



## Original article

## Genotypic characterization of soil bacteria in the Umm Al-Namil Island, Kuwait

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## ARTICLE INFO

## Article history:

Received 4 January 2021

Revised 13 March 2021

Accepted 22 March 2021

Available online 27 March 2021

## Keywords:

Genotypes

Soil bacteria

Umm Al-Namil Island

Kuwait

## ABSTRACT

Microflora is an integral part of soil ecosystem, in which bacteria are the largest group of soil microbes. This is a pioneer study for establishing baseline data on the diversity of soil bacteria among different regions in Kuwait. The aim is to understand biodiversity in different settings, how bacteria adapt to different niches in the environment as well as in different hosts. The identification of bacterial 16S rRNA molecules from environmental soil samples was investigated. Genomic Deoxyribonucleic acid DNA was extracted from 25 soil samples derived from five different test regions in the Umm Al-Namil Island, Kuwait. After amplification of bacterial 16S rRNA molecules by the Polymerase chain reaction PCR, the products were characterized and complex band patterns were obtained, indicating high bacterial diversity. A sample of the 16 s rRNA amplicons were sequenced in order to identify the species. The spatial distribution of bacterial taxa in the different soil samples was homogeneous, suggesting a stable and widespread community. Forty-nine isolates from Umm Al-Namil island were identified by comparative analysis of partial 16S rRNA gene sequences. Phylogenetic analysis was carried out in order to study the connection between the isolates to identify species. A large proportion of these isolates represent correspond to known or novel species within the *Pseudomonas* and *Bacillus* genera, which are common soil bacteria. Our results provided a reference for future studies to facilitate bacterial identification and ecological research in Kuwait.

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## 1. Introduction

## 1.1. Soil bacteria

Soil is a dynamic system with living organisms playing out a complex web of biotic and abiotic interactions. The microflora is an integral part of the soil ecosystem, in which bacteria are the largest group of soil microbes, both by total number and diversity (Gagelidze et al., 2018). Bacteria can survive in a vast range of environmental conditions (Dick, 2009) and can also live in harsh conditions including high temperature, extreme pH, and low water availability (Xie et al., 2016).

There are four functional soil bacteria groups including decomposers, mutualists, pathogens, and lithotrophs. Some groups are playing an important role in promoting plant growth, such as *Bacillus subtilis* and *Pseudomonas fluorescens*. *Azotobacter*, *Azospirillum*, *Agrobacterium*, *Gluconobacter*, *Flavobacterium* and *Herbaspirillum* are nitrogen-fixing bacteria that increase the fertility of the soil by fixing the atmospheric nitrogen (Sharma et al., 2014). Soil bacteria are also classified, according to their reliance on oxygen, into aerobic and anaerobic bacteria. Aerobacter genus is one of aerobic bacteria that is widely distributed in the soil as well as *Streptomyces* genus filamentous bacteria that gives soil an earthy smell (Lowenfels & Lewis, 2006).

Some soil bacteria are closely related to pathogenic bacteria that infect humans; therefore, it is important to understand their ecology in natural settings, and the risk posed to human health. Most soil pathogenic bacteria can be transmitted through dust and enter the body through the skin or lungs to cause certain diseases such as nocardiosis and sporotrichosis. It was found that these conditions caused by aerobic spore forming actinomycetes (Aghamirian and Ghiasian, 2009). Kuwaiti *Nocardia asteroides* isolates showed antimicrobial susceptibility against 14 antimicrobial

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Peer review under responsibility of King Saud University.



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agents (Khan et al., 2000). However, other groups of bacteria are pathogenic and causes opportunistic infections of humans such as *Pseudomonas fluorescens* (Sharma et al., 2014).

Conversely, most soil bacteria are non-pathogenic to humans, and these will compete with the pathogens for nutrients (Lowenfels & Lewis, 2006). Al-Saleh and Akbar (2015) concluded that *Pseudomonas aeruginosa* types were dominant in sites contaminated by crude oil, rather than uncontaminated sites in Kuwait. Analysis done by Khanafer et al. (2017), based on 16S rRNA-gene sequences collected from Raw, revealed the presence of *Enterobacter hormaechei* subsp. *Oharae*, *Klebsiella* spp., and *Escherichia coli*.

### 1.2. Geography of the Umm Al-Namil island

The Umm Al-Namil island is one of the smallest, uninhabited islands within Kuwait Bay in the northwestern part of the Arabian Gulf. It is far about 600 m away from the mainland of Kuwait, with 2 m elevation above sea level and oriented in a drumstick shape NE-SW direction (Fig. 1). The island is famous for its archeological relics, mainly from the Islamic era and the Bronze age (Al-Zamil et al., 2006).

The island is bounded by well-developed tidal flats. Supratidal flats consist of gypsum and anhydrite, which form most of the coastal dunes in the area and the intertidal flat is hard and accompanied by oyster mounds, whereas the subtidal flat is composed of soft muddy sand with high productivity (Al-Zamil et al., 2007). The surface sediments of the island are composed of medium-to-fine carbonate oolite sand, muddy sand, mud and biogenetic shells.

