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Properties of *Streptomyces* Bacteria from the Rhizosphere of Some Halophytes at North-East of Qatar: Al Ghariya Case Study

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Abstract: The biological activities performed by plants and microorganisms in dry and saline soil play an important role in making them thrive in these extreme environmental conditions. Our previous studies have shown the presence of *Streptomyces* bacteria in various drylands in Qatar. To understand and elucidate the roles of these bacteria in such unfavorable environments, it is important to investigate the distribution and properties of *Streptomyces* bacteria in rhizospheric soil of halophytes and compare them with non-rhizospheric soils. Therefore, in this research, four halophyte plants namely: *Caroxylon imbricatum*, *Sporobolus ioclados*, *Tamarix aphylla*, and *Tetraena qatarensis*, were chosen to investigate the properties, characteristics, and activities of *Streptomyces* isolates in these habitats. The chemical and physical properties of soil at the study area (Al Ghariya Sabkha) revealed that pH levels are almost uniform and homogenous across the Sabkha; ranging between 7.7-7.9, and salinity levels were very high at non-rhizospheric soil as compared to the rhizospheric soils, thus, all elements at the rhizospheres of the studied plants have lower concentrations than those at the non-rhizospheric soils. The colony characteristics of isolates at the rhizospheric soil of halophytes showed various types of isolates with different colony characteristics and peculiarities which indicate that a significant number of strains of *Streptomyces* bacteria have thrived under such mini-habitats of the canopy of these plants. The enzyme activities of the isolates that have been studied in the rhizospheric and non-rhizospheric soils have shown more variable isolates in the rhizosphere of the plants under investigation than those of non-rhizospheric soils. The antibacterial primary activity of the isolates of *Streptomyces* at these mini-habitats showed that most of them had clear antibacterial action against the tested strains: Gram-negative (*E. coli*), and Gram-positive (*B. subtilis*, *S. aureus*, and *S. epidermidis*). The details about these parameters and the possible use of modern approaches to identifying *Streptomyces* bacteria, and the possible roles of halophytes and their associated microbes in saline lands are also discussed in this paper. Overall, the results of this research showed that the properties and characteristics of *Streptomyces* bacteria explaining their biodiversity were high in rhizospheric soils of halophytes as compared to non-rhizospheric soils.

Keywords: Antibacterial Activity, Biochemical Characteristics, Colony Features, Elements, Halophytes, Streptomyces

1. Introduction

Microorganisms that interact with or inhabit the rhizosphere, phyllosphere, and endosphere of plants might be detrimental or beneficial for either plants and the associated microbes; such relationships can be classified as neutralism, commensalism, synergism, mutualism, amensalism, competition or parasitism [1, 2]. Notably, the best example of symbiotic relationships is shown between some plants and mycorrhiza, as the plant provides metabolites to the mycorrhiza, while the latter promotes plant growth by enhancing mineral uptake such as nitrogen and phosphorus [3]. The review article of Yasseen and Al-Thani [4] showed

some examples of microorganisms associated with various native plants from the Qatari environment, and the perspectives of the future to solve many outstanding problems regarding health, economy, and food security [5]. Living organisms that grow in Sabkhas and salt patches are highly salt-resistant, and because most microorganisms in these habitats are associated with and/or adjacent to halophytes; they have common cellular basics of physiological and biochemical mechanisms to deal with extreme environmental conditions such as high salinity and drought [6, 7]. The ecology of halophytes and their microbial inhabitants at the salt flats revealed that the total bacterial counts were considerably higher in the rhizosphere soil (coastal line and inland areas) as compared to the non-rhizosphere. Microorganisms in these habitats might play many biological roles when present in the rhizosphere of native plants, these roles include alleviating the impact of salt stress to improve food production by secreting some metabolites [5, 8, 9], and supporting the soil functions that are needed for plant growth, such as cycling of nutrients, degradation of pollutants, energy flow, and carbon utilization [10]. These studies have shown that rhizobacteria and mycorrhizae organisms can boost plant growth by stimulating the production of phytohormones and siderophores, solubilizing phosphates, and lowering ethylene levels. Moreover, such close relationships between microorganisms and their hosting plants might exchange genes by HGT (Horizontal Gene Transfer) that help produce compatible solutes and secondary metabolites including pharmaceutical products and antibiotics [6, 11-16].

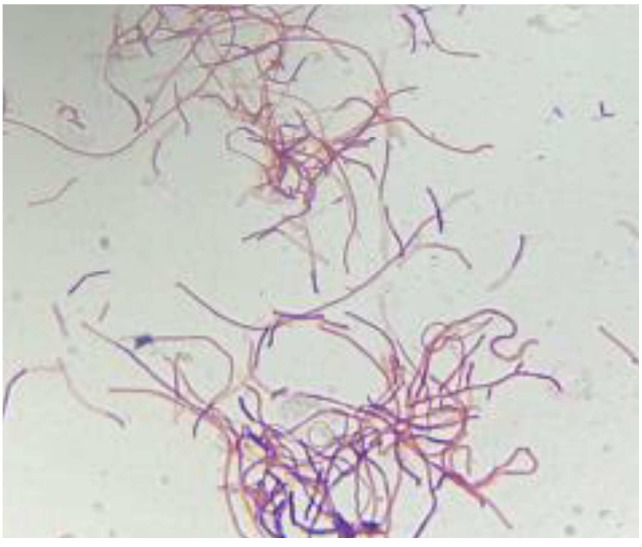


Figure 1. *Streptomyces bacteria showing the general morphology and the extensive branching aerial hyphae.*

Biological activities of Streptomyces bacteria at dry and saline lands:

Early studies on microorganisms at the desert and saline lands in Qatar revealed that the total count of *Streptomyces* spp., has accounted for about 40% of the total microbial counts [9]. Notably, over 700 species of *Streptomyces* have been described around the world [17]. Al-Thani and Mahasneh [18] studied this genus around Qatar and concluded that it was a commonly occurring microbe in drylands and Rawdahs. The general features of these bacteria are aerobic, Gram-positive, spore-forming, filamentous bacteria that produce extensive branching aerial hyphae (Figure 1) and pigmented colonies (Figure 2). However, it is interesting to report that *Streptomyces* spp., are adapted to a wide range of salinity (ECe at 25°C) from 1.2 to 40.1dSm^{-1}.

Figure 2. *Pigmented colonies of Streptomyces bacteria; white color and round colonies.*

Therefore, the identification of these species is the first important step to conduct further investigation to elucidate their biological roles at saline patches and Sabkhas. Salt marshes in Qatar include mangrove forests, coastal marshes, and inland salt flats (Sabkhas). These habitats contain the most known extremophiles such as thermophilic, halophilic, and halo-thermophilic microorganisms of bacteria and cyanobacteria [7]. The study of Al-Thani and Yasseen [19] has concluded that some Gram-positive bacilli of various shapes; singles, pairs, or short chains are found adjacent or associated with some halophytes such as *Limonium axillare*, and these microorganisms might play many roles in promoting and supporting the performance of these plants including nutrition, phytoremediation, and resistance to abiotic factors such as salinity, drought, and extreme temperatures [6]. Notably, saline habitats at the coastline and inland in Qatar and other parts of the Arabian Gulf region are rich in halophytes [16, 20-22]. The study of Fahmy and Al-Thani [23] on the microbial diversity at some Sabkhas in Qatar revealed that total bacterial counts were higher in the rhizosphere of halophyte plants as compared to non-rhizosphere soil at the coastline and inland areas. Gram-positive bacilli were predominant in the phyllospheres at the green and senescing parts of the plants, while Gram-positive cocci were predominant in isolates from rhizosphere and non-rhizosphere soils. It is interesting to report that low bacterial colonization was found in the phyllosphere of halophyte plants that have a salt extrusion mechanism than

those adopting a dilution mechanism (inclusion mechanism) to avoid salt stress [23].

The biological activities that take place at arid and saline environments include many actions by plants and microorganisms, and possibly naturally because of actions of the inhabitants at these habitats. These activities include plant, animal, and microbial adaptations, endangered species such as algae, cyanobacteria, and microorganisms might find harbor at such habitats, soil erosion and nutrient cycling can take place because of the actions of these living organisms, and finally, agricultural activities to boost crop production under these extreme conditions.

Notably, considerable pressures have increased on many human life aspects such as world population, pollution, climate change, desertification, and salinity. During the last two decades, environmentally friendly biological approaches have emerged to solve many issues facing humankind related to these aspects of agriculture, economy, and health [24]. Microorganisms may offer some kind of support to boost crops to cope with these challenges as a sustainable strategy to increase crop yields under harsh environmental conditions and to remediate polluted soil and water [25]. The report of Al-Thani and Yasseen [6] has listed and discussed the mechanisms that microorganisms can play to alleviate the extreme abiotic stresses facing plants. These mechanisms included: (1) establishment of biofilms. (2) production of polymers (e g., exopolysaccharides), (3) chemotaxis, (4) phytohormone production, (5) nitrogen fixation, (6) phosphate solubilization, (7) phytohormone-degrading enzyme production, and (8) accumulation of endogenous osmolytes.

