

## Full Length Research Paper

# Halo-thermophilic bacteria and heterocyst cyanobacteria found adjacent to halophytes at Sabkhas, Qatar: Preliminary study and possible roles

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This study was conducted to investigate the halo-thermophilic bacteria and cyanobacteria adjacent to the halophytic plants, *Suaeda virmiculata*, *Limonium axillare* and *Tetraena qatariensis*, and the microbial functionalities in Sabkhas of Qatar. These soils are alkaline and highly saline, and their moisture contents varied throughout the year. A significant presence of thermo-halophilic bacteria was found when selective media was used; however, bacterial populations were highest in soil samples taken adjacent to *L. axillare* as compared to those taken adjacent to other halophytes. They were lowest in samples taken close to *S. virmiculata*. Microscopic examinations revealed that the bacterial cells of isolated strains were Gram-positive rods with pointed ends that occurred singly, in pairs or in short chains. Most were bacilli, either *Bacillus thuringiensis* or *Bacillus cereus*. These can form endospores to survive until more favorable environmental conditions allow them to resume growth and activity. Moreover, when Sabkhan soils were transferred to the laboratory under natural conditions, only cyanobacteria grew, and some produced biofilms. The most recognizable cyanobacteria were *Anabaena* and *Nostoc*. Some produced heterocysts and akinetes, which play important roles in soil biology and nitrogen fixation. The possible roles of these microorganisms in saline environment in Sabkhan soil appear to be support of halophyte growth by alleviating salt stress and other extreme environmental conditions.

**Key words:** Halo-thermophilic bacteria, halophytes, Sabkhas, cyanobacteria, ecophysiology.

## INTRODUCTION

Wildlife, crops and even the biodiversity of the Arabian Gulf region in general and in the State of Qatar in particular, face many harsh abiotic factors which include drought, high salinity and high temperatures. Meteorological reports confirm that annual rainfall could

be well below 80 mm, electrical conductivity of soil saturated extract (EC<sub>e</sub>) could be above 200 dSm<sup>-1</sup>, and summer temperatures are known to exceed 50°C (Abdel-Bari et al., 2007; Yasseen and Al-Thani, 2013). These environmental factors can cause a host of changes in the

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**Figure 1.** Part of the study site, northeast of Qatar.

microbial community and the biodiversity in desert ecosystems and habitats (Berg et al., 2014; Classen et al., 2015; Tang et al., 2015; Zeglin, 2015). The abundance and activities of soil microorganisms are determined by these environmental factors and the presence of organic matter, pollution, etc. (Sarig et al., 1999; Katterer and Andren, 2001; Vidali, 2001; Whitford, 2002; Mohammadipanah and Wink, 2015). In fact, the microbial community is considered as a primary part of the soil biota, and it plays very important roles in nutrient availability, thereby affecting the nutrient cycle. These microorganisms rely considerably on the soil for various life activities such as nutrition, growth, pharmaceutical needs, and possibly metabolic activities (Richer et al., 2012; Gougoulas et al., 2014).

Many microbial communities and activities were recently reported to be well adapted to desert and saline environments (Mohammadipanah and Wink, 2015), and they might play various roles in alleviating the impact of harsh abiotic stresses, thereby contributing considerably to stress tolerance in plants. Such mechanisms could be associated with the activities of many living organisms (bacteria, fungi and cyanobacteria) in various parts of plant and soil systems through biological activities such as the biosyntheses of polymers, compatible solutes and plant growth-promoting hormones as well as the activation of phytohormone-degrading enzymes (Spaepen et al., 2007; D'Ippolito et al., 2011; Qurashi and Sabri, 2011; 2012; Ruppel et al., 2013; Ahemad and Kibret, 2014; Glick, 2014; Oteino et al., 2015; Shrivastava

and Kumar, 2015).