### 1.3. The soil characteristics and contamination resources

The Umm Al-Namil island has young and well-developed, light brownish grey-pale brown to brown-dark brown saline alluvial soils of poor drainage and very little vegetation (Fig. 2). The soil texture ranges from silt loam to clay loam, strongly calcareous with wind-blown sands on the surface. Reduction conditions with grey horizons and color spots were observed in some cases. The island is still under the influence of tidal action (Ergun, 1969)

and influenced by the deltaic and estuarine deposition of the Tigris-Eupharates river system.

The estuarine depositional environments include tidal channels, hardground reefal flats, tidal mudflats, spits and sand bars that are seen to interfinger with the shoreline and inner coastal deposits of the island with recent biogenic sedimentation developed around the island (Al-Zamil et al., 2007). Changes in the profiles were observed in the southern part of the island where some recurved spits have formed, and accumulation of sediment persists. These changes were also observed on the northern side of the island.

The island is impacted to various degrees by several pollutants, such as oil, petroleum hydrocarbons, trace metals, suspended particles and nutrients mostly sulphates, nitrates, and phosphates. The probable sources of these pollutants include the oil and petrochemical industry (Shuaiba Complex), power plants, and sewage and oil export activities. Residual chlorine, phosphate, temperature, and increased levels of biochemical oxygen demand (BOD) are considered indirect pollutants or indicators (Al-Ghadban et al., 2002).

### 1.4. Molecular study

For many years, most known bacteria were identified only from their 16 s ribosomal RNA gene sequence. Thus, 16 s ribosomal DNA sequence is the most used phylogenetic marker (Valadas et al., 2013). The 16S rRNA gene is still widely used because it is present in at least one copy in every bacterial genome, its conserved regions provide universal primer sites to enable simple sample identification using PCR, and its sequence provides reliable information on bacterial family, genus, or species in most cases. However, it is often not possible to distinguish closely related species or different strains belonging to a single species using this approach.

Phylogenetics has played a key role in studying the biodiversity, geographic distribution, host range, ecology, the behavior of many microorganisms (Emelianoff et al., 2008). Phylogenetic tree was used to study the evolutionary descent and relatedness in the bacteria population (Bonifassi et al., 1999). Previously this was carried

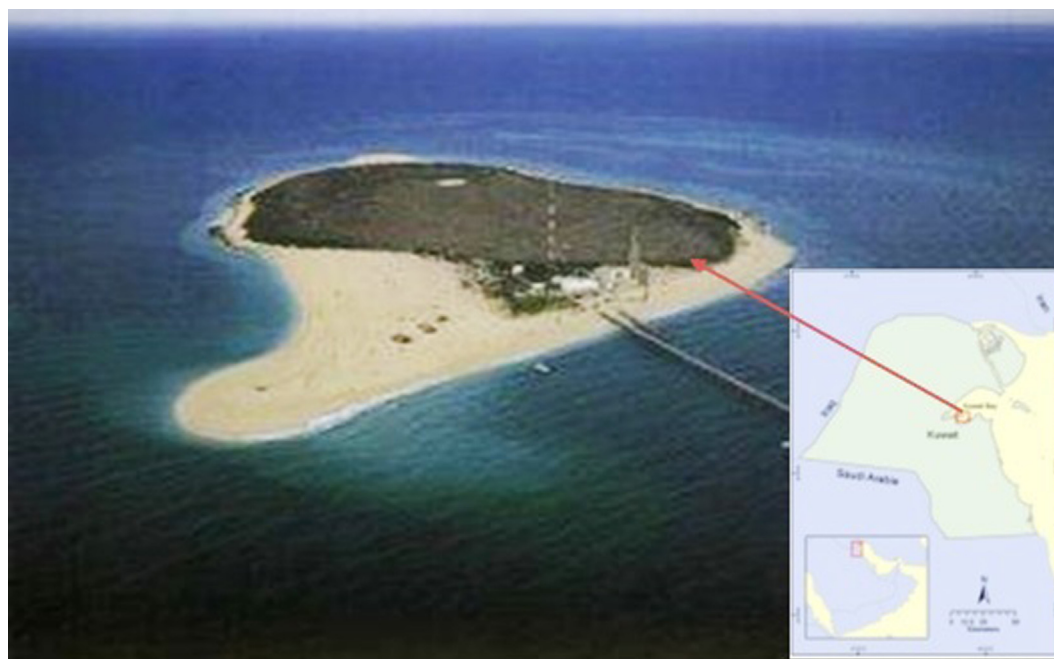


Fig. 1. The geographical location of the Umm Al-Namil Island, Kuwait.