Basically, soil is essential to provide various components of life such as nutrients, water, and others to plants, and is home to a huge number and diversity of living organisms. These components include the production of growth-stimulating hormones, stimulate the plant's immune system, and may accelerate or weaken the plant's response to severe environmental stresses [26]. Notably, recent literature revealed many other biological roles played by *Streptomyces* bacteria at dry and saline lands. These groups of microorganisms cover most of the Qatari arid lands and play many critical functions ranging from bioremediation to plant growth promotion and soil improvement. These functions maintain ecological balance and support life in these challenging environments [27-35]. Thus, the following are the main functions played in these extreme environments: (1) bioremediation and/ or phytoremediation: they can degrade a wide range of organic compounds released because of anthropogenic and /or industrial activities of oil and gas activities. Moreover, *Streptomyces* bacteria are good candidates for bio-reclamation of salt-affected soils [29], (2) nutrient cycling: *Streptomyces* can break down organic matters into simpler compounds which help to enrich the soil with essential elements for plant growth [32], (3) promotion of plant growth: *Streptomyces* produce plant growth-promoting compounds such as phytohormones and enzymes as well as enhancing nutrient availability at harsh environmental conditions [34], (4) antagonism against plant pathogens: some *Streptomyces* possess antagonistic properties against plant pathogens; by producing many secondary metabolites and antibiotic agents. Notably, saline and arid lands might make plants susceptible and vulnerable to many diseases. Secondary metabolites might have many applications; pharmaceutical and biotechnological. In saline and arid lands, *Streptomyces* might synthesize some bioactive compounds that have applications in medicine, agriculture, and other industries. *Streptomyces* bacteria have an important role in the production of antibiotics, thereby inhibiting the growth of harmful microorganisms [36], (5) nitrogen fixation: some strains of *Streptomyces* bacteria have been found to possess nitrogen fixing abilities. In nitrogen-poor arid soils like the Qatari lands [37], these bacteria could contribute to the nitrogen availability for plants [38], (6) improvement of soil structure: the substances produced by *Streptomyces* could contribute to the stability and structure of the soil. These bacteria can help in binding soil particles together, reducing erosion, and enhancing soil structure [30], and (7) survival in harsh habitats; *Streptomyces* are resistant to arid and saline soils and such traits might have crucial roles in maintaining microbial diversity and ecosystem stability in such harsh environments [39].

The present study was conducted to investigate the changes in *Streptomyces* bacteria in rhizospheric soil as compared to non-rhizospheric soil. The possible role of these bacteria in the production of antibiotics would encourage the research centers to carry out deep investigations to utilize them in industrial activities.

2. Case Study: Al Ghariya Sabkha

2.1. The Study Location

The State of Qatar is located at the western section of the Arabian Gulf between 24° 27' and 26° 10' north, and 50° 45' and 51° 40' east, it is an extension of the eastern part of the Arabian Peninsula. Al Ghariya village is located at the most north-east of the country; and as part of Al Shamal municipality. Al Ghariya with many villages around it are core of the most industrial activities of energy sources of oil and gas in Qatar, and the whole area is floating on the north basin of water and the north fields of gas as well as Al-Ryan and Al-Shaheen oil fields (Figure 3) [40].

2.2. Plant Material

Many reports have listed native plants including halophytes in North-Eastern Qatar [16, 41, 42]. Four native plants were chosen for this study, namely: *Caroxylon imbricatum**, *Sporobolus ioclados***, *Tamarix aphylla*, and *Tetraena qatarensis**** (see N. B. below). The taxonomic and morphological characteristics of these plants are described in many monographs and reports [20, 42, 43].

N.B.**Caroxylon imbricatum* [Synonyms: *Salsola imbricata* (Forssk); *Salsola baryosma* (Schult)]. ** *Sporobolus* genus is represented in the flora of Qatar by two

species, these are: *Sporobolus spicatus* and *Sporobolus ioclados* (Synonym: *Sporobolus arabicus*). ***Tetraena qatarensis* [Synonyms: *Zygophyllum hamiense* var. *qatarense* (Hadidi) Jac. Thomas & Chaudhary; *Zygophyllum qatarense* (Hadidi)].

2.2.1. Caroxylon Imbricatum

This plant is a dicot herbaceous halophyte plant found at the coastal line of Qatar. Also, it is considered an arid desert perennial plant with succulent leaves [44]. This green shrub may reach 60 cm high and spread with small shoots with new growth up to 1.5 meters. This plant can be used as animal feed and can be utilized in many industrial activities in the production of soda ash, soap, and pharmaceuticals [45]. Moreover, it is useful in medical treatments for heart diseases, cough, skin, and stomach problems. The alkaline extracts of this plant might help control obesity, Alzheimer's, and diabetes [46]. Its role in the phytoremediation of polluted and saline lands has been discussed in some recent works as a biological approach to the rehabilitation of agricultural lands [8, 16].

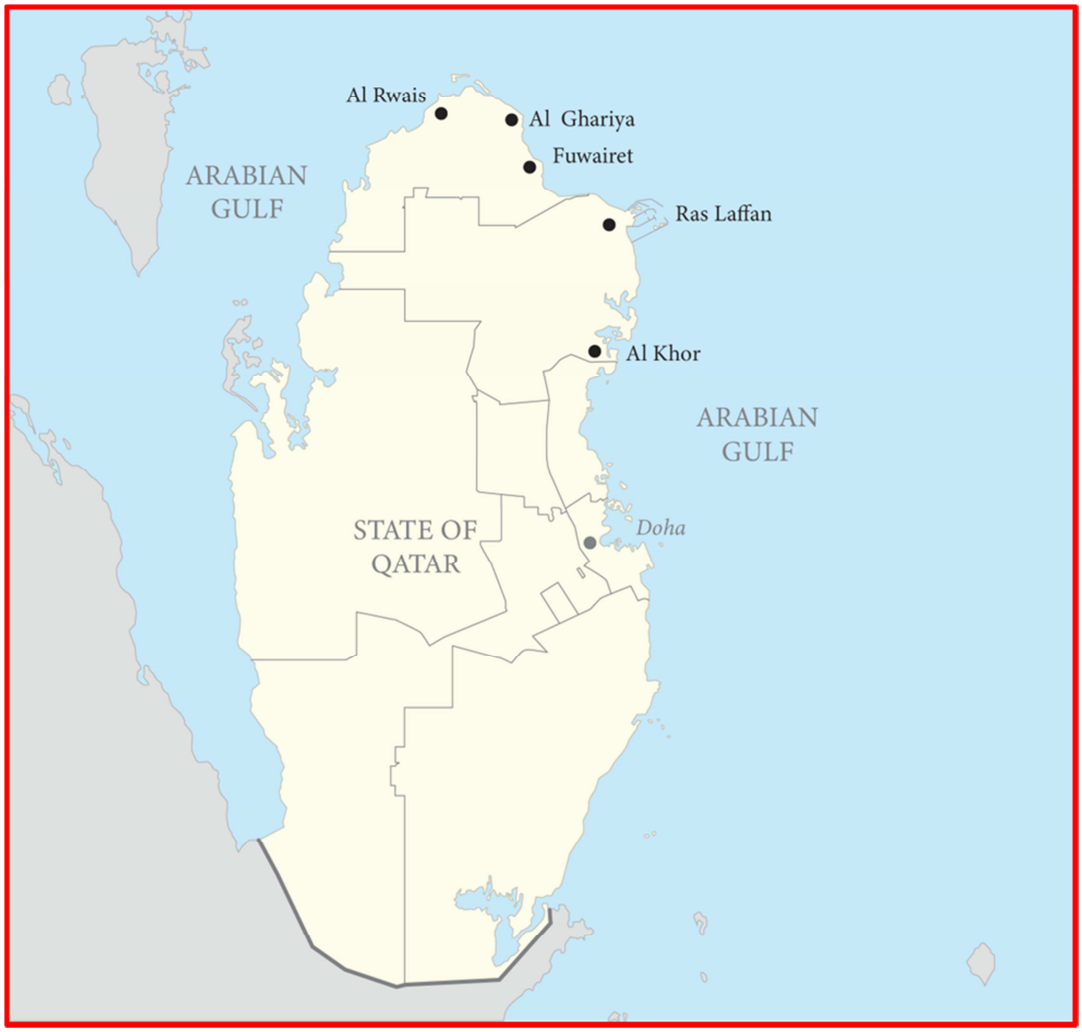


Figure 3. *Map of Qatar showing Al Ghariya village and the nearby Ras Laffan industrial area.*

2.2.2. Sporobolus Ioclados

It is a common native halophyte plant among the flora of Qatar. It is a pale yellow-green color plant; grows at saline habitats in the form of hard, spiky, stinging thorny leaf blades. Some medical uses were reported as it contains antibiotics and acts as pain relief. The high salt tolerance of this plant could encourage it to grow and thrive at coastal marshes of eastern parts of the Arabian Peninsula [47, 48]. Species of the genus *Sporobolus* proved efficient in the phytoremediation of polluted soil with petroleum hydrocarbon compounds and heavy metals [16, 49].

