

Ecology and biodiversity profile of the Genus *Streptomyces* in the soil of the State of Qatar

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بيئة و تنوع بكتيريا *Streptomyces* في تربة قطر

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لقد تمت دراسة التنوع الحيوي والبيئي لبكتيريا *Streptomyces* في 123 عينه تربه من مواقع مختلفه في دولة قطر وتم تسجيل المحتوى الرطوبي والرقم الهيدروجيني للتربه والتوصيل الكهربائي . وتبين أن المحتوى الرطوبي في العينات تتراوح بين 0.2-8.8% في حين أن الرقم الهيدروجيني كان يعيل إلى القاعديه وتراوح قيمه بين 6.7-9 ولوحظ أن التوصيل الكهربائي لعينات التربه قد تراوح أيضا بين 1.40-25 مما يشير لدرجه من الملوحة. و فيما يتعلق بالأعداد الكليه للبكتيريا فقد تراوحت بين (1.2×10^4) - (29×10^4) مستعمرة بكتيريه لكل جرام من التربه الجافه بينما العدد الكلي لبكتيريا *Streptomyces* كان بين (1×10^4) - (12×10^4) مستعمرة بكتيريه لكل جرام من التربه الجافه.

و عند دراسة المظهر الخارجي لعزلات *Streptomyces* كانت في الغالب دائريه وناعمه ومرتفعه قطرها يتراوح بين 1-5 مم مع بعض الاستثناءات وكان من الممكن عزل 340 عزله عرفت اعتمادا على الخصائص المظهرية والكيميائية التابعة لجنس *Streptomyces* وكانت جميعها قادرة على استعمال مصادر كربون مختلفه كما إن لون المستعمرة لمعظم العزلات كان ابيض (42.4%) او رمادي (18.2%) وأظهرت بقية العزلات تنوع في لون المستعمرة فمنها الشفاف والأسود والبرتقالي والوردي . كما لوحظ أن البكتيريا ذات لون المستعمرات الأبيض والبرتقالي كان سائدا في الجزء الشمالي من أراضي الدوله (المواقع 1-44) بينما البكتيريا ذات الألوان الشفافة كانت سائده في المواقع الجنوبيه (المواقع 44-85) والبكتيريا ذات الألوان

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الأسود والبي والرمادي ظهرت في المناطق الوسطى (المواقع 56-65) ولا بد من الأشاره هنا إلى أن هذه هي
الدراسة الأولى حول بكتيريا *Streptomyces* في التربة القطريه بمقدار ما هو متاح لنا في متابعة البحوث في
المنطقه .

Key Words: *Chemotaxonomic, Microbial count, Phenotypic, Streptomyces.*

ABSTRACT

Ecology and biodiversity profile of *Streptomyces* in the soil of Qatar have been studied in 123 soil samples from different sites distributed all over the country. Moisture contents of soils samples ranged between 0.2-8.8% (w/w) and pH values tended to be of the alkaline type (7.6-9). Electric conductivity (25-40.1 ms) was recorded with the highest values for sites 13, 18, 22, 50, 40 and a narrow range (0.2-7.8 ms) for the remaining sites. The total bacterial count between (1.2×10^4) – (29×10^4) c.f.u./g of dry soil, whereas the *Streptomyces* count had a narrow range of (1×10^4) – (12×10^4) c.f.u./g of dry soil.

The morphology of the isolated *Streptomyces* had a common form of colony; they were mainly spherical, smooth, elevated with diameter range between 1 - 5 mm with only a few exceptions. Thirty four of the isolates identified based on the phenotypic and chemotaxonomic characteristics, belongs to the genus *Streptomyces* and all of these were capable of utilizing various carbon sources. *Streptomyces* strains showed that 42.4 % and 18.2 % were white or grey color respectively, whereas the remaining strains showed variation in color including transparent, black, orange and pink. Moreover, the white and orange colored strains were conspicuous in the northern part of the country (sites 1-44) whereas the transparent strains were predominant in the southern part (sites 45-85). However, strains colored grey, black or brown appeared to be restricted to the midland (sites 56-65). This preliminary study represents the first about *Streptomyces* in Qatari soils as far as we know.

Introduction

Within the Domain Bacteria the genus *Streptomyces* comprises the largest number of taxa with more than 500 validly described species and subspecies [1]. The genus currently accommodates aerobic Gram-positive bacteria that has high DNA G +C % content (69-78 mole %), produces extensive branching hyphae [2,3,4]. These characteristics among others enable *Streptomyces* to be present in different environments.