**Fig. 2.** Images of the study area Umm Al-Namil Island.

out using phenotypic markers, but most phylogenies are now based on the DNA sequences. These molecular data can be represented either as a phylogenetic tree (Bonifassi et al., 1999) or else as a network if there are conflicting signals in the data due to recombination (Loidi et al., 2010).

### 1.5. Objectives

The previous research focused on understanding the mechanisms of oil degrading bacteria and thermophilic soil bacteria in different regions in Kuwait, while the changes in the population structure of soil bacteria is little known in Kuwait. Therefore, the Umm Al-Namil island, as an uninhabited island, has been chosen as the source of soil bacteria for the present study.

Identification of soil bacteria in the Umm Al-Namil island utilizing genotypic techniques is a pioneer study in Kuwait. This is to increase the future understanding of applying more advance molecular technique such as Metagenomic and the Next generation sequencing (NGS) for building up the DNA reference library of Kuwaiti bacterial soil. The results will be compared with soil bacteria collected from other urban and industrial areas in Kuwait of high human activities. This to study the relatedness of the isolates by constructing a phylogenetic tree in Kuwait.

## 2. Materials and methods

### 2.1. Soil sampling

Twenty soil samples were collected from four sites in the Umm Al-Namil Island 29°22'54" N and 47°52'1" E in DMS (Degrees Minutes Seconds), located within Kuwait Bay in the northwestern part of the Arabian Gulf. From each of the 4 sites, 5 samples were taken at 200–300 m intervals to make the 20 samples. Another 5 samples were collected from different sites out of the island as control samples. Samples were taken in March 2019. These samples were collected from a depth ranges from 5 to 10 cm below the ground surface, after removing the surface loose debris (Fig. 3). Then, the samples were numbered in a clean plastic bag and closed tightly and transferred to the research lab of the Basic Education College, where they will spread in trays to be cleaned of leaves, roots, woods, etc. The samples were sieved (2 mm mesh), and 10 g were mixed for 5 min with distilled water in bottles containing 90 ml of autoclaved.

### 2.2. Isolation of bacterial strains

After the incubation period, 0.1 ml of 10-fold dilution from each sample suspension was spread utilizing sterile glass spreading rods

over the surface of the nutrient agar. It typically contains (mass/volume): 0.5% Peptone - this provides organic nitrogen. 0.3% beef extract/yeast extract - the water-soluble content of these contributes vitamins, carbohydrates, nitrogen, and salts. The plates were incubated at 30 °C for 2–3 days and pure colonies were isolated and streaked on new plates (Fig. 4).

### 2.3. Molecular methodology “Extraction of bacterial DNA”

To extract the total genomic DNA, 300 mg of the fresh 48-hour bacterial biomass was homogenized in 100 µl of PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, USA) and 200 µl molecular water (Sigma, UK). The mixture was incubated in a water bath for 10 min at 100 °C, cooled for 2 min and then centrifuged at 14,000 × g for 3 min to collect the DNA-containing supernatant then stored at –20 °C.

### 2.4. Identification of bacterial isolates “PCR amplification of 16SrRNA and purification”

Amplifications of 16SrRNA were carried out in 50 µl reaction with 1–2 µl lyzed cell sample, 5X FIREPol Master Mix, 10 µM each primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) under the following conditions: 5 min at 95 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 2 min at 72 °C, and 1 cycle of 10 min at 72 °C.

The PCR products were separated on 1% agarose gel. Amplified DNA fragments were purified using Wizard SV Gel & PCR purification kit (Promega). The purified bacterial PCR products were sequenced using the amplification primers and the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA USA).

The PCR amplicons were then sequenced using ABI 3130xl genetic analyzer. BioNumerics v.7.1 (Applied Maths, Ghent, Belgium) was used to analyze sequences. Identification at the species level was performed by comparison with the Ribosomal Database Project database (<http://rdp.cme.msu.edu/>) and by using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### 2.5. Agarose gel electrophoresis

PCR amplification fragments and genomic DNA (gDNA) were examined by agarose gel electrophoresis in 1% TAE agarose gels (Tris-acetate-EDTA buffer). Ethidium bromide (Biorad) was added to a final concentration of 0.5 µg/ml for DNA staining. A drop of 6 X loading dye was added to a 10 µl sample of each PCR reaction which were then loaded onto the agarose gel. 10 µl of diluted 1 Kb



Fig. 3. Images of the study area soil collected areas.