2.2.3. Tamarix Aphylla

It is called Athel; and belongs to the family Tamaricaceae. It is found at various habitats including Sabkhas, wetlands, and deserts. Its size ranged from about one meter to about 17 meters in height [50, 51]. It has a peculiar characteristic; its leaves are primitive and succulent, with a joint stem. This plant plays several roles such as wind monitoring and as a medicinal plant against fungal and bacterial infections. Moreover, it is ideal to for wound healing, hair loss, cough, asthma, rheumatism, tuberculosis, and gums [52].

2.2.4. Tetraena Qatarensis

This plant is a member of the family Chenopodiaceae and is abundant at the coastal-line including salt flats and Sabkhas of Qatar. It is found in rocky and sandy lands across the country; it is spread in all disturbed areas. It is common at Al-Ghariya Sabkhas; however, some reports described it as xerophyte or xero-halophyte [43]. It is not edible to humans and avoided by animals and livestock, and little has been reported on its nutritional and medical values. Notably, this plant is found associated with some microorganisms such as soil micro-fungi including *Aspergillus fumigatus*, *Cladosporium sphaerospermum*, and *Penicillium citrinum* [53], and some endophytic and rhizospheric bacteria were reported recently [16, 54].

2.3. Soil Samples

Soil samples were collected in September 2022 from the upper and lower layers of the soil of the canopy of four halophyte plants, these soil samples represented the surface and subsurface of rhizosphere of: *Caroxylon imbricatum*, *Sporobolus ioclados*, *Tamarix aphylla*, and *Tetraena qatarensis*. Control samples were collected from the open spaces between the plants. Soil samples were kept in plastic bags and brought to the Microbiology Lab in the Department of Biological and Environmental Sciences at Qatar University, to carry out the required analyses.

The physical and chemical characteristics of soil samples were determined using standard methods. These methods were described in many reports and articles [7, 19, 40, 55]. The soil of the Al Ghariya Sabkha was classified as sandy loam-loamy sand, some soil samples were digested with acid, and minerals were analyzed in the Central Laboratory of Qatar University (ICP-OES model Optima 7300 DV). Chemical analysis of the soil was carried out in collaboration with the laboratory. The total C, H, and N, total organic components (TOC), and major and trace elements were determined using a Flash 2000 CHN analyzer, Flash 2000 NC soil analyzer, ICP-OES, and ion chromatography. More details about the methods used can be found in previous studies [56, 57].

2.4. Cultivation of Bacteria and Enzyme Tests

The methods of culture preparation for various diversity of microbial organisms were described by Bassiri [58] and used by many authors [7, 19, 59, 60]. The following media were used for various types of microorganisms: (1) Nutrient Agar (NA) for cultivation of heterotrophic bacteria; 20g of nutrient agar powder was used to prepare this media, (2) Potato Dextrose Agar (PDA) was used to cultivate fungi. 19.5g was suspended in distilled water, (3) Mac Conkey Agar (Mac C A) media were used to isolate Gram-negative bacteria: 20g of MacConkey agar powder was used, (4) Nutrient Agar + 10% NaCl: for the cultivation of halophytic and halotolerant bacteria. 20g of nutrient agar powder and 50g of NaCl were used to prepare this media, (5) Agar (Ag) for the cultivation of oligotrophic bacteria. The Ag media was prepared by dissolving 7.5g in distilled water, and (6) Nutrient (Broth) with agar for cultivating *Streptomyces* bacteria. 6.5g of NA Broth and 10g agar (HIMEDIA) were used to prepare the media. In all the above media, the powders and media components were dissolved in 500ml distilled water, then sterilized in an autoclave, and allowed to cool before being poured into the Petri dishes for bacteria cultivation. Enzyme tests were done for the identification of bacterial cultures, which included starch test, lipid test, gelatinase test, coagulation and proteolysis of casein, urea test, and nitrate reduction test. Gram staining and primary antimicrobial tests were done to look at species of *Streptomyces* bacteria and their ability to have antimicrobial action against other microbes.

2.5. Screening for Antibiotics-Producing Streptomyces

The screening for antibacterial activity was done using the cross-streak method. The antimicrobial activities of the isolates were tested by cross streak plate method employing nutrient agar medium for bacteria. Each plate was streaked with one isolate of *Streptomyces* at the center and incubated at 30°C for 3 days. After incubation, test organisms (three Gram-positive, *Bacillus subtilis*, *Staphylococcus aureus,* and *Staphylococcus epidermidis*) and (one Gram-negative *Escherichia coli*) were streaked perpendicular to the growth of the *Streptomyces* isolate and the inhibitory effect was evaluated after 48 hours of incubation at 30°C and the results were evaluated by the measurement of the inhibition zone diameter. All the test organisms used in this study were obtained from the Microbiology lab, Department of Biological and Environmental Sciences, Qatar University. More details about the methods were described by Ogundare et al. [61].

2.6. Identification and Differentiation of Bacteria Using MALDI-TOF MS

The method adopted for modern bacterial identification was used by Saleh [62]. A direct colony method with on-plate protein extraction was adopted. Briefly, a single bacterial colony from a fresh culture (incubated overnight in Luria-Bertani (LB) medium) was transferred to a MALDI Biotarget plate (48 spots). Once air-dried, 1µL of 70% formic acid was placed on the colony, followed by 1 µL of MALDI matrix solution. The matrix solution was prepared using α-Cyano-4-hydroxycinnamic acid (HCCA) in 50% acetonitrile and 2.5% trifluoroacetic acid. Once the sample spot had dried, the plate was then loaded into the MALDI-TOF MS instrument (Bruker Daltonic, Germany). Bruker Flex control software was used to compile and analyze the protein spectra, for which 240 laser shots were obtained in 40-shot steps for each spectrum and analyzed using default algorithms. The acceleration and source voltage were kept at 20 kV and 18.7 kV, respectively.

MALDI Biotyper RTC 3 software was used for comparison of the obtained protein spectra with a reference database, bacterial identification, and report generation. Flex

Analysis software was used for pre-processing (baseline correction and smoothing) of the spectra and peak identification in protein profiles. Moreover, Biotyper Compass Explorer software was used for the statistical analysis of the protein profiles (principle component analysis, PCA). The identification results were interpreted according to the manufacturer's guidelines and previous reports [63]. Briefly, a score of 1.70 – 1.99 was interpreted as highly probable genus-level identification, a score of 2.00 – 2.29 was considered as genus-level and highly probable species-level identification, and a score of 2.30 to 3.00 was interpreted as species-level identification.

3. Results and Discussion

The data of chemical and physical properties of soil at the Al Ghariya Sabkha revealed some facts as follows: (1) the levels of N, C, H, and TOC are at acceptable levels which confirm the standards set up by previous reports, studies, and monographs [40, 59, 64, 65], (2) pH levels are uniform across the Sabkha (ranged between 7.7-7.9), whether the soil is non-rhizospheric or rhizospheric of the studied halophytic plants, (3) in spite of the uniformity of pH levels, salinity levels were high at non-rhizospheric soil as compared to the rhizospheric soils at the lower layer of studied halophytes. At the upper layer, on the other hand, no big changes were noticed between the non-rhizospheric and rhizospheric soils of these halophytes (Tables 1 and 2). The reasons behind such outcomes are that at upper layers, soil might continue to accumulate salts because of high evaporation and low precipitation rates, salinity levels were lower at the rhizosphere of these halophytes, because these plants are very efficient in phytoremediation of saline soils [8, 16, 40], (4) almost all elements at the rhizospheres of the studied plants have lower concentrations than those at the non-rhizospheric soils (Tables 3 and 4).