The ecological importance of antibiotic production by soil Actinomycetes have been debated, but only limited evidence for production in natural environments was obtained [5,6]. Moreover, *Streptomyces* have been recommended for the biocontrol against the pathogenic fungi of both roots and seeds [7, 8] but they are better known as producers of commercially important clinical antibiotics [9, 10]. Due to the fact that *Streptomyces*, among other Gram-positive bacteria, are characterized by an increased resistance to desiccation [5], the arid environment like that of the soil of Qatar may provide a selective advantage for the ecology and biodiversity of the genus *Streptomyces*. In contrast to the adjacent Gulf-countries like Kuwait [11, 12] *Streptomyces* of the soil of Qatar have not been studied before. The aim of the present

study was to investigate the ecology and biodiversity of *Streptomyces* from 123 samples of soil collected from all over the State of Qatar.

Materials and Methods

Sampling

The sampling sites were selected to represent the range of the soil types of Qatar (Figure 1). Collection of soil samples was carried out along an N-S transect covering a distance of 2-km of desert land. These sites fall along the sides of main roads across the state of Qatar.

For each sample, approximately 100-g of soil was collected at a depth of 5-10 cm and in total 123 samples from different locations were obtained. Each sample was placed in a sterile plastic bag, transported in an ice-box at about 4 °C, and delivered to the Laboratory.

Sub-samples were prepared by sieving the samples and these were kept in glass ampoules of 20-g each for further analysis. Moisture content and pH of the soil samples were determined as described by Wellington and Toth (1994).

For pH determination 20g of each soil sample was dissolved into 20 ml of distilled water, was allowed to equilibrate for 1 h, and the pH value was recorded. For moisture determination, another 20g of the soil sample was placed in a pre-weighed crucible, dried at 105 °C for 24 h, re-weighed and moisture was calculated as w/w.

Bacterial strains and culture conditions

Streptomyces strains were enumerated and isolated by the dilution plate procedure [6, 10] on starch nutrient agar (SNA) consisting of soluble starch (1.0 %, w/v). A 1.0 g soil sample from each site was suspended in 9.0ml sterile nutrient broth and agitated aseptically for 5 min. Serial dilutions were made in 10-fold steps and aliquots of 0.1-ml of the appropriate dilutions were spread over the surface of the plates in triplicates. Plates prepared for counting were incubated at 37 °C for 48-h and those which were needed for the selection and isolation of different colonies were incubated for 7-10 days.

The culture conditions and maintenance was carried out according to Kieser *et al.*, (2000). All colonies on which *Streptomyces* appeared and /or these with production of the geosmin smell were selected according to their color, colony diameter, type of colony and diffused pigments for phenotypic and chemotaxonomic characterization.