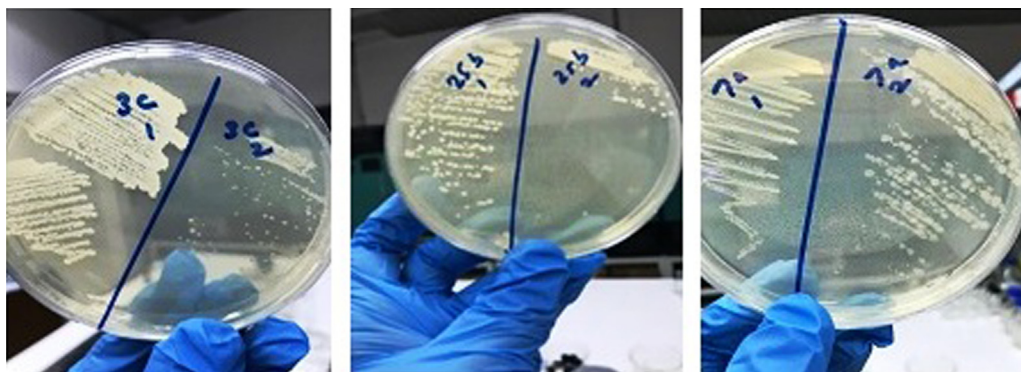


Fig. 4. Four-way streak results.

DNA standard ladder (Qiagen) was used, and the gel was run at 100 V. The gels were run with a 1X TAE buffer for 30 min. The bands were visualized using a UV illuminator and the molecular size of the observed band(s) for each sample was estimated by comparing its position on the gel with the DNA ladder.

#### 2.6. Phylogenetic analysis

The sequences were aligned using Clustal was implemented in MEGA 4.0.2 (Kumar et al., 2018). Phylogenetic trees based on 16S rRNA constructed using the Neighbor-Joining method implemented in MEGA 4.0.2. 16S rRNA tree was estimated by computing 1000 bootstrap trees (Saitou and Masatoshi, 1987). The sequences of the isolates that obtained in this study were deposited in the GenBank of NCBI. The assigned accession number for the identified bacteria are (MW714878 - MW714900).

For many years, the sequencing of 16S rRNA gene has served as an important tool for determining phylogenetic relationships between bacteria. We have therefore taken only from four regions on the island and one region from the land, from each region five samples were collected, then bacterial isolates were identified.

#### 2.7. Statistical analysis

Data was analyzed using Graphpad Prism version 9.0.0 (Windows and Mac). Results were presented as numbers and percentages.

### 3. Results

From a variety of ecological niches in the Umm Al-Namil island, 75 bacterial isolates were identified by 16S rRNA gene, only 49 isolates were clearly sequenced. The coordinates of the sampling sites are presented in Table 1. Most of the soil habitat was sandy soil. Whereas the land sits soil samples were coarse soil habitats.

From each site three different colonies were picked and purified by the four-way streak method (Fig. 4). After bacterial purification, pure colonies were inoculated in broth media, for DNA extraction. Pure colonies of isolated bacteria were obtained from the four-way streak method. DNA for each isolate has been extracted based on the method described in the methodology. The extracted DNA was purified and amplified using specific primers, the sequence for each isolate were analyzed for identification using a phylogenetic tree was constructed to study the relatedness of the samples.

Sequencing analysis version 5.2 software (Applied Biosystems, Foster City, CA, USA) was used to analyze the results. The sequences were subjected to basic local alignment search tool analysis with the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) GenBank database (Altschul et al., 1997). The sequences were then aligned automatically using ClustalX (Thompson et al. 1997) to reference the sequences obtained from GenBank, all information of the sequences were deposited in the GenBank under the accession numbers (MW714878 - MW714900) (Table 2). The table shows that 30 out of 49 isolates were the nearest match to Gammaproteobacteria, whereas 15 out of 49 isolates were the nearest match to Bacilli.

**Table 1**

Description of the soil samples and their coordinates, as determined utilizing the GPS device. The soil sample numbers (1–20) are the island soils, while the numbers (21–25) are control soil “out of the island”.

Isolate name	Habitat types	GPS Coordinates
1	Sandy soil	Lat:29.3848-Long:47.8655
2	Sandy soil with shells	Lat:29.3848-Long:47.8655
3	Muddy soil beside plants	Lat:29.3848-Long:47.8655
4	Sandy soil with shells	Lat:29.3848-Long:47.8655
5	Sandy soil with shells	Lat:29.3848-Long:47.8655
6	Sandy soil	Lat:29.3848-Long:47.8655
7	Sandy soil	Lat:29.3848-Long:47.8655
8	Sandy soil beside bushes	Lat:29.3848-Long:47.8655
9	Sandy soil	Lat:29.3848-Long:47.8655
10	Sandy soil	Lat:29.3848-Long:47.8655
11	Sandy soil beside bushes	Lat:29.3813-Long 47.8680
12	Sandy soil beside bushes	Lat:29.3813-Long 47.8680
13	Sandy soil	Lat:29.3813-Long 47.8680
14	Sandy soil	Lat:29.3813-Long 47.8680
15	Sandy soil beside bushes	Lat:29.3813-Long 47.8680
16	Sandy soil	Lat:29.3791-Long:47.8605
17	Sandy soil beside bushes	Lat:29.3791-Long:47.8605
18	Sandy soil beside bushes	Lat:29.3791-Long:47.8605
19	Sandy soil beside bushes	Lat:29.3791-Long:47.8605
20	Sandy soil with shells	Lat:29.3791-Long:47.8605
21	Land soil beside green bushes	Lat:29.3841-Long:47.8381
22	coarse soil with shells	Lat:29.3841-Long:47.8381
23	coarse soil with shells	Lat:29.3841-Long:47.8381
24	coarse soil with shells	Lat:29.3841-Long:47.8381
25	coarse soil with shells	Lat:29.3841-Long:47.8381

Phylogenetic trees were constructed using the neighbor-joining method, and branch support was gauged using 2000 bootstrap resamplings (Fig. 5). The features of 16 s rRNA that make it a useful phylogenetic tool, also mean it is useful for bacterial identification in the clinical laboratory.