Five decades ago, a pioneer study on soil bacteria and actinomycetes in the desert of Kuwait gave bases for further investigations in the field of soil microbiology in the Arabian Gulf States (Hashem and Al-Gounaim, 1973). In other studies in the area, *Streptomyces* was discovered in the Qatari desert soil (Al-Thani, 2007). Biodiversity and the roles of microorganisms in desert ecosystems is continuously investigated, and new species have been isolated from the desert and salt marshes (Al-Zarban et al., 2002a, b).

This study aimed to investigate the microbial communities close to halophytic plants such as *Suaeda*, *Limonium* and *Tetraena* and assess the possible functions of these microorganisms in the ecosystem of the Sabkhas of Qatar. Thus, the ultimate goal of successfully restoring habitats and attaining their sustainability can be achieved if extremophiles such as thermo-halophilic bacteria and cyanobacteria close to or associated with native plants and their roles in the Sabkhas ecosystem is determined.

## MATERIALS AND METHODS

### Description of the study sites and climatic data

This study was conducted in the Sabkhas area (Figure 1) in northeast Qatar (Figure 2) at 25°38'13.59" N latitude and 51°30'02.31" E longitude. The Qatari climate is arid or semi-arid,



Figure 2. Map of Qatar.

the annual mean air temperature is 27°C, and the average annual free water evaporation ranges from 1750 to 2150 mm. The average minimum temperature of 22°C is attained in January, and the average maximum of 47°C is reached in July. Thus, the area has a desert climate with cool winters and hot dry summers. The average annual precipitation is 81 mm, and the rain falls normally during the winter and spring months (November to April).

### Sampling

The site is dominated by desert shrubs, but only three native halophytes were chosen for this study: *Suaeda virmiculata*, *Limonium axillare* and *Tetraena qatarense* (previously known as *Zygophyllum qatarense*). All grow in the same ecosystem but differ in their ecophysiological adaptations. Soil samples were collected using a soil auger 5 cm in diameter at random depths between 0 and 10 cm under selected individual plants on 30<sup>th</sup> April 2016 (representing the end of the wet season and the beginning of the dry season). Ten individual plants from each species were chosen, and soil samples were collected 5 m apart adjacent to these plant species. Controls were obtained from the open spaces between the plants. Each soil sample was placed in a well-sealed polyethylene bag and then transported to the laboratory. Soil type, microbiological and physicochemical analyses were conducted after removing stones, roots and organic debris, and sieving soil samples

through a 2-mm mesh.

### Soil analysis

Each sample was subjected to three analyses.

#### Soil moisture (%SM)

Soil moisture content was determined by drying 10 g of the sample at 105°C for 48 h and re-weighing, then calculating gravimetric soil moisture.

#### Soil salinity (EC)

Salinity was measured in a suspension of soil in distilled water (1:10) using an autoranging EC/temperature meter. Expressed as  $\text{dSm}^{-1}$ , soil salinity was evaluated according to the method described by Landon (1991).

#### Soil pH

Soil pH was measured using a glass electrode inserted into a 1:2 (w/w) soil: water mixture.

**Table 1.** Soil properties around the roots of the studied plants (mean  $\pm$  standard deviation and range across 10 samples).

Parameter	<i>Suaeda vermiculata</i>	<i>Limonium axillare</i>	<i>Tetraena qatarense</i>	Control
Soil water content (%)	18.17 $\pm$ 2.22 (15.11 - 22.89)	21.50 $\pm$ 1.06 (19.76 - 22.89)	17.27 $\pm$ 4.63 (8.88 - 22.09)	20.67 $\pm$ 2.80 (16.47 - 25.30)
pH of soil suspension	8.44 $\pm$ 0.11 (8.21 - 8.58)	8.37 $\pm$ 0.21 (8.07 - 8.72)	8.56 $\pm$ 0.10 (8.41 - 8.70)	8.53 $\pm$ 0.08 (8.42 - 8.66)
EC of soil suspension (dSm <sup>-1</sup> )	14.02 $\pm$ 2.45 (10.62 - 18.50)	17.37 $\pm$ 1.51 (15.33 - 19.62)	16.51 $\pm$ 3.45 (8.83 - 19.83)	16.67 $\pm$ 1.62 (14.74 - 18.50)

### Microbiological analysis

The procedure used was described by Bassiri (2014). A plate count method was used to estimate the population of heterotrophic bacteria, in colony-forming units (CFUs). Nutrient agar (NA) and nutrient agar plus 2 M NaCl (2MNA) were prepared and separately inoculated with 200  $\mu$ L of a 10-fold soil dilution (using sterilized distilled water). After incubation at 35 and 60°C for 7 and 3 days, respectively, bacterial populations were determined for each plate. All cultures were performed in duplicate.