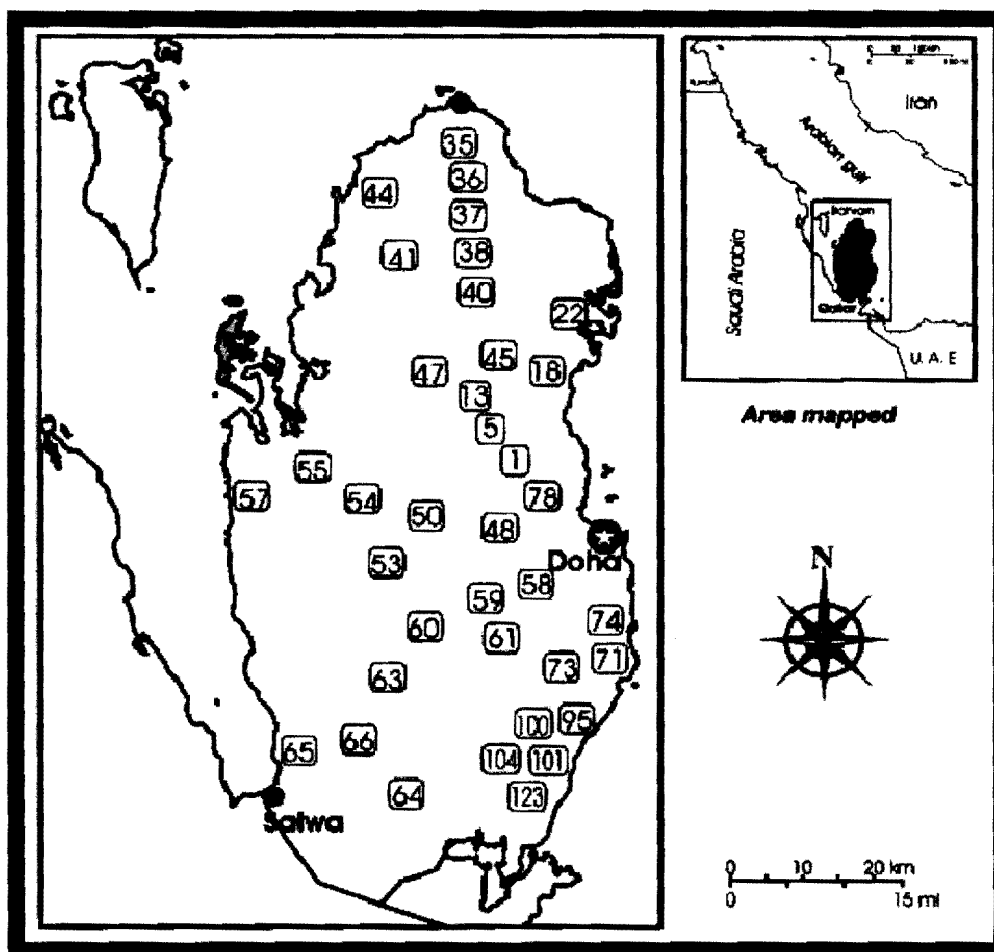


Figure 1. The Location Of Sampling Sites Of The Soil Of Qatar As Numbered In Tables 1,2 & 3.

Phenotypic and biochemical characterization

Cell morphology and spore formation were examined by phase-contrast microscopy (Leitz Diaplan) from culture at different growth stages. The Gram-positive test was conducted to ensure the prokaryotic reaction of the selected isolates. For phenotypic characterization, conventional physiological and cultural tests were performed as described previously (15,16). Growth on various standard bacteriological media was tested by using nutrient agar, yeast extract agar, malt extract agar, meat extract agar, starch-nutrient agar (SNA) and peptone agar following the manufacturer's instructions (Scharlau, Spain). Growth was studied at 3 different temperatures (4, 37, 55 °C) on starch-nutrient agar (1.0 % starch). The ability of strains to grow under aerobic conditions but not otherwise was also investigated. For the carbon utilization tests, the strains growth ability was tested after a 1-2 weeks period of incubation at 37 °C on starch-nutrient agar (1.0 % starch) with 1.0% of each substrate (glucose, xylose, lactose, sucrose, sorbitol, manitol, lactose and starch).

Biochemical tests were conducted and included: amylase, oxidase, catalase, urease, nitrate-reduction, H₂S production, and indole productions. The hydrolysis tests for starch, casein and gelatin were conducted separately in nutrient broth supplemented with 1 % of each. Once the generic identification was completed, the *Streptomyces* strains were given the accession numbers of Qatar Culture Collection. The doubted, strains were dispatched to the NCIMB LTD, Aberdeen, U.K. for confirmation of species identity.

Results And Discussion

Some physical and chemical characteristics of the soils in the studied sites are showed in Table 1. The range of moisture content varied between 0.2-8.8 % and therefore the moisture appeared to be similar in all sites; as the State of Qatar has dry land with the soil generally characterized by sedimentation of fine materials which vary between silty clay loams to sandy loam texture. Under such circumstances the distributions of *Streptomyces* have a selective advantage where all Gram-positive bacteria showed desiccation-resistant capabilities in arid communities [5]. Therefore, one has to expect that the annual precipitation, but not soil-moisture, would causally relate to the type of the predominant microbial community under such circumstances

Table 1: Physical and chemical characteristics for individual sampling sites of the soil of Qatar.

1	Duheal	Brown	1.9	8.2	7.8
5	Nueam	Brown	1.1	8.3	1.6
13	Waseel	Brown	5.2	7.6	35.1
18	Sumisma	Brown	4	7.9	38.1
22	Thakhera	White	1.3	9.5	32.1
30	Ka'ban	White	1.9	8.6	2
41	Querea	Brown	3.5	7.8	26.5
44	Zebara	Brown	5.2	8.6	2.9
48	Dokhan road	Brown	1.7	8.4	1.8
50	Jameleh road	Brown	3.4	7.8	25
56	Zakreet	White	5.4	8.6	2.5
58	Salwa road	White	1.5	8.6	2.5
59	Al-Saylyah	White	5.3	8.5	1.2
60	rashid road	Brown	0.3	8.7	0.3
61	Satalit Station	Brown	0.3	8.9	0.2
64	Sudanthil	Brown	0.2	8.2	0.7
65	Abu-Samra 1	Grey	8.8	8.6	2.6
66	Abu-Samra 2	Brown	0.7	8.5	1.2
72	Wakra City	Brown	1.1	8.3	2.1
78	North Khalifa	Grey	1.2	7.7	5.5
80	Old Ghanem	Grey	0.4	8.3	3.1
83	al-Jesra	Grey	3	8.2	12.4
85	Al-Sulta	Grey	4.4	8.2	40.1

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The soil of the studied sites appeared to be alkaline as pH values varied within a narrow range of 7.6-9.8. This range is quite favorable for the growth and proliferation of *Streptomyces*. In contrast to the pH and moisture content, the electric conductivity of the studied soils showed some differences between the sites.