Forty-nine isolates from the soil were identified by 16S rRNA gene sequence analysis. Many of these isolates belong to groups of globally distributed soil bacteria, including well-characterized Gammaproteobacteria, Bacilli, Actinobacteria and Betaproteobacteria.

The results indicated that Umm Al-Namil soil contained at least 10 dominant genera of soil bacteria; *Pseudomonas sp*, *Bacillus sp*, *Planococcus sp*, *Brevibacillus sp*, *Comamonas sp*, *Rheinheimera sp*, *Acinetobacter sp*, *Microbacterium sp*, *Massilia sp* and *Kocuria sp*.

The largest groups are *Pseudomonas sp* and *Bacillus sp*. Clade 1 in the phylogenetic tree grouped mixed isolates of (*Brevibacillus sp*, *Planococcus sp*, *Bacillus sp*, *Kocuria sp*, *Microbacterium sp*, *Comamonas sp*, *Acinetobacter sp* and *Pseudomonas sp*. Clade 2 contained a group of *Bacillus sp* such as *B. atrophaeus*; this species has been used extensively in biomedicine as an indicator for heat and chemical-based decontamination regimens of the environment “Bioremediation”. Clade 3 in the phylogenetic tree contained *Pseudomonas spp.* and *Bacillus* are not closely related, as one is Grm -ve and the other is Gram + ve.

Gammaproteobacteria and Bacilli are shown in all collective regions comparing to others (Fig. 6). However, Gammaproteobacteria is significantly higher than bacilli. Actinobacteria and Betaproteobacteria are found in small quantities among few regions as seen.

#### 4. Discussion

The Umm Al-Namil is a shallow and smallest island located within the Kuwait Bay in the northwestern part of the Arabian Gulf. The island, as an uninhabited island, has been chosen as the source of soil bacteria for the present study. It is influenced by the deltaic and estuarine deposition of the Tigris-Euphrates river system. The soils are wet most of the year due to a high-water

table, with texture ranges from silt loam to clay loam, strongly calcareous with wind-blown sands on the surface. The beach sediment consists of fine-medium oolitic to biogenic sand with a high percentage of coarse grain clastics and shell fragments.

Twenty-five soil samples were collected from four sites on the island for soil bacteria identification, utilizing genotypic techniques. The results were compared with soil bacteria collected from other urban and industrial areas in Kuwait of high human activities. This helped in studying the relatedness of the isolates by the construction of phylogenetic trees.

The spatial distribution of bacterial taxa in soil from different samples at this site was homogeneous hence being a stable and widespread community. Forty-nine isolates were obtained from the island and identified by a comparative analysis of partial 16S rRNA gene sequences. Phylogenetic analysis was carried out to study the relation between the isolates to identify the species.

For many years, most of the known bacteria are identified only from their 16 s ribosomal RNA gene sequence. Thus, 16 s ribosomal gene sequence is the mostly used phylogenetic marker (Valadas et al., 2013). A study was done to isolate micro-organisms that are considered to have biotechnological potential from the soil present in the Altiplano highland of the Atacama Desert (Maza et al., 2019). Next generation sequencing of the 16S rRNA gene was used to examine the soil bacterial diversity which was compared with the results obtained from the culture experiments performed in their study. This indicates that 16S rRNA gene is still widely used because it is presented in at least one copy in every bacterial genome, its conserved regions provide universal primer sites to enable simple sample identification using PCR, and its sequence provides reliable information on bacterial family, genus, or species in most cases. However, it is often not possible to distinguish closely related species or different strains belonging to a single species using this approach.

In order to study the evolutionary descent and relatedness in the bacteria population, phylogenetic tree was used in many studies (Bonifassi et al., 1999). Previously this was carried out using phenotypic markers, but most phylogenies are now based on the DNA sequences. These molecular data can be represented either as a phylogenetic tree (Bonifassi et al., 1999) or else as a network if there are conflicting signals in the data due to recombination (Loidi et al., 2010). Phylogenetics has played a key role in studying the biodiversity, geographic distribution, host range, ecology and behavior of many microorganisms (Emelianoff et al., 2008). A study was conducted (Suleiman et al., 2017) for identifying the species *Vachellia pachyceras* (known as the Lonely tree) and its unidentified relative specimens *V. tortilis* and *V. gerrardii* in Kuwait where Phylogenetics was one of the approaches used for the analyses. As in our study, Clustal and MEGA softwares were used for multiple sequence alignment and construction of phylogenetic trees respectively. Neighbor-Joining method was mainly implemented in our study for constructing the trees and bootstrap analysis (of 1000 bootstrap replications) was used in both the studies for assessing the branch support.

