Journal of Environmental Sciences, 2021; Vol. 50, No. 3: 70-78

Journal of Environmental Sciences

JOESE 5

Population structure of soil bacteria in the Kuwaiti governorates, using 16S rRNA sequencing

Fadaa Alowna,*, Ahlam Alsharidaha and Mayada Kansourb

a.Public Authority for Training and Applied Education (PAAET). Department of Science-College of Basic Education, Kuwait.

b. Kuwait University, Faculty of Science, Biological Sciences Department, Kuwait.

* Corresponding author, e-mail address: fadaaalown@gmail.com.

Reprint

Volume 50, Number 3: 70-78

(2021)

http://Joese.mans.edu.eg

P-ISSN 1110-192X

e-ISSN 2090-9233

Journal of Environmental Sciences, 2021; Vol. 50, No. 3: 70-78

Journal of Environmental Sciences

JOESE 5

ISSN 2090-9233

Journal homepage http://Joese.mans.edu.eg

Original Article

Population structure of soil bacteria in the Kuwaiti governorates, using 16S rRNA sequencing

Fadaa Alowna,*, Ahlam Alsharidaha and Mayada Kansourb

a.Public Authority for Training and Applied Education (PAAET). Department of Science-College of Basic Education, Kuwait.

b. Kuwait University, Faculty of Science, Biological Sciences Department, Kuwait.

* Corresponding author, e-mail address: fadaaalown@gmail.com.

Article Info

Article history:

Received 17/ 8 /2021

Received in revised form 30/10/2021

Accepted 22/11/2021

Keywords: *Bacilli, Kuwaiti Governorates, phylogenetic analysis*.

Abstract

This research represents the first biogeography study of soil bacteria in Kuwait, by analyzing the 16S rRNA gene for 53 isolated bacteria collected from the six Kuwaiti governorates in summer and winter. No significant differences were found in bacterial communities among seasons, indicating that seasonal variation in temperature had little influence on bacterial community. The isolated soil bacteria were studied using phylogenetic analysis, that help in building up the genetic database of the bacterial Kuwaiti isolates. Twenty-eight bacterial taxa were identified by 16S rRNA gene sequencing. The results showed that most of bacteria species belong to Bacilli., while Gammaproteobacteria, Actinobacteria and Betaproteobacteria are less abundant. Cronobacter sakazakii, Enterobacter cloacae, Stenotrophomonas pavanii, Serratia rubidaea, Mixta theicola, and three different species of Pseudomonas were detected. No significant seasonal differences were found. Whole genome sequencing is required to understand how these taxa might be adapted to their different environments.

1. Introduction

16S rDNA gene sequencing is a commonly used tool to identify environmental and clinical bacteria, without the need to culture (Mignard and Flandrois, 2006). However, this tool cannot provide any information regarding antibiotic resistance. Traditionally the diversity of soil bacteria is assayed by phenotypic methods (Torsvik et al.,1990), but 99.5%-99.9% of some group of bacteria such as the fluorescent soil bacteria cannot be observed by culturing on media, therefore most bacteria will be excluded from the work if culturing is required (Torsvik et al., 1990).

Regarding the bacterial community in the soil, especially the heterotrophic bacteria (which is responsible for the mineralization of the organic matter content in the soil) these bacteria share the consumption of the organic carbon and releases the CO2 by soil respiration. Moreover, decomposition of the soil organic matter by microorganism's activities may also release CO2 which also make changes in the microbial community. Soil microbial communities are very complex, and it is an extremely useful ecosystem for economic sustainability as well as for global nutrient cycling (El Shahed et al., 2008).

Environmental surveys around the world increase our understanding of the microbial ecology and evolution by determining how microbes interact with different physical and chemical factors. This will provide an evidence on the tolerance of the microbes to different environmental changes, such as temperature and pH (Lozupone and Knight, 2007).

Phylogenetics is the study of evolutionary descent and relatedness (Gregory, 2008). 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 (Gregory, 2008) or else as a network if there are conflicting signals in the data (Huson and Bryant, 2006). Phylogenetics have played a key role in studying the biodiversity, geographic distribution, host range, ecology, behavior and coevolution of pathogenic bacteria (Stock, et al., 2009).