For instance, there was a wide range of conductivity (25-40.1 ms) with the highest values for sites 13, 18, 22,50,40 respectively and a narrow range (0.2-7.8 ms) for the remaining sites.

The total bacterial and the *Streptomyces* contents are given in Table 2. The total bacterial count showed a wide range (1.2×10^4)–(29×10^4) c.f.u./g of dry soil with the maximum and minimum values at sites 72 and 59 respectively.

This indicates a difference on the distribution of the total bacterial population around the country. In contrast, *Streptomyces* count showed a very narrow range of (1×10^4)–(12×10^4) c.f.u./g of dry soil with the highest values (10×10^4)–(12×10^4) c.f.u./g of dry soil at sites 78 and 80 respectively, indicating that the *Streptomyces* counts of the soil of Qatar are distributed almost evenly around the country.

This is comparable to that reported (1.3×10^4) – (1.15×10^5) c.f.u /g for the Brazilian soils [6].

The total count of *Streptomyces* in the present study accounted to about 30-40 % of the total bacterial count suggesting that members of this group are the predominant bacteria in Qatari soils. Similar results were reported before for other parts of the World [17,6,10].

Table 2: Total bacterial count, *Streptomyces* count (c.f.u / g of dry soil) and colony color frequencies (%).

1	Duheal	8	1	80	-	-	-	20	-
5	Nueam	6	1	90	5	-	-	5	-
13	Waseel	7	1	80	-	-	-	-	20
18	Sumisma	9	1	80	-	-	-	20	-
22	Thakhera	6	1	10	-	5-	-	10	30
30	Ka'ban	1.3	1	80	-	20	-	-	-
41	Querea	7	1	10	-	-	-	90	-
44	Zebara	7	2	30	-	-	50	20	-
48	Dokhan road	10	1	-	10	20	60	10	-
50	Jameleh road	10	1	5	-	-	95	-	-
56	Zakreet	5	1	40	10	10	30	-	10
58	Salwa road	8	2	5	-	-	70	-	25
59	Al-Saylyah	1.2	5	5	-	15	70	-	10
60	rashid road	3	1	20	40	-	20	-	20
61	Satalit Station	9	4	30	5	5	45	15	-
64	Sudanthal	6	4	25	10	5	35	-	25
65	Abu-Samra 1	6	3	30	-	60	5	-	5
66	Abu-Samra 2	18	5	50	5	5	35	5	-
72	Wakra City	29	1	40	5	10	40	5	-
78	North Khalifa	10	10	10	-	-	90	-	-
80	Old Ghanem	12	12	10	-	-	90	-	-
83	al-Jesra	8	1	10	-	50	40	-	-
85	Al-Sulta	8	6	5	-	-	90	5	-

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The isolated *Streptomyces* strains possessed a common form of colony; they were mainly spherical, smooth, slightly elevated with a diameter range between 1 - 5 mm except, for the 3 strains Q1B, Q61A, Q64B where the colony diameter reached 10 mm.

Based on the phenotypic and chemotaxonomic characterization, 34 isolates were identified as belonging to the genus *Streptomyces* (Table 3) and all the isolated strains were aerobic, Gram-positive, non-acid-fast, non-motile and with an optimal-growth obtained on starch-nutrient agar at 37 °C.

All strains were capable of the utilization of the various carbon sources including glucose, xylose, lactose, succrose, sorbitol, manitol, lactose, starch.

All the isolated 34 *Streptomyces* strains have been deposited under their accession number of Qatar Culture Collection in the Microbiology and Biotechnology Laboratory.

However 10 other strains, isolated from the industrial area of Qatar (sites 95-123) were kept separately for further studies (Table 3)

Table 3: Morphological characteristics of the isolated strains of *Streptomyces* during growth on nutrient agar.

