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Diversity and Antimicrobial Potential of Culturable Actinobacteria from Desert Soils of Saudi Arabia

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Abstract

The present investigation Saudi Arabian desert actinobacteria have been studied for discovery of novel actinobacteria and also useful new sources for bioactive metabolites. Soil samples were collected from 10 different places in Riyadh province of Saudi Arabia. The maximum actinobacterial count $21.8x10^3$ was recorded in Hazlullah soil sample and minimum $1.6x10^3$ was recorded in Al-Kharj soil samples. Totally 134 morphologically distinguished culturable actinobacteria were isolated from 10 soil samples and screened their antimicrobial potential. Altogether 134 isolates, 16 actinobacterial isolates only exhibited the antimicrobial activity. However, 6 isolates were potential against Gram-positive, 5 were potential against Gram-negative and 14 were potential against yeast like pathogenic fungi. Nevertheless, only three isolates namely DA3-7, DA3-12 and DA7-2 showed broad spectrum antimicrobial activity.

Key words: Desert actinobacteria, culturable, antimicrobial, cross streak method

INTRODUCTION

Screening of microbial natural products continues to represent an important route to the discovery of novel chemicals for the development of new therapeutic agents and for evaluation of the potential of lesser-known and/or new bacterial taxa [1]. Among the microorganisms, actinobacteria gain special importance, as they are the most potent source for the production of antibiotics and other bioactive secondary metabolites. It has been estimated that approximately one-third of the thousands of naturally occurring antibiotics have been obtained from actinobacteria [2]. The antibiotic resistance and decrease in the rate of discovery of new antimicrobial compounds draws the attention of scientists to try to investigate unexplored habitats for novel actinobacteria as possible candidates of new antimicrobials. The recent discovery of novel primary and secondary metabolites from taxonomically unique populations of extremophilic actinobacteria suggest that, these organisms could add a new dimension to microbial natural product research [3-6].

In Saudi Arabia, the desert habitats representing about 95% of the land and they are fewer study in actinobacteria research [7-9]. Therefore, we were interested to screen the Saudi Arabian desert actinobacteria as a new source for production of novel active compounds. The present investigation highlights the isolation of actinobacteria from the Saudi Arabian desert habitats and screening for their antimicrobial potential.

MATERIALS AND METHODS

Soil samples collection

Totally ten soil samples were collected from desert environment in and around Riyadh province, Saudi Arabia (Fig. 1). Samples were collected from the top 5 cm of soil using a sterile scoop and placed in sterile polyethylene bags. All the samples were labelled, sealed and brought to the laboratory. The samples were processed immediately on reaching the laboratory and permanently stored in refrigerator at 4°C.

Isolation of actinobacteria

Sample treatment

Heat treatments were performed for all soil samples. One hundred gram of soil samples were taken in sterile petri dishes separately. The samples were placed in hot air oven at 70°C for 10 min. The treated samples were used for actinobacteria isolation.

Media and culture condition

The medium used for actinomycete isolation and enumeration were starch-casein agar (SCA) the composition of the media (g/l): starch 10g, casein 0.3g, KNO_3 2g, NaCl 2g, K_2HPO_4 2g, $MgSO_4$-$7H_2O$ 0.05g, $CaCO_3$ 0.02g, $FeSO_4$-$7H_2O$ 0.01g and agar 18g [10] and minimal medium (MM) the following of the composition of media (g/l): glucose 0.5g, yeast extract 0.5g, K_2HPO_4 1g, $MgSO_4$-$7H_2O$ 0.5g, NaCl 0.5g, Agar 18g and microelement stock 1ml [11] were employed supplemented with

