences data in GenBank and a phylogenetic tree was constructed using the Neighbor-joining method in MEGAX. Clear clustering and a high degree of homology with related defined species in NCBI were found (Fig. 13). The homology of the isolates to related defined species indicates that these fungal species have not been exposed to different environmental factors, or otherwise that would stimulate additional genetic diversity that are commonly indicated as concerted evolution (Ganley and Kobayashi, 2007). BLAST search revealed that some isolates obtained from different locations had 100% identity. For example:

Table 3
Detailed distribution of isolated bacterial genera and species.

Bacterial genera and species	n (%)
<i>Bacillus</i>	
<i>B. amyloquifasciens</i>	1 (0.4%)
<i>B. axarquiensis</i>	1 (0.4%)
<i>B. cereus</i>	1 (0.4%)
<i>B. simenis</i>	1 (0.4%)
<i>B. sp.</i>	4 (1.7%)
<i>B. subtilis</i>	7 (3.1%)
<i>B. valensis</i>	1 (0.4%)
<i>Lactobacillus murinus</i>	6 (2.6%)

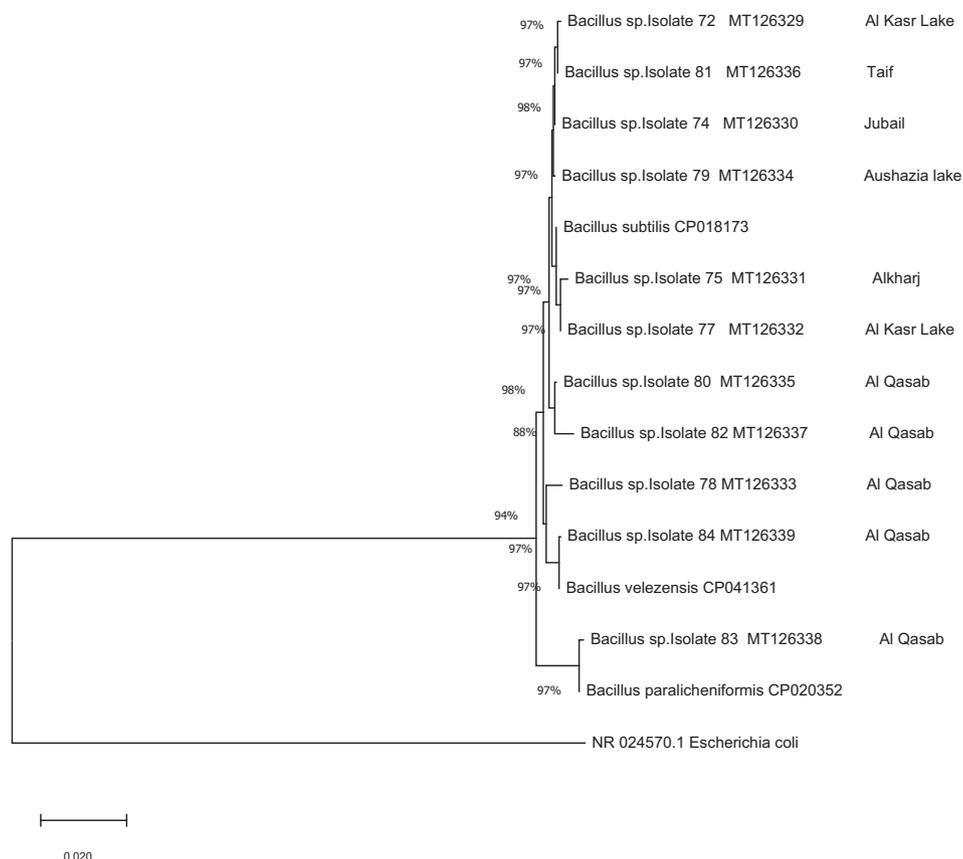


Fig. 12. Phylogenetic relationships among Bacterial isolates were used for constructing the phylogenetic tree including the corresponding species, *Bacillus paralicheniformis* (CP020352), *Bacillus velezensis* (CP041361) and *Bacillus subtilis* (CP018173). 16S rDNA sequences were aligned using ClustalW. A Neighbor-joining method was used to build the tree with 1000 bootstraps by MEGA X. The GenBank accession No. of the 16S rDNA sequences used for phylogenetic tree analysis are indicated at the end of each branch (given the MT Numbers). The *Escherichia coli* (GenBank accession number NR 024570) is used as outgroup. Numbers shown next to the branches display percentage of bootstrap values. Scale bar specified nucleotide substitutions per site.

Alternaria spp. (GenBank accession numbers MN995511, MN995504, MN995536, MN995547, MN995553 and MN995541) were isolated from AlQasab, Al-Aushazia, Jubail, Al-Madina and Tabuk, respectively. These strains were 100% alike to each other. Similarly, Sterflinger and Prillinger (2001) isolated *Phoma exigua* var. *foveata* strain based on ITS1 sequences from two different areas in Austria (rock in Vienna city and the quarry). Alsohaili and Bani-Hasan (2018) demonstrated different species of *Aspergillus* including *A. terreus*, *A. ochraceus*, *A. flavus* and *A. ustus* indicating their presence in special conditions different from that of the currently studied areas. It was well documented that in global soils, the most fungal phyla detected were 90% of *Ascomycota* and *Basidiomycota* (Tedersoo et al., 2014). However, *Aspergillus niger*, *Aspergillus tubingensis*, *Alternaria tenuissima*, *Alternaria gaisen*, *Rhizopus stolonifer*, and *Penicillium citrinum* were not isolated. Generally, fungal biodiversity including *Ascomycota*, basal fungi, and *Basidiomycota*, *Dothideomycetes*, *Pezizomycetes*, and *Sordariomycetes* were not isolated from the study locations. Such isolates were isolated from Jordanian and Saudi Arabian deserts in a previous study (Murgia et al., 2019). Osmophilic fungi *Aspergillus*, *Alternaria*, and *Penicillium* were also isolated, but not *Cephaosporium*, *Acremonium* and *Botryotrichum* as were reported by Abdel-Hafez (1982c). Many other fungal species that were reported to grow in the sandy deserts of Saudi Arabia were not isolated including halophilic fungi *Drechslera* and thermophilic and thermotolerant fungi, such as *Humicola grisea* var. *thermoidae*, *Chaetomium therrnophile* var. *copropile*, *C. thermophile* var. *copropile*, *A. nidulans* and *C. thermophile* var. *dissitum* (Abdel-Hafez, 1981).