Most of the isolates identified in our study belonged to well-characterized soil bacterial groups such as Gammaproteobacteria, Bacilli, Actinobacteria and Betaproteobacteria. In a study that was performed to assess the bacterial composition in chitin-enriched soils (Das et al., 2010), Gammaproteobacteria and Bacilli were discovered to be the predominant classes which is similar to our paper.

Regardless of the sampling site, our study has determined the isolated soil bacteria most frequently belonging to the *Bacillus* and *Pseudomonas* groups. Some of the *Bacillus* species determined were *B. atrophaeus*, *B. tropicus* etc. that are found to improve plant growth in heavy metal contaminated soil (Efe, 2020). *P. peli*, *P. caspiana*, *P. taiwanensis*, *P. xanthomarina* etc. were the Pseu-

**Table 2**  
Information of the microbial isolates from the studied soil samples.

Isolate No.	Total bases	Subdivision	Nearest GenBank match	Similarity %	Bases compared	GenBank accession no.
1b	288	Gammaproteobacteria	<i>Pseudomonas caspiana</i>	100	292/294	MW714891
1c	293	Gammaproteobacteria	<i>Pseudomonas caspiana</i>	100	295/296	MW714891
2a	285	Gammaproteobacteria	<i>Pseudomonas peli</i>	100	285/285	MW714896
2b	294	Bacilli	<i>Bacillus atrophaeus</i>	100	294/294	MW714879
2c	288	Gammaproteobacteria	<i>Pseudomonas caspiana</i>	100	288/288	MW714891
3a	310	Bacilli	<i>Bacillus tropicus</i> strain	100	310/310	MW714883
3b	298	Gammaproteobacteria	<i>Pseudomonas taiwanensis</i>	100	298/298	MW714899
3c	296	Bacilli	<i>Bacillus atrophaeus</i> strain	99	298/299	MW714879
4a	299	Gammaproteobacteria	<i>Pseudomonas hunanensis</i>	100	299/299	MW714894
4b	300	Gammaproteobacteria	<i>Pseudomonas songnenensis</i>	99	304/306	MW714897
4c	281	Gammaproteobacteria	<i>Pseudomonas coleopterorum</i>	100	281/281	MW714892
5a	303	Gammaproteobacteria	<i>Pseudomonas taiwanensis</i>	100	303/303	MW714899
6a	297	Gammaproteobacteria	<i>Pseudomonas songnenensis</i>	100	297/297	MW714897
6b	287	Gammaproteobacteria	<i>Pseudomonas hunanensis</i>	100	287/287	MW714894
8a	280	Gammaproteobacteria	<i>Pseudomonas taiwanensis</i>	100	280/280	MW714899
9a	315	Bacilli	<i>Bacillus pacificus</i>	100	315/315	MW714881
9b	317	Bacilli	<i>Bacillus pacificus</i>	100	317/317	MW714881
10a	295	Actinobacteria	<i>Kocuria himachalensis</i>	100	295/295	MW714886
10b	298	Betaproteobacteria	<i>Massilia yuzhufengensis</i>	100	296/298	MW714887
11b	292	Bacilli	<i>Bacillus Mojavensis</i>	98	304/310	MW714880
12a	278	Bacilli	<i>Brevibacillus laterosporus</i>	100	282/282	MW714884
12b	290	Bacilli	<i>Bacillus simplex</i>	100	290/290	MW714882
13a	297	Gammaproteobacteria	<i>Pseudomonas stutzeri</i>	100	297/297	MW714898
13b	284	Gammaproteobacteria	<i>Pseudomonas stutzeri</i>	100	297/297	MW714898
14a	296	Gammaproteobacteria	<i>Pseudomonas caspiana</i>	99	300/302	MW714891
14b	287	Actinobacteria	<i>Microbacterium hydrocarbonoxydans</i>	99	289/290	MW714888
15a	291	Bacilli	<i>Bacillus tropicus</i>	99	293/294	MW714883
15b	292	Bacilli	<i>Bacillus tropicus</i>	100	292/292	MW714883
16a	294	Bacilli	<i>Bacillus tropicus</i>	100	294/294	MW714883
18a	282	Bacilli	<i>Brevibacillus laterosporus</i>	100	282/282	MW714884
18b	290	Bacilli	<i>Bacillus simplex</i>	100	290/290	MW714882
19a	281	Gammaproteobacteria	<i>Pseudomonas hunanensis</i>	100	281/281	MW714894
19b	272	Gammaproteobacteria	<i>Pseudomonas hunanensis</i>	99	278/281	MW714894
20a	288	Bacilli	<i>Planococcus kocurii</i>	100	288/288	MW714888
20c	298	Gammaproteobacteria	<i>Pseudomonas taiwanensis</i>	100	298/298	MW714899
21a	249	Betaproteobacteria	<i>Comamonas jiangduensis</i>	98	259/264	MW714885
21b	288	Gammaproteobacteria	<i>Pseudomonas entomophila</i>	100	288/288	MW714893
21c	292	Bacilli	<i>Bacillus tropicus</i>	100	292/292	MW714883
22a	280	Gammaproteobacteria	<i>Pseudomonas hunanensis</i>	100	280/280	MW714894
22b	278	Gammaproteobacteria	<i>Rheinheimera mesophila</i>	99	280/281	MW714900
22c	294	Gammaproteobacteria	<i>Pseudomonas alcaligenes</i>	100	294/294	MW714890
23a	267	Gammaproteobacteria	<i>Acinetobacter oryzae</i>	99	275/279	MW714878
23b	285	Gammaproteobacteria	<i>Pseudomonas entomophila</i>	100	285/285	MW714893
24a	301	Gammaproteobacteria	<i>Pseudomonas moorei</i>	100	301/301	MW714895
24b	293	Gammaproteobacteria	<i>Pseudomonas entomophila</i>	99	295/296	MW714893
25a	301	Gammaproteobacteria	<i>Pseudomonas stutzeri</i>	100	301/301	MW714898
25b	298	Gammaproteobacteria	<i>Pseudomonas stutzeri</i>	100	298/298	MW714898