16S ribosomal DNA sequence is the most used phylogenetic marker, as it is universally present in bacteria, and a high level of conservation makes it possible to design PCR primers that work on a wide range of taxa (Vandamme, et al., 1996). However, it

is often not possible to distinguish closely related species or different strains which belong to a single species using this approach.

1.1 Background

This study specified for identifying different types of isolated soil bacteria from different regions among Kuwait governorates, isolation was done in July 2017 and March 2018. The isolated soil bacteria from different regions were studied using phylogenetic analysis.

Since climatic conditions and soil type may influence the distribution of bacteria and the associated host (Lelie et al., 2015), the genotype of collected samples in each region among two seasons were correlated. The effect of soil moisture on bacterial growth was studied by estimating the incorporation of thymidine and leucine. Soil temperature is also an important environmental factor. Optimum microbial growth in the soil occurs at 25-30°C.

Many studies in Kuwait have focused on a specific type of soil bacteria, such as hydrocarbon, thermophilic, halophilic bacteria. The ecological attributes and the drivers determining soil bacteria composition and distribution is limited. This project represents the first biogeography study of soil bacteria in Kuwait, by analyzing the 16S rRNA gene in 54 isolated bacteria collected from the six Kuwaiti governorates; Kuwait City (Alasimah), Hawalli, Farwaniyah, Mubarak Al-Kabeer, Ahmadi and Jahra Governorates (Fig. 1). This will help in building up the genetic database of the bacterial Kuwaiti isolates.

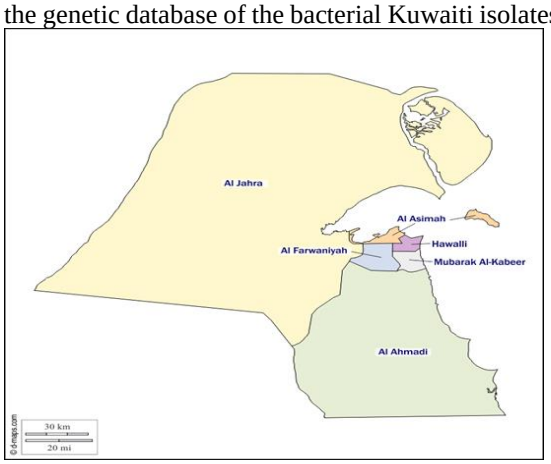


Figure 1. Locations and names of the Kuwaiti governorates. G1 (Al-Asimah), G2 (Hawally), G3 (Al-Farwaniyah), G4 (Mubarak Al-Kabeer), G5 (Al-Ahmadi), and G6 (Al-AJahra).

1.2 Geography

The relief of Kuwait is generally flat or gently undulating, broken only by occasional low hills and shallow depressions. The land (desert) begins to rise slightly in the southwest, along the border with Saudi Arabia. The elevations range from sea level in the east to 951 feet (290 metres) above sea level at Al-Shiqāyā peak, in the western corner of the country. Elsewhere in coastal areas, large patches of salty marshland have developed. Throughout the northern, western, and central sections of Kuwait, there are desert basins, which fill with water after winter rains; historically these basins formed important watering places, refuges for the camel herds of the Bedouin.

Kuwait has no permanent surface water, either in the form of standing bodies such as lakes or in the form of flows such as perennial rivers. Intermittent water courses (wadis) are localized and generally terminate in interior desert basins. Little precipitation is absorbed beyond the surface level, with most being lost to evaporation. Except in the new green belt of Kuwait city and in a few desert oases such as Al-Jahra, where cultivation and irrigation are carried out, the vegetation consists of scrub and low bushes (and ephemeral grass in the spring). Halophytes (salt-loving plants) grow on the marshy stretches along the coast. The harsh climate limits mammals to the occasional gazelle, fox, or civet (Britannica, 2021).

1.3 Climate of Kuwait

The climate of Kuwait is desert, with a huge temperature difference between winter and summer. If there is enough rainfall, the desert turns green from mid-March to the end of April. But during the dry season, between April and September, the heat is severe daytime temperatures ordinarily reach 44°C and on occasion approach 54°C. The winter is more agreeable (frost can even occasionally occur in the interior, though never on the seacoast).