Cyanobacteria were examined using a light microscope; after transferring soils from Sabkhas, sterilized distilled water was added and the mixture was exposed to 3-4 weeks of natural light and temperature conditions in the laboratory.

## RESULTS AND DISCUSSION

### Ecophysiology of halophytes at Sabkhas

The soils of the Sabkhas in Qatar are mostly sandy loam with rock and gypsum, and they are dry during the summer and highly saline throughout the year. An evaluation of soil moisture, pH and salinity (Table 1), revealed the soils to be alkaline (pH 8.5), dry (absolute water content of less than 22%), and highly saline (up to 17.5 dSm<sup>-1</sup>) under natural environmental conditions, which promote halophytes like *S. vermiculata*, *L. axillare*, *T. qatarense* and possibly others to grow and thrive in such habitats. However, EC measurements can differ depending on the methods used. For example, the EC of a saturated soil extract (EC<sub>e</sub>) exceeds 200 dSm<sup>-1</sup> among Sabkha soils if the method described by Richards (1954) is used. In fact, adopting one particular method relies on the estimated moisture content in the soil, but the moisture content of soils from the Sabkhas in Qatar vary between summer and winter (Al-Busaidi et al., 2006).

The taxonomic descriptions and habitats of the studied plants have been well-documented (Abdel-Bari, 2012). *Suaeda* spp. are represented in the local flora of Qatar by two species: *Suaeda aegyptiaca* and *S. vermiculata*. The latter is common among coastline vegetation by the edges of mangrove forests between the tidal zone and higher grounds. Specifically, it is widespread along the eastern coastline of the Qatar peninsula. *Limonium* spp. are represented by a single species, *L. axillare*, in the

Qatari flora. This species is widespread on the coastline of the Sabkhas and is usually associated with *Halopeplis*. It is common in central Qatar and Doha and appears after the seasonal rains. *T. qatarense* (syn. *Zygophyllum qatarense*) is widespread; it is the most common plant in Qatar and occurs in all types of habitats, both the rocky and sandy soils at the coastline and inland, forming large communities. It can grow in saline soils and in the Sabkhas. The primary features of the studied plants are shown in Table 2. All are succulents found in moist, saline and dry habitats; however, only *L. axillare* has salt glands, and it has lower water content than the other two, which have no salt glands. It seems that *S. vermiculata* and *T. qatarense* have more succulent habits of diluting the extra salts absorbed from the environment, while *L. axillare* extrudes extra salt using salt glands (Yasseen and Abu-Al-Basal, 2008).

In fact, the physiological and biochemical characteristics of the halophytes examined in this study were not measured; however, the data reported by Abdel-Bari et al. (2007) and many others (Yasseen and Al-Thani, 2007; Yasseen and Abu-Al-Basal, 2008; Yasseen and Al-Thani, 2013; Yasseen, 2016) showed that these plants accumulate substantial amounts of Na<sup>+</sup> and Cl<sup>-</sup> to achieve a water balance between the plants and their environment by lowering the solute and water potentials of their tissues (Flowers and Yeo, 1986; Yasseen and Abu-Al-Basal, 2008).