domonas species isolated which are recorded as Potential Biological Agents for Agricultural and Environmental Uses, some of this strain was considered as a promising resource for agriculture and bioremediation of contaminated soils (Crovadore et al., 2016). *Pseudomonas alcaligenes* is a Gram-negative aerobic bacterium used for bioremediation purposes of oil pollution, pesticide substances, and certain chemical substances, as it can degrade polycyclic aromatic hydrocarbons. It can be a human pathogen, but occurrences are very rare.

Such studies can significantly expand the knowledge about bacterial identification and occurrence of specific species in different types of soil which are relevant in expanding the view of the increase in database storage of various types of bacteria present in Kuwait soil. This might allow considering the opportunity to consider soil as an important source of different types of bacteria which can survive in suboptimal conditions, as the condition found on the island.

Another study from Saudi Arabia was done to identify soil samples from different habitats. They found high diversity in microbial communities that varied in soil habitat. Although the molecular identification of the collected isolates was classified to two

bacterial genera, *Bacillus* and *Lactobacillus*, results showed high specificity of some microbes to a specific area. Generally, their work in the study revealed no stability in the bias of variations of soil variables in relation to microbial (Alotaibi et al., 2020).

## 5. Conclusion

This work is a pioneer study aimed to generate baseline data on the bacterial communities and diversity in soil samples from Umm Al-Namil Island in Kuwait using 16S rRNA sequencing. This is to understand the biodiversity present in different settings, how bacteria adapt to different niches in the environment as well as in different hosts.

The results indicate that the most abundant phylotypes were members of the Gammaproteobacteria and Bacilli. The prevalence of potentially pathogenic species was low. The data from different samples showed consistency in the dominance of *Pseudomonas* spp. and *Bacillus* species. Future work could focus on whole genome sequencing these strains for further detailed characterization, and for using metagenomics to assay the diversity within the sam-