The frequent winds from the northwest are cool in winter and spring and hot in summer. Southeasterly winds, usually hot and damp, spring up between July and October; hot and dry south winds prevail in spring and early summer. The “shamāl” is a northwesterly wind common during June and July, causes dramatic sandstorms.

Annual rainfall averages only from 25 to 180 mm, chiefly between October and April, though cloudbursts can bring more than 2 inches (50 mm) of rain in a single day (Britannica, 2021).

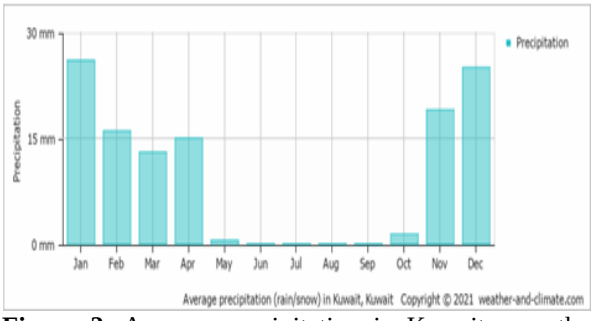


Figure 2. Average precipitation in Kuwait over the year. (Data from weather station: Kuwait, Kuwait). https://www.climatestotravel.com/climate/kuwait

1.4 Soils

The soil of Kuwait governorates is mainly sandy in texture with little organic matter and scarce plant nutrients to support plant growth and development. Most of soils are shallow in depth from continuous wind erosion and the lower calcareous layers that appear on the topsoil. Over-grazing and vehicle

movement through the desert soil enhanced soil erosion and mobility. Soil water holding capacity is low (7%) with high infiltration rate (50-100 cm/h). Evaporation is high (3,000 mm/y), particularly in the summer periods (Mahdi and Majda, 2002). Ten soil types are mapped by Aziz and Al-Ali (2014) characterizing the soils of Kuwait. These are clay, clayey-sand, gypsum, pebble, pebble-gypsum, rocky, and salt-Gypsum, sand dunes, sand, and sandy limy soil types.

This study is the first biogeographical study, that aims to identify different types of isolated soil bacteria from the Kuwaiti governorates. This will help in building up the genetic database of the bacterial Kuwaiti isolates. The main objective of this study was to improve our understanding about biodiversity of soil bacteria in the six Kuwaiti governorates, the following specific aims:

1. Evaluate the environmental parameters that control the distribution of soil bacteria.
2. Detect the difference of the bacterial communities in the six governorates.
3. Identify the dominant group of bacteria in the soil.

2. Materials and Methods

2.1 Site selection and soil sampling

One hundred and twenty soil samples were collected from the six governorates mentioned before, 10 samples from each governorate were collected in July 2017 temperature (50-55°C) and 10 samples from each governorate were collected in March 2018 temperature (18-20°C), 54 samples were successfully analyzed. The samples from each governorate have different vegetation types and a wide range of soil types and environmental characteristics. Samples were numbered in clean plastic bag and closed tightly and transferred to the research lab. Soil samples were sieved through 2-mm mesh to homogenize and remove roots, plants detritus and stones. These samples were collected from a depth ranges from 5-10 cm below the ground surface, then 10 grams were mixed for 5 min. in bottles containing 90 ml of autoclaved distilled water.

2.2 Bacteria isolation and purification

0.1ml 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 contribute 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**.**

2.3 Bacterial DNA Extraction

To extract the total genomic DNA for each sample, 300 mg of the fresh 48-h 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, then cooled for 2 min and finally centrifuged at 14,000 x g for 3 min to collect the DNA-containing supernatant extractions

2.4 PCR amplifications and purification

The 16S rRNA genes were amplified by polymerase chain reaction (PCR). The mixture contained puReTaq Ready-To-Go PCR Beads (Amersham Biosciences, UK), 1 µl (25 ng) of DNA template, 1 µl each of the universal primer combinations GM5F (5′-CCTACGGGAGGCAGCAG-3′) and 907R (5′-CCGTCAATTCMTTTGAGTTT-3′) (Santegoeds et al., 1998). The reaction volume was completed to 25 µl with molecular water.