Table 1. *Physical and chemical properties of the upper layer of the soil of halophytes at Al Ghariya Sabkha.*

Properties	Control	*S. ioclados*	*T. aphylla*	*T. qatarensis*
ECe (dSm^{-1}) *	4.80	4.98	4.38	2.20
Salinity (ppt)**	2.40	2.40	2.70	1.10
pH	7.70	7.90	7.90	7.90
N%	0.15	0.20	0.19	0.15
C%	3.92	4.03	3.76	3.42
H%	0.84	1.81	1.12	0.96
TOC%	0.22	0.43	0.83	0.30

N.B. No data for the upper layer soil of *C. imbricatum*

N.B. * dS/m: decisiemens per meter; dS/m is the unit of measuring salinity of saturated soil extracts at 25°C, Levels of ECe: < 0.40: non-saline, 0.40-0.80: slightly saline, 0.81-1.20: moderately saline, 1.21-1.60: saline, 1.61-3.20: Strongly saline, > 3.20: very strongly saline.

**ppt: part per thousand (g/kg); is the unit of measuring salinity in saline solutions, levels of ppt: <0.5: fresh water (non-saline), 0.5-1.0: slightly saline, 1.0-2.0: moderately saline, 2.0-10.0: strongly saline, 10.0-35.0: very strongly saline, <35.0: extremely saline.

Table 2. *Physical and chemical properties of the lower layer of the soil of halophytes at Al Ghariya Sabkha.*

Properties	Control	*C. imbricatum*	*S. ioclados*	*T. aphylla*	*T. qatarensis*
ECe (dSm^{-1})*	4.8	4.80	2.7	1.4	1.4
Salinity (ppt)**	2.4	2.4	1.3	2.0	0.7
pH	7.7	8.1	7.9	7.9	7.9
N%	0.15	0.12	0.2	0.17	0.18
C%	3.92	3.14	3.63	3.50	3.36
H%	0.84	1.94	1.51	1.25	1.15
TOC%	0.22	0.25	0.44	0.43	0.33

Look at the N.B. of Table 1.

Recent studies [8, 16] showed that many halophytes including those involved in the current study are efficient in removing elements found in the saline soils, (5) the colony-forming unit (CFU/g) at the non-rhizospheric soil as a control treatment was ranged between 2.2 x 10^3-1.5 x 10^4. Such a wide range might be a response and reflection of the influence of the environmental conditions on the soil and the biota in the Sabkha. However, the data of rhizospheric soils of some of the studied plants such as *S. ioclados* and *T. qatarensis* showed CFU/g, almost at the range of non-rhizospheric soil, while *T. aphylla* showed high counts beyond the acceptable figures (Table 5). The role of these plants on the rhizospheric bacteria will be discussed later.

Table 3. *The mineral content (µg/g dry soil) of the upper layer of halophytes soil at the Ghariya Sabkha.*

Elements	Control	*S. ioclados*	*T. aphylla*	*T. qatarensis*
Al	410.19: 10,000-300,000 µg/g (Below normal) *	532.82	163.94	31.32
B	130.99: 5-30 µg/g (Above normal) ***	108.38	94.87	104.53
Ba	20.07: 15-3500 µg/g (Normal) **	15.09	14.86	19.62
Ca	13011.23: 400-500 µg/g (Above normal) ***	18384.39	12015. 01	7475.49
Cd	0.39: 0.01-1.00 µg/g (Normal) **	0.29	0.27	0.26

Elements	Control	*S. ioclados*	*T. aphylla*	*T. qatarensis*
Co	3.09: 15-30 µg/g (Below normal) *	2.02	1.85	2.39
Cr	9.09: About 40 µg/g (Below normal) *	6.30	7.02	8.49
Cu	7.61: 2-100 µg/g (Below normal) *	5.69	5.12	5.81
Fe	1644: 20,000-550,000 µg/g (Below normal) *	1572.21	1171.08	951.30
K	2647.50: above 20, 000 µg/g (Below normal) *	1523.70	1210.49	1076.0
Li	8.36: 1 -200 µg/g (Normal) **	4.89	4.51	4.32
Mg	4695.88: 500-5000 µg/g (Normal) **	4371.38	3623.79	3521.31
Mn	112.20: 20-3000 µg/g (Normal) **	71.90	58.92	70.41
Na	3277.21: <40 µg/g (Very high) ***	1227.48	1788.98	305.68
Ni	17.06: 20-30 µg/g (Below normal) *	11.34	8.87	11.68
Pb	3.89: 15-40 µg/g (Below normal) *	2.73	1.82	2.16
Sr	1008.55: about 400 µg/g (Above normal) ***	830.14	742.37	784.79
Zn	16.1010-300 µg/g (Normal) **	9.70	8.46	11.10

*Below normal, **Normal, ***Above normal,
N.B. No data for the upper layer soil of *C. imbricatum*

Table 4. *The mineral content (µg/g dry soil) of the lower layer of halophytes soil at the Ghariya Sabkha.*

Elements	Control	*C. imbricatum*	*S. ioclados*	*T. aphylla*	*T. qatarensis*
Al	410.19: 10,000-300,000 µg/g (Below normal) *	40.07	330.17	161.16	63.14
B	130.99: 5-30 µg/g (Above normal) ***	119.47	98.82	108.95	81.15
Ba	20.07: 15-3500 µg/g (Normal) **	14.17	13.63	12.13	12.40
Ca	13011.23: 400-500 µg/g (Above normal) ***	6580.92	15571.50	13629.59	7485.65
Cd	0.39: 0.01-1.00 µg/g (Normal) **	0.23	0.24	0.28	0.21
Co	3.09: 15-30 µg/g (Below normal) *	1.66	1.70	1.63	1.41
Cr	9.09: About 40 µg/g (Below normal) *	4.94	5.38	5.81	5.02
Cu	7.61: 2-100 µg/g (Below normal) *	5.78	4.61	6.77	3.76
Fe	1644: 20,000-550,000 µg/g (Below normal) *	869.44	1114.80	1447.43	1072.90
K	2647.50: above 20, 000 µg/g (Below normal) *	837.04	941.20	1079.73	653.30
Li	8.36: 1 -200 µg/g (Normal) **	3.69	4.00	4.44	3.31
Mg	4695.88: 500-5000 µg/g (Normal) **	3626.08	4160.50	4588.52	2535.95
Mn	112.20: 20-3000 µg/g (Normal) **	62.39	61.93	56.01	46.69
Na	3277.21: <40 µg/g (Very high) ***	202.32	406.98	967.48	174.84
Ni	17.06: 20-30 µg/g (Below normal) *	10.21	9.62	8.68	8.09
Pb	3.89: 15-40 µg/g (Below normal) *	2.32	1.87	1.76	1.39
Sr	1008.55: about 400 µg/g (Above normal) ***	665.58	722.71	734.22	614.13
Zn	16.1010-300 µg/g (Normal) **	8.24	8.40	8.24	6.70

*Below normal, **Normal, ***Above normal

Table 5. *Colony-forming unit (CFU/g) at non-rhizospheric soil (control) and at rhizospheric soils (upper and lower layers) of three halophytes using NA culture media.*

Layer of rhizosphere	*S. ioclados*	*T. aphylla*	*T. qatarensis*
Upper	3.00×10^3	3.20×10^4	4.80×10^3
Lower	4.50×10^3	3.90×10^4	4.50×10^3

The CFU/g at the non-rhizospheric soil (control) of the studied halophytes ranged between 2.2×10^3 -1.5×10^4.
Only one figure of CFU/g for *C. imbricatum* for the lower layer: 5.32×10^4, this figure indicated that the count is higher than the range shown by the non-rhizospheric soil.

3.1. Diversity of Streptomyces Bacteria

Previous studies on microorganisms showed that *Streptomyces* bacteria predominated in the Qatari habitats, and species of this genus showed wide variation of morphological and biochemical characteristics [9, 18, 23]. Notably, the current study revealed a limited number of species of this genus in the non-rhizospheric soils, only four isolates at this mini-habitat of the halophytes under investigation were recognized, these isolates showed limited characteristics of colony features which included: aerial mycelium color, soluble pigment, reverse color, and the form of the whole colony. The colors of aerial mycelia were recognized as white, dark brown, and burgundy, the soluble pigment was brown-yellowish, the reverse colors were white and dark brown, while the form of the whole colony at all colonies was irregular (Table 6).

Table 6. *Four colony characteristics of selected isolates of Streptomyces from the non-rhizospheric soil [59].*

Isolates	Aerial mycelium color	Soluble pigment	Reverse color	Form of whole colony
1	White	-	White	Irregular
2	Dark brown	Brown yellowish	Dark brown	Irregular
3	Burgundy	Brown yellowish	Dark brown	Irregular
4	Burgundy	Brown yellowish	Dark brown	Irregular

-No, N.B. Isolates 3 & 4 showed variable growth in the nutrient agar from moderate growth to heavy growth, all these bacteria have Gram positive test.