In fact, these plants absorb ions that are abundant in their growth media, and the range of these concentrations exceeds the range set by Chapman and Pratt (1961) for normal plant growth. These ions at such high concentrations could inhibit or impair protein transporters, leading to nutrient imbalances (low K<sup>+</sup> and Mg<sup>2+</sup>); however, the acceptable Ca<sup>2+</sup> concentrations found in the shoot systems in these plants could support resistance mechanisms by providing various functions (Nilsen and Orcutt, 1996; Orcutt and Nilsen, 2000; Mengel et al., 2001) such as maintaining internal membrane structures, regulating ion homeostasis for stomatal control, activating enzymes such as ATPase and other proteins (Yasseen and Abu-Al-Basal, 2008). Other physiological and biochemical variables were negatively affected under these soil and environmental



**Table 2.** Primary features of the studied plants and thermo-halophilic bacterial (THB) counts of bacteria found adjacent to three halophytic plants.

Variable	Plant species		
	<i>S. vermiculata</i>	<i>L. axillare</i>	<i>T. qatariense</i>
Family	Chenopodiaceae	Plumbaginaceae	Zygophyllaceae
Habitat	Moist saline soils, Sabkhas	Coastline and Sabkhas	All types of habitats, dry-saline soils
Life form	Undershrub	Undershrub	Undershrub
Halophyte/Xerophyte	Halophyte / Xerophyte	Halophyte	Xerophyte
Succulence	Shoots and leaves	Large fleshy leaves	Leaves and petioles
Plant water content* (%)	77 - 88	64 - 72	80 - 88
Salt glands	No salt glands	Salt glands on both leaf surfaces	No salt glands
THB (cfu-g <sup>-1</sup> x 10 <sup>2</sup> )**	0.4	7.2	0.65

\*The range among ten individuals of each species is given. \*\*THB (cfu-g<sup>-1</sup> x 10<sup>2</sup>) for the control was 3.3.

conditions, which included photosynthetic pigments, total soluble sugars and nitrogen as compared to glycophytes and crop plants grown under normal soil conditions (Alhadi et al., 1999; Abdel-Bari et al., 2007). Moreover, the studied plants showed various abilities to accumulate compatible organic solutes, such as proline, to provide various physiological and biochemical functions (Yasseen, 2016).

### Microbiology and possible roles

The arid salty soils of the study location contain a considerable number and variety of bacterial communities, but little is known about microbial functionalities in Qatari soils. Investigations of various groups of bacterial extremophiles were performed using NA at 35°C, 2 M NaCl (2MNA) at 35°C, NA at 60°C and 2 M NaCl (2MNA) at 60° to isolate heterotrophic, halophilic, thermophilic and thermo-halophilic bacteria, respectively. Incubation clearly showed that the bacterial populations of heterotrophic ( $2.5 \times 10^4$  cfu-g<sup>-1</sup> dry soil), halophilic ( $1.5 \times 10^4$  cfu-g<sup>-1</sup> dry soil) and thermophilic ( $3 \times 10^2$  cfu-g<sup>-1</sup> dry soil) bacteria did not differ between the samples taken adjacent to the halophytes. Normally, such outcomes can be attributed to the use of a NA medium that is not selective; therefore, the types and numbers of colonies were nearly the same. However, when selective media were used, such as 2MNA at 60°C, selective for extremophiles, populations of thermo-halophilic bacteria were highest in soils adjacent to *L. axillare* and lowest in soils adjacent to *S. vermiculata* (Table 2).

The bacterial cultures shown in Figures 3 and 4 indicate that colonies of most strains were 1 to 2 mm in diameter, circular, smooth or mucoid, slightly raised and non-pigmented. However, some pigmented colonies were found in halophilic cultures incubated at 35°C. Selected isolated colonies were characterized by Gram stain, shape and morphology, and were observed using a light microscope. To obtain a diverse assortment of isolates,