Amplification was done in Veriti Thermal Cycler (Applied Biosystems, USA) by a touch-down PCR in which the initial denaturation was at 95 °C for 5 min, and the annealing temperature started at 65 °C and decreased by 1 °C every cycle to 55 °C, at which additional 15 cycles were carried out. Denaturation was at 94 °C for 1 min, and primer extension at 72 °C for 1 min..The PCR products were purified using QIA quick PCR purification kit (Qiagen, USA) in order to remove the Taq polymerase, primers and dNTPs.

2.5 Processing of sequencing data

Partial sequencing of the 16S rRNA genes was performed using BigDye version Terminator Kit (Applied Biosystems, USA); 20 ng of the PCR product was added to 2 µl of the Big Dye v 3.1 terminator and 2 µl of Big Dye Terminator v 1.1, v 3.1 5X sequencing buffer; l µl of either 907R or GM5F was added to the mixture, and the final volume was brought up to 10 µl with molecular water. Labeling was completed in Veriti Thermal Cycler (Applied Biosystems, USA) using one cycle of 96 °C for l min, then 25 cycles of l min at 96 °C, 5 s at 50 °C and 4 min at 60 °C. The pure template DNA samples were processed in 3130xl genetic analyzer (Applied Biosystems, USA).

2.6 Phylogenetic analysis

Sequencing analysis version 5.2 software (Applied Biosystems, USA) was used to analyze the results. Sequences were subjected to basic local alignment search tool analysis with the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) GenBank database (Al tschul et al., 1997). The sequences were deposited in the GenBank under the accession numbers (MW680907-MW680934). A phylogenetic tree was constructed using neighbor-joining bootstrap proportions, based on 2000 replicates.

2.7 Statistical analysis

Graphpad Prism version 9.0.0 (Windows and Mac) was used for the data analyzing. Results were presented as numbers and percentages.

Table 2. Information related to 16S rRNA gene sequencing of the microbial isolates

Isolate no.	Total bases	Subdivision	Nearest GenBank match	Similarity %	Bases compared	GenBank accession no.
1	549	Betaproteobacteria	*Achromobacter mucicolens*	100	549/549	MW680907
2	547	Bacilli	*Bacillus amyloliquefaciens*	100	547/547	MW680908
3	561	Bacilli	*Bacillus cereus*	100	561/561	MW680909
4	546	Bacilli	*Bacillus halosaccharovorans*	100	546/546	MW680910
5	558	Bacilli	*Bacillus mojavensis*	100	558/558	MW680911
6	546	Bacilli	*Bacillus nakamurai*	100	546/546	MW680912
7	546	Bacilli	*Bacillus piscis*	100	546/546	MW680913
8	423	Bacilli	*Bacillus proteolyticus*	99	431/435	MW680914
9	548	Bacilli	*Bacillus simplex*	100	548/548	MW680915
10	549	Bacilli	*Bacillus subtilis*	100	549/549	MW680916
11	547	Bacilli	*Bacillus tequilensis*	100	547/547	MW680917
12	549	Bacilli	*Bacillus tropicus*	100	549/549	MW680918
13	561	Bacilli	*Bacillus wiedmannii*	100	561/561	MW680919
14	529	Actinobacteria	*Cellulosimicrobium cellulans*	100	529/529	MW680920
15	530	Betaproteobacteria	*Comamonas thiooxydans*	99	540/545	MW680921
16	539	Gammaproteobacteria	*Cronobacter sakazakii*	100	539/539	MW680922
17	514	Actinobacteria	*Dietzia papillomatosis*	99	520/523	MW680923
18	551	Gammaproteobacteria	*Enterobacter cloacae*	100	551/551	MW680924
19	549	Bacilli	*Lysinibacillus boronitolerans*	100	549/549	MW680925
20	539	Bacilli	*Lysinibacillus fusiformis*	100	539/539	MW680926
21	552	Bacilli	*Lysinibacillus pakistanensis*	100	552/552	MW680927
22	552	Bacilli	*Lysinibacillus sphaericus*	100	552/552	MW680928
23	547	Gammaproteobacteria	*Mixta theicola*	100	547/547	MW680929
24	553	Gammaproteobacteria	*Pseudomonas indoloxydans*	100	553/553	MW680930
25	549	Gammaproteobacteria	*Pseudomonas putida*	99	553/555	MW680931
26	548	Gammaproteobacteria	*Pseudomonas songnenensis*	100	548/548	MW680932
27	555	Gammaproteobacteria	*Serratia rubidaea*	100	555/555	MW680933
28	551	Gammaproteobacteria	*Stenotrophomonas pavanii*	100	551/551	MW680934

Table 3. Bacterial isolates identities in each governorate at different seasons.