On the other hand, the colony characteristics of isolates at the rhizospheric soil of *Caroxylon imbricatum* showed various types of isolates with different colony characteristics and peculiarities which indicate that significant number of strains of *Streptomyces* bacteria thrive under such mini-habitat of the canopy of the halophyte plant (Table 7). The root system of *C. imbricatum* might exudate and release various amounts of substances that could help accelerate and promote the growth of these strains [66]. Two mechanisms were suggested to phytoremediate heavy metals: (1) phyto-extraction in which plants absorb elements from contaminated soil and accumulate them in various plant organs, and (2) phyto-stabilization in which metals are immobilized by many methods including: adsorption and precipitation [67].

Some studies found that the rhizosphere of *Caroxylon imbricatum* could release substances or create conditions that encourage the growth of certain *Streptomyces* strains [16]. In fact, the specific mechanisms and effects would depend on the types of *Streptomyces* present in the rhizosphere and the chemical signals exchanged between the plant and microorganisms [68]. This interaction could potentially benefit the plant by improving its nutrient uptake or protecting it from soil-borne pathogens. Recent work of Baeshen et al. [69] revealed a huge number of microbes at the rhizosphere of some halophytes such as *Tamarix aphylla* and *Halopeplis perfoliate*, these microbes included bacteria groups such as Actinobacteriota, Proteobacteria, and fungi phyla such as Ascomycota and Basidiomycota.

Table 7. *Fifteen colony characteristics of selected isolates of Streptomyces from the rhizospheric soil of Caroxylon imbricatum [59].*

Isolates	Aerial mycelium color	Soluble pigment	Reverse color	Form of whole colony
1	Grey-white edge	Brown yellowish	Light brown	Irregular
2	Grey-white edge	Grey	Grey	Round
3	Grey	-	Grey	Round
4	Grey	-	Beige	Round
5	Grey	Grey	Grey	Round
6	Grey	Grey	Burgundy	Round
7	White	-	Grey	Round
8	Grey	-	Burgundy	Irregular
9	Grey	Grey	Grey	Irregular
10	Grey-white edge	-	Grey	Irregular
11	Grey	Grey	Burgundy	Irregular
12	White	-	Burgundy	Irregular
13	Grey	-	Grey	Irregular
14	Burgundy	-	Burgundy	Irregular
15	Grey	-	Burgundy	Round

-No

Regarding the colony characteristics of isolates of the rhizospheric soil of *T. aphylla*; they were different from both non-rhizospheric soil and the rhizospheric soil of *C. imbricatum*. However, still limited number of isolates were found under the canopy of *T. aphylla*, and some recent reports showed that this plant has a great effect on various types of fungi and bacteria. The leaves and flowers of these plants proved to have a great impact on six pathogenic fungi such as: *Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium solani, Aspergillus niger*, *Penicillium digitatum*, and *Penicillium tubesulum*, and some bacterial strains such as *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Kliebsiella pneumoniae*, *Salmonella typhi*, and *Staphylococcus aureus*. Therefore, further investigation is needed to look at the effect of the plant extracts of *T. aphylla* on the *Streptomyces* bacteria at the Qatari habitats. Notably and on some rare occasions, the aerial mycelium color and the form of the whole colony were almost like those of the non-rhizospheric soil and the rhizospheric soil of *C. imbricatum*. This means that the growth of some isolate species was encouraged under the influence of substances released from the root system of *T. aphylla* (Table 8).

The data of other studies on *Sporobolus ioclados* and *Tetraena qatarensis* confirmed the above conclusion as shown in Tables 9 and 10 [60]. New outcomes regarding *Streptomyces* isolate from the rhizospheric soil of these halophytes. The data showed that upper layers of the rhizospheric soils of these halophytes have a limited number of isolates, while lower layers contain a significant number of isolates which indicates that *Streptomyces* species thrive in such soils. The reason behind such a significant number of isolates at the lower layer of some halophytes such as: *Caroxylon imbricatum*, *Sporobolus ioclados*, and *Tetraena qatarensis* can be explained by low salinity reported at the rhizosphere. Such low salinity levels could be created by great salt absorption, accumulation, and extrusion at various plant organs and tissues.

Table 8. *Six colony characteristics of selected isolates of Streptomyces from the rhizospheric soil of Tamarix aphylla [59].*

Isolates	Aerial mycelium color	Soluble pigment	Reverse color	Form of whole colony
1	Grey-white edge	Yellowish	Yellowish	Irregular
2	Grey-white edge	-	Grey	Round
3	Grey	-	Beige	Round

Isolates	Aerial mycelium color	Soluble pigment	Reverse color	Form of whole colony
4	White	Yellowish	Beige	Irregular
5	White-Grey edge	Grey	Grey	Round
6	Grey	-	-	Round

-No, N.B. Isolates 3 & 4 showed variable growth in the nutrient agar from moderate growth to heavy growth, all these bacteria have Gram positive test

Table 9. *Ten colony characteristics of selected isolates of Streptomyces from the rhizospheric soil of Sporobolus ioclados [60].*

Isolates	Layer	Color	Form	Edge	Elevation
1	Upper	Grey	Circular	Entire	Raised
2	Upper	Grey	Circular	Entire	Raised
3	Lower	Yellow	Circular	Entire	Umbonate
4	Lower	White-grey	Irregular	Undulate	Raised
5	Lower	Dark-grey	Irregular	Undulate	Raised
6	Lower	Grey	Circular	Entire	Raised
7	Lower	Grey	Circular	Entire	Raised
8	Lower	Dark yellow	Irregular	Undulate	Umbonate
9	Lower	Yellow	Irregular	Undulate	Flat
10	Lower	Brown	Irregular	Undulate	Raised

Table 10. *Fourteen colony characteristics of selected isolates of Streptomyces from the rhizospheric soil of Tetraena qatarensis [60].*

Isolates	Layer	Color	Form	Edge	Elevation
1	Upper	White-grey	Circular	Entire	Flat
2	Upper	Grey	Irregular	Lobate	Flat
3	Upper	Grey	Circular	Entire	Umbonate
4	Upper	Grey	Irregular	Lobate	Flat
5	Lower	Grey	Circular	Entire	Raised
6	Lower	Dark grey	Circular	Entire	Raised
7	Lower	Grey	Circular	Entire	Convex
8	Lower	Grey	Circular	Entire	Raised
9	Lower	Grey	Circular	Entire	Raised
10	Lower	White grey	Circular	Entire	Flat
11	Lower	Grey	Irregular	Lobate	Umbonate
12	Lower	Grey	Irregular	Lobate	Raised
13	Lower	Light red	Circular	Entire	Raised
14	Lower	Grey	Circular	Entire	Raised

3.2. Biochemical Tests

The study of enzyme activities has been considered an important step to identify some microorganisms. These are part of biochemical tests to differentiate between groups and could be used to identify some bacteria genera. Multiple tests were used previously to identify these groups at the Al-Dhakhira Sabkhas in Qatar [23]. In the current study, only seven tests were used, many of which were adopted previously: (1) Urease (Urea analysis) (URE), (2) Starch analysis (STA), (3) Nitrate reduction (NO_3-R), (4) Casein (CAS), (5) Gelatin liquefaction (GEL), (6) Lipid (LIP), and (7) DNA. The data of these parameters for non-rhizospheric soil are shown in Table 11, four isolates from this soil of Al-Ghariya Sabkha showed negative results test except for STA. All the isolates of STA parameter were positive or high positive which indicates the ability of some *Streptomyces* to resist salinity, as high salinity levels were observed at non-rhizospheric soils as compared to the rhizospheric soils of the halophytes under investigation.

Table 11. *The biochemical tests of Streptomyces isolates from the non-rhizospheric soil.*

Isolates	URE	STA	NO_3-R	CAS	GEL	LIP	DNA
1	-	+	-	-	-	-	-
2	-	+++	-	+	-	-	-
3	-	+	-	-	-	-	-
4	-	+	+	-	-	-	-

URE: Urea hydrolysis, STA: Starch hydrolysis, NO_3-R: Nitrate reduction, CAS: Casein, GEL: Gelatin liquefaction, LIP: Lipase activity, DNA: DNase activity, Level of reactions: - No reaction, + Positive, ++ Moderate positive, +++ High positive.

On the other hand, the data of biochemical tests of six isolates that were chosen randomly from rhizospheric isolates of the halophyte plants under investigation are shown in Tables 12, 13, 14, and 15. The following discussion is dedicated to looking at the changes in rhizospheric isolates as compared to non-rhizospheric soil.