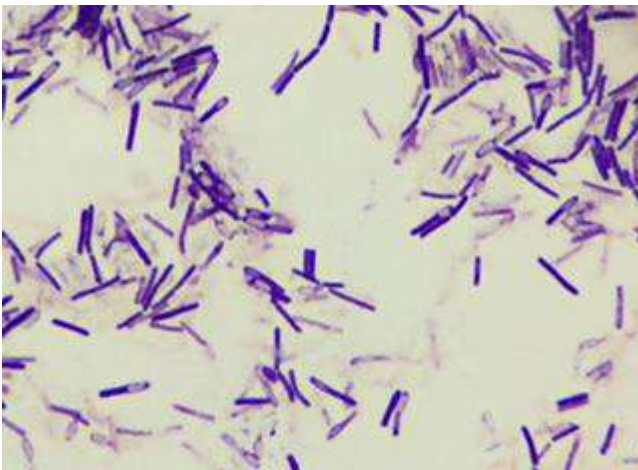
**Figure 3.** Culture of halophilic bacteria grown in nutrient agar in 2 M NaCl at 35°C.



**Figure 4.** Thermophilic bacteria cultured using nutrient agar and incubated at 60°C. The white colonies are thermophilic *Streptomyces*, which predominate in desert soils.



**Figure 5.** Gram positive bacilli, non-spore-forming long bacteria isolated from nutrient agar in 2 M NaCl after incubation at 60°C for 2 days. Magnification 1000x (scale, 10 µm).



**Figure 6.** Gram-positive spore-forming single bacilli: thermophilic bacteria incubated in nutrient agar at 60°C for 2 days. Magnification 1000x (scale, 10 µm).

colonies that displayed different morphologies such as off-white, orange-red or cream colors, non-circular colony shapes, and mucoid textures were chosen. Microscopic examinations revealed that cells of isolated strains were Gram-positive rods with pointed ends that occurred singly, in pairs, or in short chains. In fact, most isolates belonged to the genus *Bacillus*; they were rod-shaped, Gram-positive, non-spore-forming long bacteria (Figure 5) isolated from 2MNA incubated at 60°C for 2 days. Some were spore-forming (Figure 6), and almost all strains formed endospores after growth on NA at 60°C. Spores were ellipsoidal or sometimes spherical (0.7 to 1.5 µm in diameter) and were located at a central to subterminal position. The frequency of spore-forming



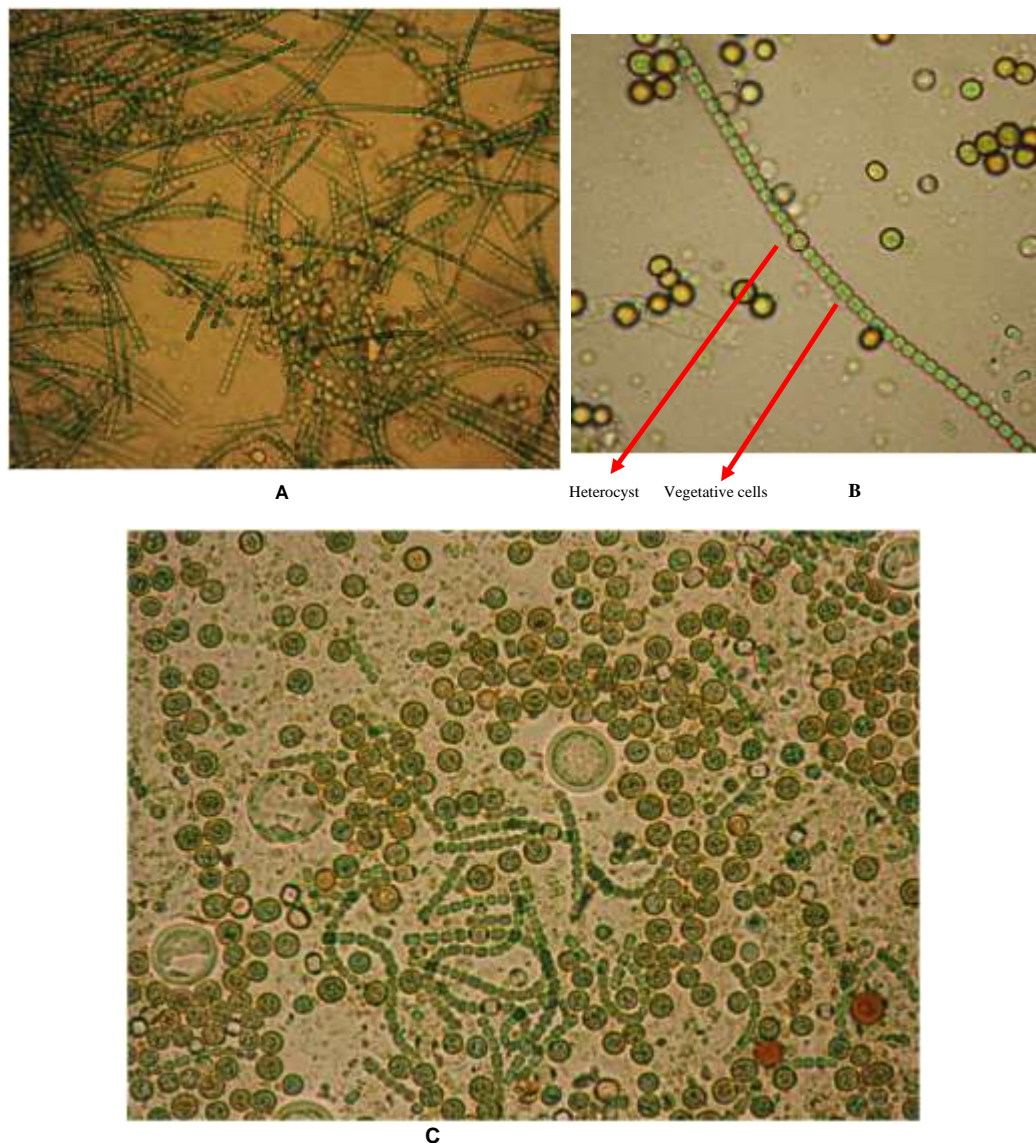
**Figure 7.** Cyanobacteria grew after soils were transferred to the laboratory, the mixture of soil and sterilized distilled water was left under natural light and temperature for 3 to 4 weeks. Some produced a biofilm on the inner surface of the glass bottle, producing bubbles of oxygen.