Governorates	Bacterial isolates at Winter	Bacterial isolates at Summer
G1	*Bacillus proteolyticus* *Pseudomonas putida* *Lysinibacillus pakistanensis* *Bacillus cereus* *Bacillus amyloliquefaciens*	*Bacillus cereus* *Bacillus simplex* *Bacillus nakamurai* *Enterobacter cloacae*
G2	*Bacillus tropicus* *Bacillus subtili* *Bacillus cereus*	*Bacillus cereus* *Cellulosimicrobium cellulan* *Lysinibacillus boronitolerans* *Bacillus tequilensis* *Bacillus halosaccharovorans* *Bacillus amyloliquefaciens*
G3	*Lysinibacillus pakistanensis* *Lysinibacillus pakistanensis* *Comamonas thiooxydans* *Bacillus mojavensis*	*Lysinibacillus fusiformis* *Mixta theicola* *Bacillus subtilis* *Lysinibacillus pakistanensis* *Bacillus cereus*
G4	*Achromobacter mucicolens* *Lysinibacillus boronitolerans* *Stenotrophomonas pavanii* *Stenotrophomonas pavanii* *Bacillus nakamura* *Dietzia papillomatosi* *Bacillus cereus*	*Bacillus subtili* *Bacillus cereus* *Cronobacter sakazaki*
G5	*Bacillus wiedmannii* *Bacillus wiedmannii* *Bacillus simplex* *Lysinibacillus pakistanensis* *Bacillus cereus* *Pseudomonas indoloxydans*	*Lysinibacillus boronitolerans*
G6	*Bacillus wiedmannii* *Serratia rubidaea* *Bacillus tropicus* *Lysinibacillus sphaericus* *Bacillus cereus* *Bacillus cereus*	*Bacillus piscis* *Bacillus cereus* *Bacillus cereus* *Bacillus subtilis* *Pseudomonas songnenensis*

3. Results and Discussion

One hundred twenty soil samples were collected from the six Kuwaiti governorates, denoted G1(Al-Asimah), G2 (Hawally) , G3 (Al-Farwaniyah), G4 (Mubarak Al-Kabeer), G5 (Al-Ahmadi), G6 (Al-AJahra). The soil almost has the same profile of the governorates from G1 to G5, that is mainly sandy with salt and gypsum accumulations, while the governorate G6 is characterized by sandy to loamy soil with gypsum and carbonate cement (Aziz and Al-Ali, 2014). The soil of governorate no. G4 was covered by urban and industrial activities. The identified isolates are illustrated in Figure (4).

Al-Saleh and Akbar (2015) stated that both Bacillus sp and pseudomonas sp are dominant in oil contaminated soil of Kuwait, such as the Sulaibikhat. The contaminating pollutants include hydrocarbons, heavy metals, and suspended particles. Study in Kuwait identified a two strains Bacillus velezensis and Bacillus subtilis as HC-degrading strain isolated from Kuwait Bay and from mangroves rhizosphere, respectively (Yateem and Al-Sharrah, 2011). Soil contained Bacillus stearothermophilus was found in oil contaminated desert soil samples, collected in summer seasons, that are capable to grow at extreme temperature obligatory at high temperature and on crude oil as a sole source of carbon and energy (Sorkhoh., et al 1993).

The data shows that 42 out of 54 bacterial isolates were successfully identified by 16SrRNA gene. These isolates belong to four subdivision as shown in Table 1. The table shows that 41 out of 54 isolates were the nearest match to Bacilli, whereas 9 out of 54 isolates were the nearest match to Gammaproteobacteria. The rest of the 54 sample were identified equally as Betaproteobacteria and Actinobacteria.