1	Duheal	<i>Streptomyces</i>	Q1B	White	s,e	2-10
5	Nueam	<i>Streptomyces</i>	Q5B	White	s,e	2-4
		<i>Streptomyces</i>	Q5C	Pink	s,e	2-5
13	Waseel	<i>Streptomyces</i>	Q13B	White	s,e	1-2
		<i>Streptomyces</i>	Q13C	Violet	s,e	2-5
30	Ka'ban	<i>Streptomyces</i>	Q30A	White	s,e	2-4
		<i>Streptomyces</i>	Q30C	Violet	s,e	1-2
		<i>Streptomyces</i>	Q30D	Transparent	f,i	1-3
41	Querea	<i>Streptomyces</i>	Q41A	White	s,e	2-4
56	Zakreet	<i>Streptomyces</i>	Q56A	White-grey	s,e	1-3
		<i>Streptomyces</i>	Q56E	White	s,e	1-3
		<i>Streptomyces</i>	Q56F	Grey	s,e	1-3
		<i>Streptomyces</i>	Q56G	Black	s,e	1-3
59	Al-Saylyah	<i>Streptomyces</i>	Q59A	Black-grey	s,e	1-2
		<i>Streptomyces</i>	Q59B	Orange	s,e	1-3
60	rashid road	<i>Streptomyces</i>	Q60B	White	s,e	1-5
		<i>Streptomyces</i>	Q60C	Pink	s,e	2-4
		<i>Streptomyces</i>	Q60F	Grey-black	s,e	2-4
		<i>Streptomyces</i>	Q60G	Grey-black	s,e	2-4
		<i>Streptomyces</i>	Q60H	Grey-white	s,e	2-4
61	Satalit Station	<i>Streptomyces</i>	Q61A	Grey-black	s,e	1-10
		<i>Streptomyces</i>	Q61B	Pink	s,e	1-2

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		<i>Streptomyces</i>	Q61D	White	s,e	2-5
64	Sudanthil	<i>Streptomyces</i>	Q64A	White	s,e	-
		<i>Streptomyces</i>	Q64B	Black-grey	s,e	4-8
65	Abu-Samra 1	<i>Streptomyces</i>	Q65C	Black	s,e	1-2
		<i>Streptomyces</i>	Q65D	Black	s,e	1-2
66	Abu-Samra 2	<i>Streptomyces</i>	Q66A	Transparent	f,i	1-2
		<i>Streptomyces</i>	Q66B	White	s,e	1-4
		<i>Streptomyces</i>	Q66C	Red	s,e	1-2
72	Wakra City	<i>Streptomyces</i>	Q72D	White	s,e	-
		<i>Streptomyces</i>	Q78A	Transparent	f,i	-
78	North Khalifa	<i>Streptomyces</i>	Q80A	White	s,e	-
80	Old Ghanem	<i>Streptomyces</i>	Q80B	Transparent	f,I	-
89	Sudan	<i>Streptomyces</i>	Q89	Grey-White	s,e	2-5
95	Umsaeed*	<i>Streptomyces</i>	Q95	White	s,e	2-5
97	Umsaeed*	<i>Streptomyces</i>	Q97	Golden	s,e	2-5
100	Umsaeed*	<i>Streptomyces</i>	Q100	Grey	s,e	2-5
102	Umsaeed*	<i>Streptomyces</i>	Q102	Grey	s,e	2-5
104	Umsaeed*	<i>Streptomyces</i>	Q104	Grey-Pink	s,e	2-5
105	Umsaeed*	<i>Streptomyces</i>	Q105	Grey-Pink	s,e	2-5
106	Umsaeed*	<i>Streptomyces</i>	Q106	Grey	s,e	2-5
107	Umsaeed*	<i>Streptomyces</i>	Q107	Grey	s,e	2-5
123	Umsaeed*	<i>Streptomyces</i>	Q123	Grey-White	s,e	2-5

Colony form: elevated (e), spherical (s), irregular (I) and flatten (f).

* Umsaeed= Industrial area of Qatar.

Colony diameter was given in range (min. - max.)

The frequency of the *Streptomyces* according to the color of colonies (Table 4) showed a unique pattern of distribution between different sites: white and orange colonies were common in the northern part of the country (sites 1-44), transparent strains were predominant in the southern part (sites 44-85) and grey, black or brown coloured colonies were (sites 56-65) restricted to the midlands of the country. This indicates the presence of a limiting ecological factor(s) (other than moisture and pH) responsible for *Streptomyces* distribution. This necessitates further studies to elucidate the effect of such assumed factors?

Table 4: Frequency of the color-lines of the isolated *Streptomyces* strains.

Serial No	Color-line	Number isolates	Frequency (%)
1	White	14	42.4
2	Grey	6	18.2
3	Pink	4	12.1
4	Orange	1	3
5	Red	2	6.1
6	Black	3	9.1
7	Transparent	3	9.1
Total	-	33	100

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