Table 12. *The biochemical tests of Streptomyces isolates from the rhizospheric soil of Caroxylon imbricatum.*

Isolates	URE	STA	NO_3-R	CAS	GEL	LIP	DNA
1	-	-	+	-	-	-	-
2	-	+	+	-	-	-	-
3	-	++	-	-	-	-	-
4	+	++	+	+	-	-	-
5	-	-	-	-	-	-	-
6	-	+	-	-	-	-	-

URE: Urea hydrolysis, STA: Starch hydrolysis, NO_3-R: Nitrate reduction, CAS: Casein, GEL: Gelatin liquefaction, LIP: Lipase activity, DNA: DNase activity, Level of reactions: - No reaction, + Positive, ++ Moderate positive, +++ High positive.

Table 13. *The biochemical tests of Streptomyces isolates from the rhizospheric soil of Sporobolus ioclados.*

Isolates	URE	STA	NO_3-R	CAS	GEL	LIP	DNA
1	-	-	-	+	-	++	-
2	+	-	+	-	+	++	-
3	+	+	-	+	-	+	-
4	-	-	-	+++	-	+	-
5	+	-	+	-	-	-	-
6	-	-	-	-	-	-	-

URE: Urea hydrolysis, STA: Starch hydrolysis, NO_3-R: Nitrate reduction, CAS: Casein, GEL: Gelatin liquefaction, LIP: Lipase activity, DNA: DNase activity, Level of reactions: - No reaction, + Positive, ++ Moderate positive, +++ High positive.

Table 14. *The biochemical tests of Streptomyces isolates from the rhizospheric soil of Tamarix aphylla.*

Isolates	URE	STA	NO_3-R	CAS	GEL	LIP	DNA
1	-	+	-	+	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	+	-	++	-
4	+++	-	-	-	-	-	-

Isolates	URE	STA	NO_3-R	CAS	GEL	LIP	DNA
5	+++	-	-	-	-	-	-
6	-	+	-	-	-	-	-

URE: Urea hydrolysis, STA: Starch hydrolysis, NO_3-R: Nitrate reduction, CAS: Casein, GEL: Gelatin liquefaction, LIP: Lipase activity, DNA: DNase activity, Level of reactions: - No reaction, + Positive, ++ Moderate positive, +++ High positive.

Table 15. *The biochemical tests of Streptomyces isolates from the rhizospheric soil of Tetraena qatarensis.*

Isolates	URE	STA	NO_3-R	CAS	GEL	LIP	DNA
1	+	+	+	-	-	-	-
2	-	+	-	-	-	++	-
3	+	-	+	+++	+	-	-
4	-	-	+	-	-	++	-
5	-	++	-	+	+	++	-
6	+	-	+	-	-	-	-

URE: Urea hydrolysis, STA: Starch hydrolysis, NO_3-R: Nitrate reduction, CAS: Casein, GEL: Gelatin liquefaction, LIP: Lipase activity, DNA: DNase activity, Level of reactions: - No reaction, + Positive, ++ Moderate positive, +++ High positive.

URE is used to test for some bacteria that can produce urease. This enzyme catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The formation of ammonia alkalizes the medium and the change in pH is detected by the change in color from light orange (at pH 6.8) to pink (at pH 8.1). Therefore, the negative test results URE indicate that urease-producing bacteria are absent such as *Proteus* spp. A positive test of this parameter means that some bacteria are present in the media that can produce the urease enzyme. Thus, species of *Streptomyces* may be capable of producing urease, however, not all of them necessarily do, and the presence and absence of urease production can vary among different strains and species within the genus. Therefore, the URE test can be a useful tool for identifying and distinguishing between different *Streptomyces* strains based on their urease-producing capabilities. Salinity as a harsh environmental factor could inhibit many microorganisms including some strains and species of this genus or inactivate the enzyme urease, otherwise, these strains depend on other sources of nitrogen [9, 70, 71]. Table 11 showed that the URE test was negative, such a result could indicate the absence of *Streptomyces* strains that are capable of producing urease. On the other hand, some positive results of the URE test were obtained that clearly showed other *Streptomyces* strains thrived in the rhizospheric soil of the halophytes under investigation.

The starch analysis (STA) test is used to identify bacteria that can hydrolyze starch (amylose and amylopectin) using the enzymes a-amylase and oligo-1,6-glucosidase. Thus, this test helps to distinguish between bacteria that produce the enzyme amylase, which can degrade starch and those that do not. For example, it has often been used to differentiate the species of some bacterial genera such as *Clostridium* and *Bacillus*. *Streptomyces* bacteria are known to produce various extracellular enzymes, including amylases, which can be involved in the degradation of complex organic compounds like starch. Therefore, the starch hydrolysis test can be a useful tool for identifying and characterizing *Streptomyces* strains based on their ability to hydrolyze starch. The data of STA in Table 11 indicated that most of the isolates from non-rhizospheric soil showed positive results while one of these isolates was highly positive. Similar results were obtained from the rhizospheric soil of *Caroxylon imbricatum*, on the contrary, most isolates from the rhizospheric soils of *Sporobolus ioclados*, *Tetraena qatarensis*, and *Tamarix aphylla* showed negative results. Such outcomes might be a normal response to the effect of the fallen leaves and fruits of these plants on the microbial activity of the soil, *Tamarix* spp. is a good example such conclusion [72].

The NO_3-R test has been used to differentiate bacteria that are able to reduce NO_3 such as Enterobacteriaceae from those that cannot. NO_3-reduction test may be one among other tests, mentioned above, that can be conducted to differentiate *Streptomyces* bacteria from other bacteria groups. The data of NO_3-reduction showed that the number of isolates that showed positive results increased at the rhizospheric soils of *Caroxylon imbricatum*, *Sporobolus ioclados*, and *Tetraena qatarensis*, however, *Tamarix aphylla* showed negative results, the allelopathic effect of this plant might be the reason behind such results [72].

Casein (CAS) is just one of many biochemical tests used to identify bacteria, as *Streptomyces* is a genus that can produce various enzymes and secondary metabolites including proteases like caseinase. Therefore, the Casein test can be a valuable tool for identifying and differentiating *Streptomyces* species based on their ability to break down casein. Al-Thani and Yasseen [9] reported some results that a limited number of bacteria isolates could hydrolyze the milk protein in the rhizosphere soils. The data of the current study revealed that most isolates from the non-rhizospheric and rhizospheric soils gave negative results (Table 11), however, some others gave positive results and even highly positive, such outcomes need further investigation using a modern approach.

The Gelatin liquefaction (GEL) test is a microbiological test used to differentiate between bacterial genera based on their ability to produce gelatinase; the enzyme that hydrolyzes or breaks down gelatin. In fact, GEL test is useful to differentiate certain bacteria strains such as species in the genera *Staphylococcus* and *Clostridium* [9, 23]. This test is not specific to *Streptomyces*, but it is one among many other tests and techniques used in microbiology for bacterial identification and classification. However, GEL test can be useful in the identification and differentiation of bacteria in clinical microbiology, particularly in the identification of certain pathogenic bacteria. Notably, not all bacteria produce gelatinase, so this test is used to differentiate bacteria that have this enzyme activity, therefore, other alternative tests or methods for the same purpose can be used. All the GEL tests for *Streptomyces* isolates in the non-rhizospheric soils showed negative results, also, the rhizospheric soils of the halophytes under investigation showed the same negative results except a few positive results which need further investigation (Tables 13 and 15).

Lipid (LIP) tests are often used in microbiology; based on the theory that lipids are organic molecules that constitute the

cell membranes of bacteria, and different bacteria species may have distinct lipid compositions. There are many methods and techniques adopted for bacterial identification which include: Gram staining, lipid extraction, FAME analysis, phospholipid analysis, MALDI-TOF mass spectroscopy, and lipid staining [73-78]. Notably, bacterial identification is rarely based solely on lipid tests, and typically, a combination of tests including morphology, staining characteristics, biochemical tests, molecular techniques, and genetic analysis are used accurately to achieve reliable results. The idea of this test is to detect the ability of bacteria to produce exoenzyme lipase and hydrolyze lipids. Tables 11 and 12 show that all isolates at non-rhizospheric soil and the rhizospheric soil of *Caroxylon imbricatum* gave negative results, while rhizospheric soils of *Sporobolus ioclados*, *Tamarix aphylla*, and *Tetraena qatarensis* gave positive results. Such outcomes need further investigation to look at the type of bacteria species that can hydrolyze lipids.

The DNase test alone is not a definitive method for detecting the presence of bacteria or identifying specific bacteria species. Thus, for bacteria detection and identification, various other techniques, and tests such as culture methods, PCR, DNA sequencing, biochemical assays, and immunological assays are commonly used. DNase can be employed in different ways to aid in bacterial identification and analysis, these include DNase test for *Staphylococcus* species, identification of unknown bacteria, and research and DNA degradation studies. Regarding *Streptomyces* bacteria: positive DNase: these bacteria are capable of producing DNase which hydrolyzes the DNA in the agar, creating a zone around the bacteria colony, negative DNase: these bacteria do not produce DNase, and there will be no clear zone around the colony. From the data in Tables 11-15, no *Streptomyces* isolate produced DNase at the non-rhizospheric or rhizospheric soils of the halophyte plants under investigation. Therefore, further investigation is needed to identify those isolates.