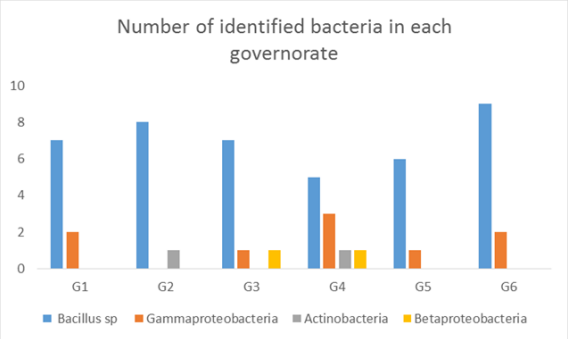
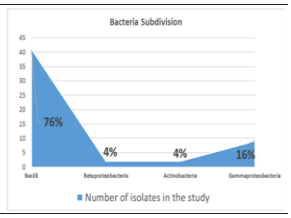


Figure 3. Distribution of the identified soil bacteria groups in the six governorates.

Out of 120 samples 28 bacterial isolates were identified as shown in Table 3. The sequences were aligned automatically using ClustalX (Thompson et al. 1997) to reference the sequences obtained from GenBank, all information of the sequences was deposited in the GenBank under the accession numbers (MW680907 - MW680934) (Table 2).

Table 1. Numbers of identifying bacteria groups.

Subdivision	Number of isolates
Bacilli	42
Betaproteobacteria	2
Actinobacteria	2
Gammaproteobacteria	9



The results of the 16S rRNA analysis shows that no differences in the distribution of bacteria groups in the two seasons (Figure 5).

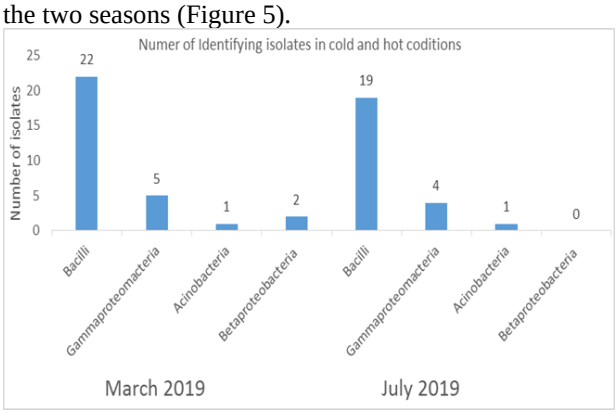


Figure 4. The distribution of bacterial groups in cold (March) and hot (July) temperatures.

As we know that soil bacterial diversity is massive, and the composition and diversity of soil bacteria can be affected by a wide range of biotic and abiotic factors (Fierer and Jackson, 2006). However, our understanding of the factors that structure soil bacterial across sites is limited. The results show that Bacilli was the dominant in the six governorates in both summer and winter (Table 3). Phylogenetic tree was constructed using neighbor-joining method. The branch support was gauged using 2000 bootstrap re-samplings (Fig. 6).

The largest groups are Bacilli and Gammaproteobacteria, respectively. These groups were found in all collective sites in Kuwaiti soil. However, Bacilli is significantly higher than Gammaproteobacteria.

Actinobacteria and Betaproteobacteria were found in relatively low diversity and abundance among the government. 16S sequencing was performed and phylogenetic tree was constructed to identify the 54 isolates.

A significantly high degree of identity in the Bacilli strains (100 % similarity) when examined for sequence alignment using BLASTN 2.2.1 for the identification of the 16S rRNA gene sequence. 16S rRNA was the used for characterization of the samples, which is genomic approach to study the identity of bacteria based upon 16S rRNA gene (Lane et al., 1985). This gene is a conserved among prokaryotic cells, which is perfect to use as primer site for samples gene amplification using PCR (Lin & Schwarz., 2003).

Depending on the climatic changes it has been found that the soil respiration has some fluctuations depending on the warming and cooling of the temperature (Kuffner et al., 2012). However, our study represents controversial results, that the distribution of soil bacteria does not affected by climate changes during winter and summer.