cells was higher among thermophilic than halophilic cells. Thermo-halophilic bacteria (as extremophiles) are those living organisms that are boosted and can survive in the extreme environmental conditions normally found in the Arabian Gulf region.

The severe environmental conditions of the desert in the Arabian Gulf regions have not only negatively impacted and imposed a great deal of constraints on the microbial community, they differentiate the types of microbial species. Some Gram-positive bacilli can form endospores to endure the severe periods until more favorable conditions allow them to resume growth. Cyanobacteria grew in these Sabkhas when soils were transferred to the laboratory and settled under natural environmental conditions, and some produced a biofilm on the inner surface of the glass bottle, producing bubbles of oxygen (Figure 7). These cyanobacteria were examined and confirmed to be *Anabaena* and *Nostoc* (Figure 8).

Interestingly, thermo-halophilic bacteria counts were high adjacent to the halophyte *L. axillare* as compared to the others, bearing in mind that this plant is well adapted to saline environments and has salt glands to extrude extra salts (Yasseen and Abu-Al-Basal, 2008). This plant is predominant at the coastal line and Sabkhas, while *S. vermiculata* and *T. qatarse* can be found in other habitats of inland areas in addition to the Sabkhas (Abdel Bari et al., 2007). Such outcomes might reflect the roles that these bacteria play in the life of halophytes at harsh environments of the Arabian Gulf region. More





**Figure 8.** *Anabaena* and *Nostoc* genera are filamentous with heterocysts and akinetes. These were the most common cyanobacteria found after Sabkha soil incubation under natural conditions. Magnification 10x (A); 400x (B and C).