3.3. The Anti-Bacterial Primary Test

Streptomyces bacteria serve as a valuable tool in antibiotic research and development, providing a natural source of antibiotics and insights into their production; and assisting in the fight against antibiotic-resistant bacterial strains. The saline soil collected from the rhizosphere of halophytes at Sabkhas might have some bacteria including *Streptomyces* that could be a source of antibiotics. Two isolates from non-rhizospheric soils and five isolates were chosen randomly from rhizospheres of the halophytes under investigation. Antibacterial primary tests of *Streptomyces* isolated from Al Ghariya Sabkha were done, and the results showed clearly that many isolates from the rhizospheres of the halophytes have clear antibacterial action against the test strains used in this study (Table 16).

Table 16. *Antibacterial primary test of Streptomyces isolates against four test strains: Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis*.*

Soil sample	Isolates	Test strain			
		B. subtilis	***E. coli****	***S. aureus***	***S. epidermidis***
Non-rhizospheric	1	-	-	-	-
	2	-	-	-	-
*Caroxylon imbricatum***	1	-	No growth	-	No growth
	2	-	-	-	-
	3	-	No growth	-	-
	4	++	++	++	-
	5	-	+	+	+
*Sporobolus ioclados***	1	++	+	+	+
	2	++	++	+++	+++
	3	-	-	+++	-
	4	+	++	++	+
	5	+++	+	-	++++
*Tamarix aphylla***	1	+	-	+	-
	2	-	-	-	No growth
	3	-	+	+	+
	4	-	No growth	-	No growth
	5	+	++	++	+
*Tetraena qatarensis***	1	+++	++++	++++	++++
	2	-	-	-	-
	3	++	++	+	++++
	4	-	-	-	-
	5	+	++	++	++

**E. coli* (Gram-negative), all others are Gram-positive
***Rhizospheric soils of halophytes under investigation
Inhibition zone range: -(absent), + (1.5-2.5cm) (weak), ++(2.5-3.5cm) (moderate), +++(3.5-4.5cm) (high), ++++(4.5-5.5cm) (extremely high)

The chemical and physical properties of the Sabkha soil revealed some facts about the harsh environmental conditions facing the biota at this habitat. Notably, the pH of the Sabkha soil ranged between 7.7-7.9, as the normal pH value for normal plant growth is 7; considered optimum for the growth of *Streptomyces* bacteria [79]. However, the pH values from

7.7-7.9 are considered moderate alkaline suitable for the growth of *Streptomyces* bacteria; which provides these bacteria and halophytes with essential nutrients. In fact, most antibiotics are optimally produced at a pH close to 7.0 [79], and salinity levels in terms of ECe of soil samples ranged from 0.9-4.9 dSm^{-1} at this Sabkha, thus some soil samples with low salinity levels could be suitable for antibiotics production, while some of these soil samples, especially at the upper layer, might not be suitable for the growth of *Streptomyces* bacteria [55, 80]. A limited number of strains of these bacteria live in non-rhizospheric soils. Less salinity levels in rhizospheric soils might encourage many *Streptomyces* isolates of these bacteria to prove active in anti-bacterial action against test strains [81]. Therefore, the cultivation of halophytes at the saline lands and Sabkhas could achieve more one goal: (1) alleviate the salt stress leading to land acclimatation for the cultivation of crops, (2) phytoremediation of polluted and saline soils, (3) antibiotic production could be another objective as *Streptomyces* bacteria proved efficient in the production of antibiotics, which raises the interest of scientists and research centers to conduct serious research works leading to manufacturing antibiotics, and (4) many enzymes are produced by animals, plants, and microorganisms at the soil biota [82]; such process is considered as the most accurate indicator of changes in microbial activities in the soil [83]. Soil microorganisms mainly synthesize extracellular enzymes such as β glucosidase, urease hydrolase, phosphatase, glycosylating enzymes, cellulase, amylase and many more, so soil enzymes play a vital role in biodegradation and soil fertilization [84, 85].

3.4. Identification and Differentiation of Isolates

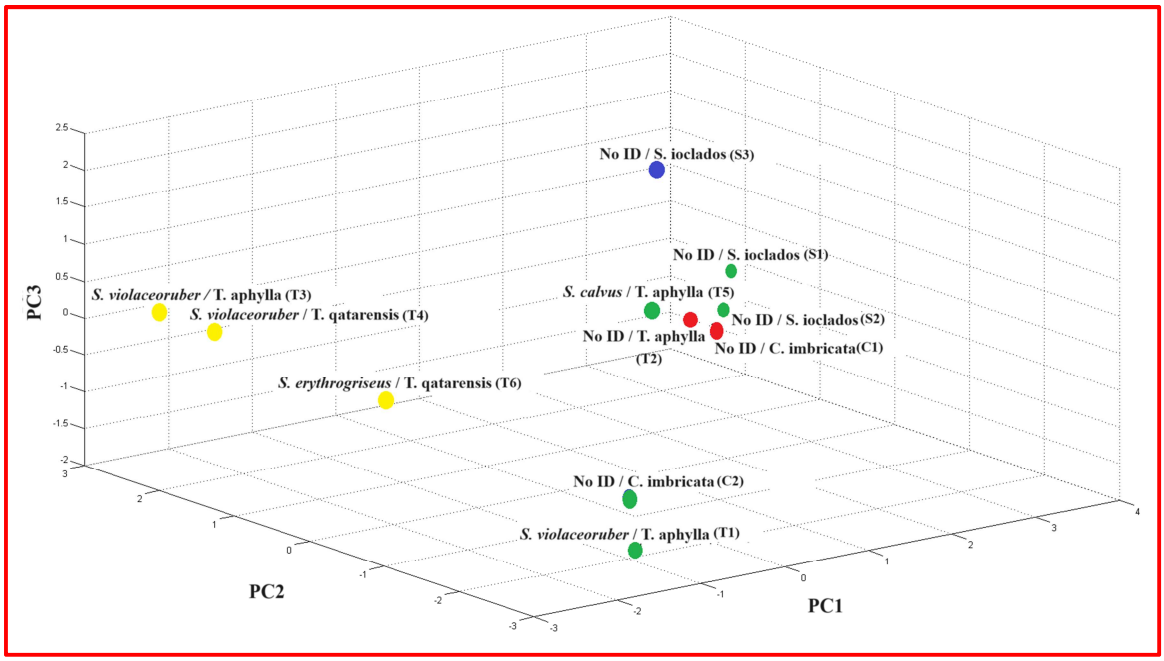
The traditional methods of bacteria identification that are reported in this work have been used for many years and are still relevant today. However, these methods have several inherent limitations and defects, including limited accuracy, and more time consumption. Therefore, modern approaches have been suggested as more advanced techniques like MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectroscopy), and molecular methods such as PCR and DNA sequencing. These methods offer advantages in terms of speed, accuracy, and ability to identify a broader range of bacteria including those that are difficult to culture or have atypical characteristics. Thus, the MALDI-TOF MS technique was used to identify and differentiate the isolates based on their protein profiles. There were four strains isolated from the rhizosphere of *Tamarx aphylla* that were analyzed using this technique. Two out of four were identified as *Streptomyces violaceoruber* (with a score of 1.74, and 1.89) and one was identified as *Streptomyces calvus* (score of 1.70). One of the four strains could not be identified using this method. Similarly, two strains isolated from rhizosphere soil of *Caroxylon imbricatum,* and three of *Sporobolus ioclados* were also not identified by MALDI-TOF MS. However, two strains isolated from the lower soil layer of *Tetraena qatarensis* were identified as *Streptomyces erythrogriseus* (1.74), *Streptomyces violaceoruber* (1.79). The results of identification showed that the MALDI-TOF MS analysis requires further robustness in the commercial database as some of the *Streptomyces* strains could not be identified. This is consistent with the previous literature. In another study, Yarbrough et al. [86] also concluded that the database of Bruker Biotyper needs to be updated for accurate identification of *Streptomyces* isolates as only 3 out of 10 isolates could be identified using different methods. Therefore, to improve the rapid identification of *Streptomyces* strains, Loucif et al. [87] developed the in-house library and then identified the unknown strains. The use of an in-house database helped to identify 100% of the unknown isolates with scores as high as 2.8. In this research, we were able to identify 5 out of 11 isolates up to the species level (Table 17). Nevertheless, the protein profiles generated by MALDI-TOF MS analysis can be further analyzed statistically to develop differentiation among the strains [63, 88]. Therefore, in this research, principle component analysis (PCA) was performed to develop clusters of closely related isolates and to understand the differences between the strains. Moreover, PCA helped to differentiate the strains at species and sub-species levels. PCA 3D diagram plotted in Figure 4a shows the differences between the strains with respect to principle components 1, 2, and 3 (PC1, PC2, and PC3). The total variation explained by PC1, PC2, and PC3 is about 68% (Figure 4b). The distance between the two points shows the level of differences between their protein profiles. PCA analysis resulted in 4 groups of a total of 11 isolates. One of the groups colored in yellow consists of two species of *S. violaceoruber* isolated from the lower soil layer of *T. aphylla* and *T. qatarensis* plants and one species of *S. erythrogriseus*.