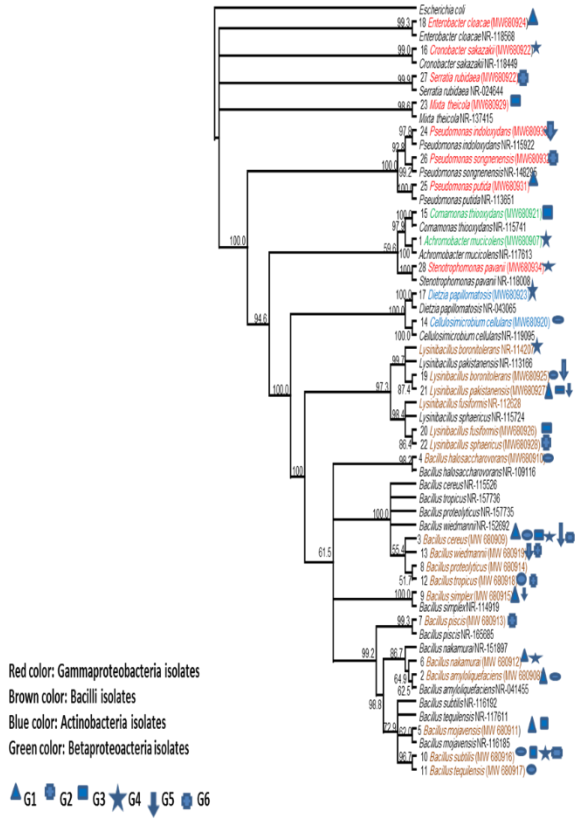


Figure 5 Phylogenetic tree based on 16S rRNA gene partial sequences showing the phylogeny of isolated bacteria (Table 1). Values shown on each node of the phylogenetic trees are bootstrap values. A total of 2,000 bootstrap replicates were performed.

Conclusions

Strains of soil bacteria from different soil samples of the six Kuwaiti governments were successfully

isolated. Twenty-eight bacteria isolate from soil samples was tested initially by 16S rRNA gene sequencing. The results showed most of bacteria species belong to Bacilli (76%), while the rest are Gammaproteobacteria (16.6%), Actinobacteria and Betaproteobacteria (3.7 %). By comparison with a similar study on a small Kuwaiti island, called Umm Al-Namil, the results revealed that Gammaproteobacteria and Bacilli are the dominant species (Alown et al., 2021), but the bacterial isolates species in Gammaproteobacteria group was different. In this study Cronobacter sakazakii, Enterobacter cloacae, Stenotrophomonas pavanii, Serratia rubidaea, Mixta theicola, and three different species of Pseudomonas were detected. By contrast, in the previous study of Alown et al. (2021), only Pseudomonas sp was found belonging to Gammaproteobacteria in the soil of Umm Al-Namil. Interestingly, all of the identified isolates group well known as soil bacteria that has been identified in other region such as Saudi Arabia (Alotabi et al., 2020), Bacillus subtilis strains also was identified and characterized from oil contaminated soil in Kuwait by 16S rRNA sequencing (Al-Sharidah et al., 2000). Whereas Pseudomonus spp was found in both oil contaminated and non-contaminated soil samples in Kuwait (Al-Saleh & Amber, 2015).

No significant differences were found in bacterial communities among seasons, indicating that seasonal variation in temperature had little influence on bacterial community among six governorates. The study recommended by further research to study the whole genomic sequences and evaluate their adaptation to different environments.

Acknowledgements

The authors are indebted to Prof. Edward Feil, Professor of Bacterial Evolution, University of Bath Department of Biology and Biochemistry, for language editing. Special gratitude's are directed to Kuwait University project GS01/02 for analyzing the bacterial sequencing.

4. References:

Alotaibi, M.O., Sonbol, H.S., Alwakeel, S.S., Suliman, R.S., Fodah, R.A., Abu Jaffal, A.S., Alothman, N. I., Mohammed, A.E. (2020). Microbial Diversity of Some Sabkha and Desert Sites in Saudi Arabia. Saudi Journal of Biological Sciences, 27: 2778–2789.

Alown, F., Alsharidah, A., Shamsah, S. (2021). Genotypic Characterization of Soil Bacteria in the Umm Al-Namil Island, Kuwait. Saudi Journal of Biological Sciences, doi: https://doi.org/10.1016/j.sjbs.2021.03.060, In press.

Al-Saleh, E. and Akbar, A. (2015). Occurrence of Pseudomonas aeruginosa in Kuwait Soil. Chemosphere, 120: 100-107.

Aziz, M.A. and Al-Ali, J.M. (2014). Application of Geoinformatics on Mapping the Desertification Sensitivity in Kuwait. Bulletin of the Egyptian Geographical Society 87, 28-53.