There were many newly discovered fungal species that we could isolate which grow on neutral to alkaline pH and with minimal soil moisture. These include *Ascobolus*, *Aureobasidium*, *Embellisiam Emericellam*, *Eremodothis*, *Gymnoascus*, *Hypocreales*, *Llthothelium*, *Madurellam*, *Microascus*, *Mycelophthora*, *Myzostoma*, *Paraphaeosphaeria*, *Phoma*, *Pleospora*, *Pleosporales*, *Preussia*, *Pseudochaetosphaeronema*, *Sirodesmium*, *Sporomia*, *Thielavia* and *Westerdykella*. On the other hand, six *Bacillus* species and *Lactobacillus murinus* were isolated. In comparison to the study of Al-Yemeni and Hashem (2006), different soil characteristics and soil depth in the current study might explain the variations in isolated bacterial strains between the two studies. Another factor that has to be considered for these variations in bacterial species is the influence of the host and the benefit from surrounding plant growth that may have selected beneficial microbes to promote their growth under these extremely non-hostile environments, such as the deserts of Saudi Arabia, as explained by Eida et al. (2018).

4. Conclusion

Results from the current study indicated high diversity in microbial communities in different areas that varied in soil physicochemical characteristics in Saudi Arabia. Higher fungal diversity than bacteria might indicate the ability of fungi to withstand extreme conditions since they were isolated from Sabkha as well as desert areas. Our current investigation revealed high specificity of some microbes to a particular area. Overall, there is no consistency in the trend of variations in soil variables in relation to microbial

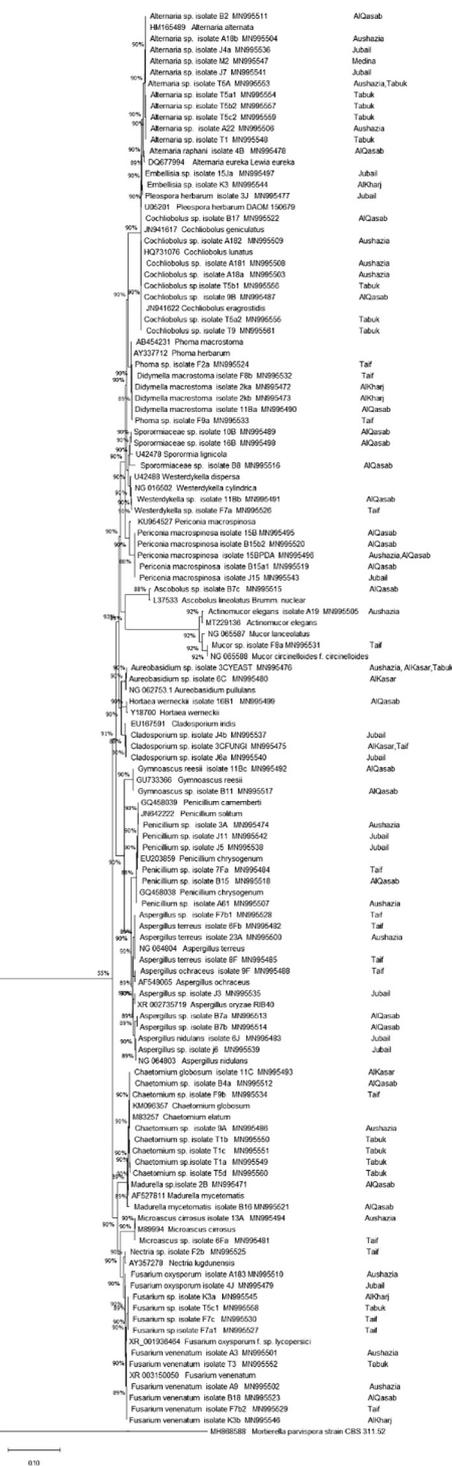


Fig. 13. Phylogenetic tree for fungal species isolated from nine sites in Saudi Arabia based on 18S rDNA sequences were used for constructing the phylogenetic tree (including the corresponding species from NCBI, indicated with GenBank accession number at the tip of each branch). 18S rDNA sequences were aligned using MUSCLE. A Neighbor-joining tree was conducted using MEGAX with 1000 bootstrap replicates according to Kimura 2-parameter model. The GenBank accession No. of the 18S rDNA sequences isolates in this study are indicated at the end of each branch (given the MN Numbers). The *Mortierella parvispora* strain CBS 311.52 (GenBank accession number MH868588) was used as outgroup. Numbers shown next to the branches display percentage of bootstrap values. Scale bar specified nucleotide substitutions per site.

abundance. From this ground it would be necessary to proceed with further experimental work in the future to explore the possible factors involved in controlling the microbial occurrence and distribu-

tion pattern. The significance of such information will be of great value for understanding the microbial habitat ecology and, in turn, it would be easy to formulate special protocol to address such microbes. Furthermore, investigations on the uncommon fungal species isolated separately might be useful in provision of valuable biological materials of medicinal importance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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