Table 17. *The results of MALDI-TOF MS identification.*

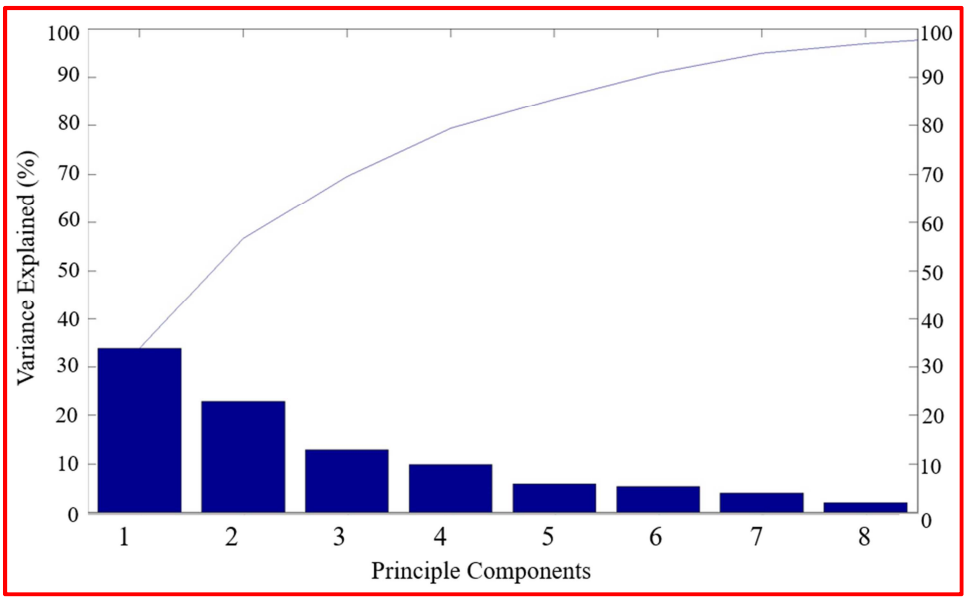
Isolates (Table 12-15)	Plant species	Bacterial identification	Score	PCA code
Table 12: 1	*Caroxylon imbricatum* (Lower layer) **	No identification possible		C1
Table 12: 6	*Caroxylon imbricatum* (Lower layer)	No identification possible		C2
Table 13: 2	*Sporobolus ioclados* (Lower layer)	No identification possible		S1
Table 13: 3	*Sporobolus ioclados* (Lower layer)	No identification possible		S2
Table 13: 6	*Sporobolus ioclados* (Lower layer)	No identification possible		S3
Table 14: 1	*Tamarix aphylla* (Upper layer) ***	*Streptomyces calvus*	1.70	T5
Table 14: 3	*Tamarix aphylla* (Upper layer)	*Streptomyces violaceoruber*	1.74	T1
Table 14: 4	*Tamarix aphylla* (Lower layer)	No identification possible		T2

Isolates (Table 12-15)	Plant species	Bacterial identification	Score	PCA code
Table 14: 6	*Tamarix aphylla* (Lower layer)	*Streptomyces violaceoruber*	1.89	T3
Table 15: 1	*Tetraena qatarensis* (Upper layer)	*Streptomyces erythrogriseus*	1.74	T6
Table 15: 5	*Tetraena qatarensis* (Lower layer)	*Streptomyces violaceoruber*	1.79	T4

*Code of isolates from tables 12-15, **Lower layer: 10-30cm subsurface deep,
***Upper lower: 1-10cm of the surface deep.



a



b

Figure 4. *PCA analysis of the protein profiles of the isolates (a) PCA 3D; (b) Variance explained by the PCA.*

Another group colored in green contains *S. calvus* from *T. aphylla*, *S. violaceoruber* from *T. aphylla*, and 3 unidentified isolates. Another two unidentified isolates (colored in red) are also placed near the green colored group demonstrating their similarities with other isolates. This shows that all the 5 not identified strains also belong to closely related species of *Streptomyces* as they are placed very close to *S. calvus*. One not identified isolated (from *S. ioclados*) is grouped separately showing its differences with other strains and its potential to be a different species. The dendrogram plotted in Figure 5 also confirms the differentiation of isolates among each other and among different groups of strains.

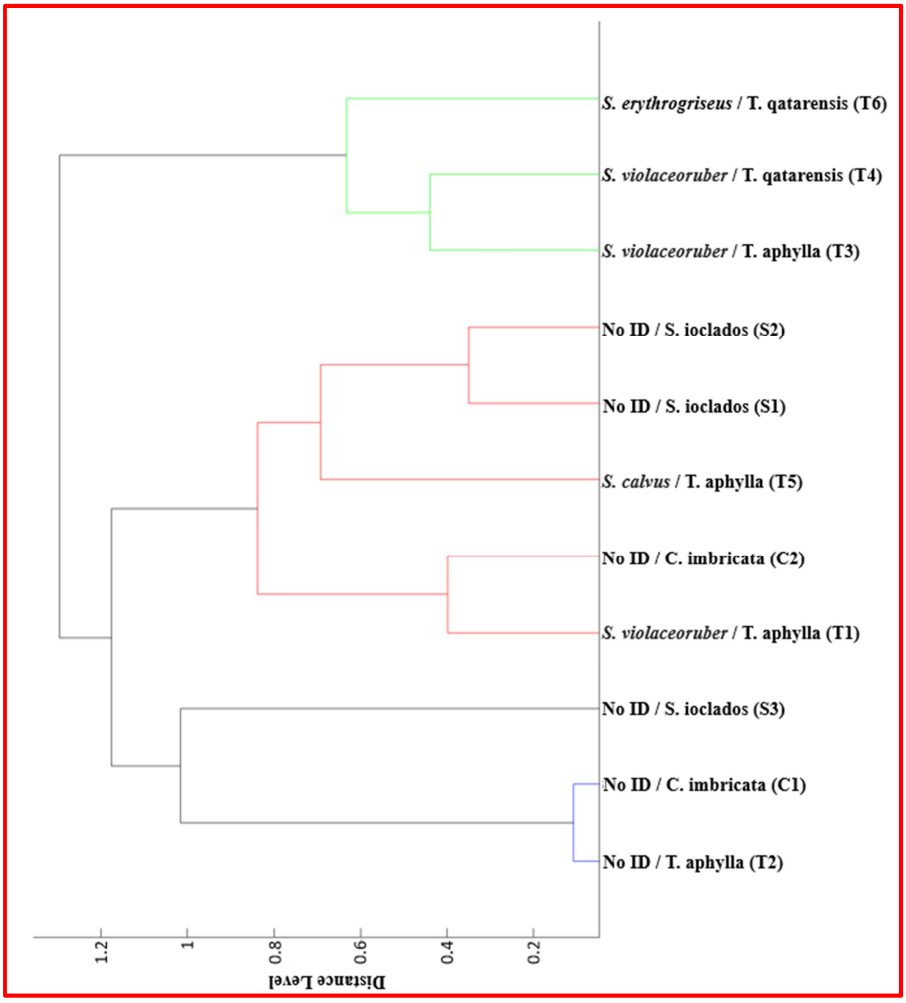


Figure 5. *PCA Dendrogram of the Streptomyces strains.*

4. Conclusion

Biological solutions to many problems facing mankind have been suggested recently, as a new approach to solve many problems of environmental stresses including salinity and impacts of pollution on health and agriculture. The gene bank of native plants and the associated microorganisms could be experimental materials that can be used for modern biotechnology. *Streptomyces* bacteria proved efficient in remedying polluted soils by facilitating mineral absorption by halophytes and producing antibiotic compounds at low salinity levels around the rhizosphere. MALDI-TOF MS helped to identify 5 out of 11 tested isolates up to a species level and its combination with PCA provided further differentiation among the strains based on the protein profiles. However, it was concluded that the commercial database for MALDI-TOF MS requires further improvement for the identification of *Streptomyces* sp. Further investigation and deep research are needed using modern gene technology to solve many problems in health and agriculture.

Conflicts of Interest

The authors declare no conflicts of interest regarding this work.

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