El Shahed, M.S., Youssef, N.H., Spain, A.M., Sheik, C., Najar, F.Z., Sukharnikov, L.O., Roe, B.A., Davis, J.P., Schloss, P.D., Bailey, V.L., Krumholz, L.R. (2008). Novelty and uniqueness patterns of rare members of the soil biosphere. Appl. Environ. Microbiol. 74(17), 5422-8.

Al-Sharidah, A., Richard, A., Golecki, J., Dierstein, R., Tadros, M. (2000). Isolation and characterization of two hydrocarbon-degrading Bacillus subtilis strains from oil contaminated soil of Kuwait. Microbiological Research 155(3). 157-164.

Al tschul S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zheng, Z., Miller, W. & Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research 25, 3389-3402.

Fierer, N. and Jackson, R. (2006). The diversity and biogeography of soil bacterial communities. PNAS, 103 (3) 626-631. https://doi.org/10.1073/pnas.0507535103

Gregory, T.R., 2008. Understanding Evolutionary Trees. Evolution: Education and Outreach 1(2), 121-137.

Huson, D.H., and Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23(2), 254-267.

Iovieno, P. and Baath, E. (2008). Effect of drying and rewetting on bacterial growth rates in soil. Fems Microbiology Ecology 65(3), 400-407.

Khalaf, F. I., Gharib, I. M. and Al-Hashash, M.Z. (1984). Types and characteristics of the Recent Surface Deposits of Kuwait, Arabian Gulf. Journal of Arid Environments 7,9-33.

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., Sessitsch, A. (2012). Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. FEMS Microbiology Ecology 82(3), 551–562.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L. and Pace, N.R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci., 82, 6955-6959.

Lelie, D.V., Jack A., and Gilberta, E.H. (2015). The Soil Microbiome Influences Grapevine-Associated Microbiota. mBio 6(2), 1-10.

Lin, M. and Schwarz, J.R. (2003). Seasonal shifts in population structure of Vibrio vulnificus in an estuarine environment as revealed by partial 16S ribosomal DNA sequencing. FEMS Microbiol. Ecol., 45, 23-27

Lozupone, C.A., and Knight, R. (2007). Global patterns in bacterial diversity. Proc. Natl. Acad. Sci. 104(27), 11436–11440.

Mahdi, A. and Majda, S. (2002). Soil properties and characteristic in Kuwait for agricultural development. Thai National AGRIS Centre, Kasetsart University.

Mignard, S. and Flandrois, J.P. (2006). 16S rRNA sequencing in routine bacterial identification: A 30-month experiment. Journal of Microbiological Methods 67(3), 574-581.

Pietikainen, J., Pettersson, M., and Baath, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. Fems Microbiology Ecology 52(1),49-58.

Santegoeds, C.M., Ferdelman, T.G., Muyzer, G. & Beer, D. (1998). Structural and functional dynamics of sulfate-reduction populations in bacterial biofilms. Applied and Environmental Microbiology 64, 3731-3739

Sorkhoh, N.A., Ibrahim, A.S., Ghannoum, M.A. (1993). High-temperature hydrocarbon degradation by Bacillus stearothermophilus from oil-polluted Kuwaiti desert. Appl Microbiol Biotechnol 39, 123–126. https://doi.org/10.1007/BF00166860

Stock, S., Vandenberg, J. and Glazer, I. (2009). Insect Pathogens. Molecular Approaches and Techniques, CAB Intl.

Thompson, S.A., mani, E.V., Lindell, A.H., King, C. J & McArthur, J. V. (2007). Novel tetracycline resistance determinant isolated from an environmental strain of Serratia marcescens. Applied and Environmental Microbiology 73(7), 2199-2206.

Torsvik, V., Goksoyr, J. and Daae, F.L. (1990). High Diversity in DNA of Soil Bacteria. Environmental microbiology 56(3), 782-787.

Vandamme, P., Pot, B., Gillis, M., Vos, P., Kersters, K & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiology and Molecular Biology Reviews 60(2), 407p.

Yateem, A. and Al-Sharrah, T. (2011). Enhanced Hydrocarbons Degradation in the Rhizosphere of Mangrove Plants by a Halophilic Bacillus Subtilis Subtilis Strain, Soil and Sediment Contamination 20:3, 261-273, DOI: 10.1080/15320383.2011.560980.