investigation is needed to look at the ecological aspects of these bacteria and the type of relationships with these halophytes. Microorganisms isolated from the selective NA media used with these soil samples belonged to the *Bacillus* genus. *Bacillus thuringiensis* is reportedly the most common bacilli isolated from Qatari soils, followed by *Bacillus cereus* (Umlai et al., 2016). Microorganisms in soil adjacent to or associated with halophytes play many possible important roles supporting and promoting their growth through various mechanisms and methods (Yuan et al., 2016; Al-Thani and Yasseen, 2017). These roles have been discussed widely across the world as a result of the extreme desert environment, but microbial flora's influential roles in governing key life bioprocesses of

surface and subsurface soils is very interesting, which cover various aspects of economic, agriculture and health sectors. In fact, during the last two decades, a huge number of articles and reports of experimental work have suggested many possible mechanisms and methods adopted by microorganisms in alleviating the harsh abiotic stresses facing plants in general and crops in particular (Al-Thani and Yasseen, 2017). These mechanisms include: (a) establishing a biofilm (Qurashi and Sabri, 2012), (b) producing polymers (exopolysaccharides) (Qurashi and Sabri, 2011), (c) chemotaxis (D'Ippolito et al., 2011), (d) accumulating endogenous osmolytes such as compatible organic solutes (Fernandez-Auni3n et al., 2010; Shrivastava and

Kumar, 2015; Vilchez et al., 2016 ), (e) producing phytohormones and the enzymes degrading them (Spaepen et al., 2007; Kazan, 2013; Glick, 2014), (f) fixing nitrogen (Ahemad and Kibret, 2014), (g) solubilizing phosphate (Oteino et al., 2015), (h) secreting various regulatory chemicals around the rhizosphere (Ahemad and Kibret, 2014), (i) removing organic (petroleum hydrocarbons) and inorganic (heavy metals) contaminants from soil and water (Yasseen, 2014), (j) producing antibiotics (Ahmed et al., 2013; Bizuye et al., 2013) and (k) increasing carbon utilization efficiency (Gougoulas et al., 2014).

Recent evidence suggested that all the above mechanisms could be utilized by these microorganisms to support and help native plants in their adaptation to severe environments, allowing them to resist abiotic stresses and promote the growth and productivity of adjacent plants through many methods (Hanin et al., 2016). For example, some bacterial strains can form a biofilm as a strategy to improve growth in crops such as wheat and chickpeas under severe environmental conditions (Afrasayab et al., 2010; Qurashi and Sabri, 2012). Moreover, Kasim et al. (2016) concluded that *Bacillus* bacteria (such as *Bacillus amyloliquifaciens*) could provide a biofilm to alleviate salt stress around the root systems of crops such as barley. Other methods might be at work in such a system such as the production of polymers (exopolysaccharides) adopted by some bacteria to protect native plants against various types of stresses such as salinity, drought and extreme temperatures (Nicolson et al., 2000; Qurashi and Sabri, 2011; 2012; Aanniz et al., 2015; Sardaria et al., 2017).

Additionally, cyanobacteria found in Sabkhan soils produce heterocysts, which play significant roles in nitrogen fixation during nitrogen shortages in such environments (Redfield et al., 2002), especially those in Qatar (Ashore, 1991), to support halophytes coping with saline environments (Yasseen and Abu-Al-Basal, 2008). Akinetes, on the other hand, are produced by certain filamentous cyanobacteria to survive the harsh environments caused by high salinity, high temperatures and hard desiccation (Hori et al., 2003).

## Conclusion

Having recognized the presence of bacilli and cyanobacteria adjacent to or associated with native plants in the Qatari peninsula, the roles of these microorganisms in the performance of these plants were concentrated on, and their aspects, including nutrition, pollution, production of antibiotics, etc were evaluated. One possible approach is to examine the origins of metabolic pathways in microorganisms and native plants and determine whether these pathways originate from native plants or microorganisms. Alternatively, some plant genes might have originated from prokaryotes. This

would require a thorough investigation at the molecular level, and horizontal gene transfer must be considered a possibility. Further identification of the molecular approach is needed for the bacterial strains found in the Sabkhas of Qatar.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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[http://www.sas.upenn.edu/LabManuals/biol275/Table\\_of\\_Contents\\_files/10-Antibiotics-New.pdf](http://www.sas.upenn.edu/LabManuals/biol275/Table_of_Contents_files/10-Antibiotics-New.pdf).
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