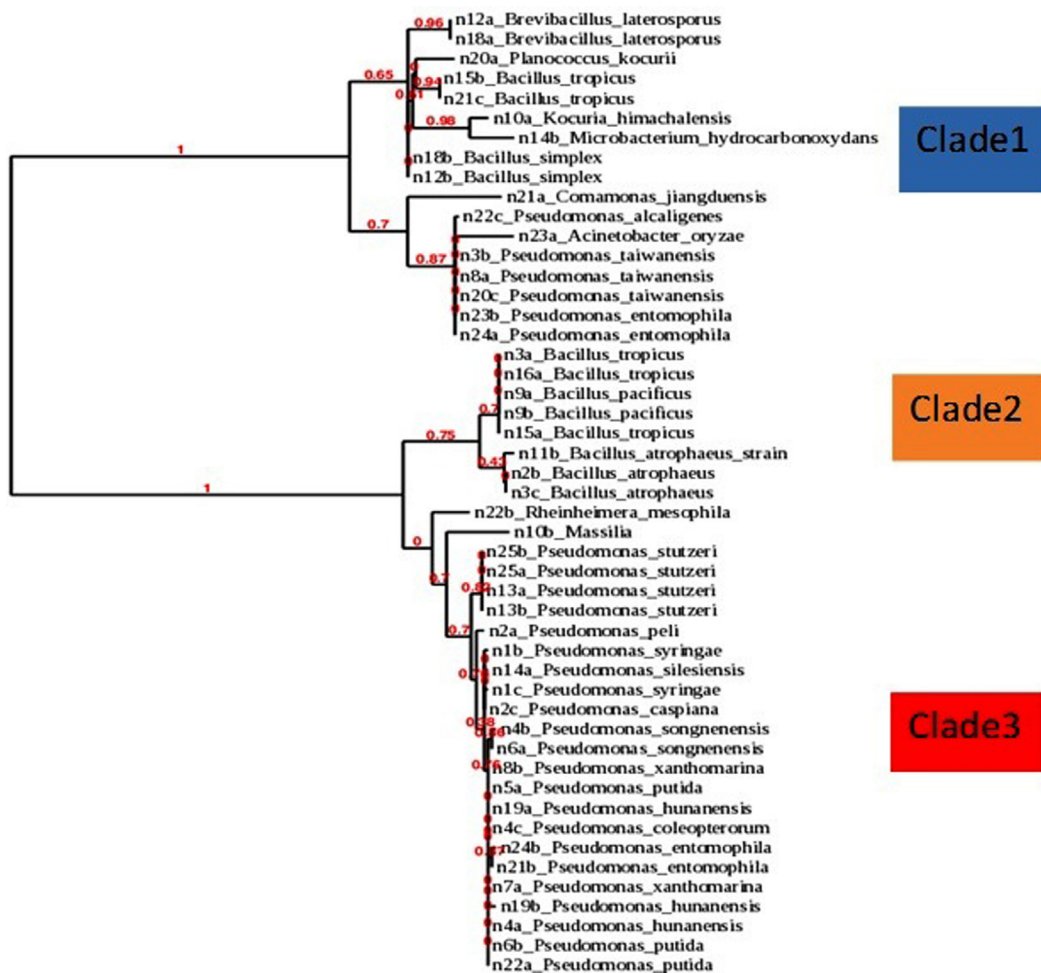


Fig. 5. Neighbour-joining tree based on 16S rRNA gene sequences was used to build up the tree with 1000 bootstraps for the 49 isolates.

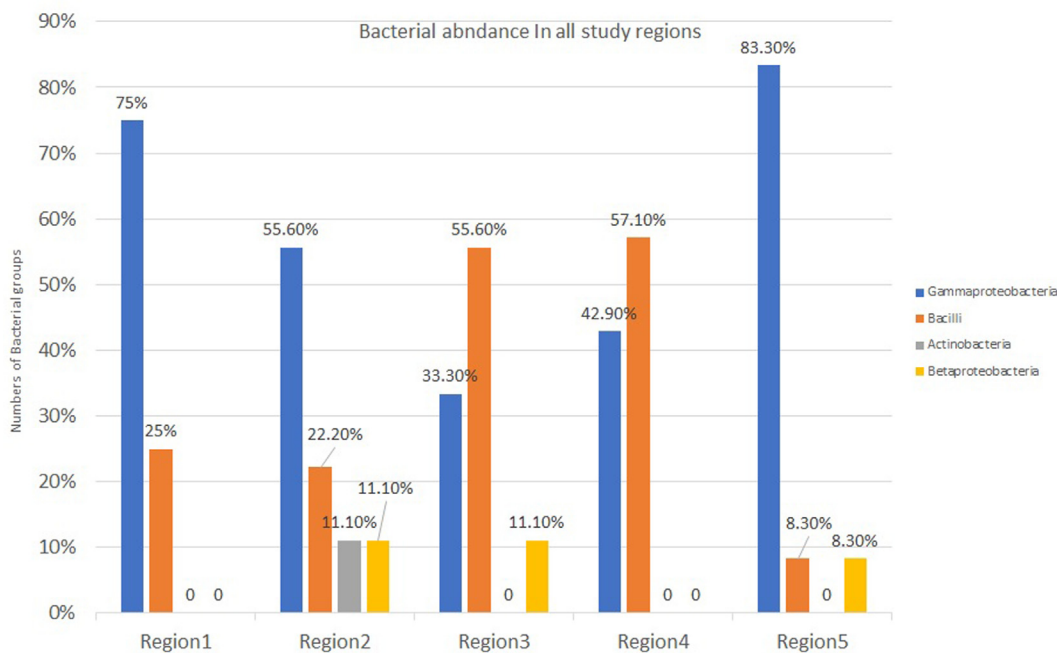


Fig. 6. Bacterial abundance in all study regions.

ples without introducing the biases due to culturing. Nevertheless, this represents an important first step in assaying bacterial diversity in Kuwaiti soils.

### 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.

### Acknowledgements

The authors are grateful to the Research Administration in the public authority for applied education and training (PAAET) who funded this research project (Project No BE-19-04). The authors are also indebted to the Science Department for providing the facilities in the laboratory, where all the analytical and experimental work was carried out. The authors appreciate the thorough review given by an anonymous reviewer, which improved the quality of the manuscript. The authors would like to thank the General Facility at Kuwait University Project GS01/02 for genetic analysis facility.

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