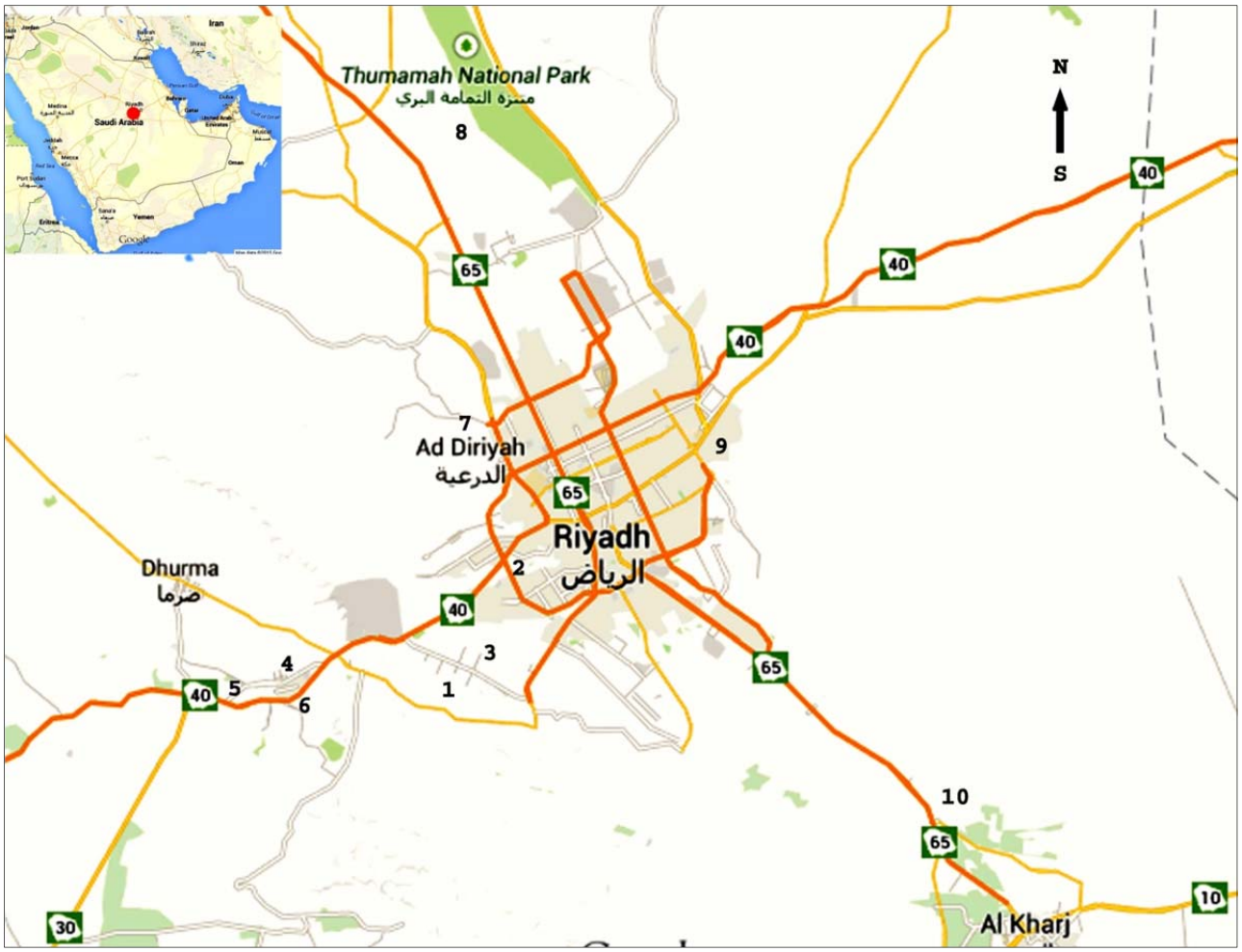

Fig. 1. A map showing the sampling stations

1 - Al-Qarinah, 2 - Al-Rabeya, 3 - Dahyat Namar, 4 - Hazlullah, 5 - Al-Muzahmiyah, 6 - Al-Uraija, 7 - Ad Diriyah, 8 - Thumamah, 9 - Janadriyah, 10 - Al-Kharj

Amphotericin B 20 µg/ml and Nalidixic acid 10 µg/ml to prevent the fungal and bacterial growth. The prepared mediums were sterilized at 121°C in 15 lbs pressure for 15 min and were poured into the sterile Petri dishes. The collected soil samples 5 g were taken in each sample separately in 100 ml Erlenmeyer flask containing 50 ml of distilled water and diluted upto 10^{-4}. From 10^{-3} and 10^{-4} dilution 0.1 ml of the sample was spread over the agar plates in triplicates. The inoculated plates were incubated at 28°C for seven to ten days. Numbers of presumptive actinobacteria and total culturable bacteria were counted and the results expressed as mean colony forming units (cfu) per gram dry weight soil. Stock cultures were prepared for each isolates by transferring mycelium and spores from each of the purified isolates into cryotubes containing 1.5 ml 20% (w/v) sterile glycerol solution [12] and stored at -20°C.

Screening of antimicrobial potential

Antimicrobial potential of actinobacterial isolates were determined by streak plate method, using the following test microorganisms obtained from the American Type Culture Collection (ATCC): *Klebsiella pneumoniae* ATCC 13882, *Enterococcus faecalis* ATCC 49532, *Candida albicans* ATCC 2091, *Saccharomyces cerevisiae* ATCC 9763, *Escherichia coli* ATCC 10536, *Proteus vulgaris* ATCC 33420, *Salmonella enterica* ATCC 13311, *Cryptococcus neoformans* ATCC 90113, *Pseudomonas aeruginosa* ATCC 27883, *Staphylococcus aureus* ATCC 6538P. Actinobacterial isolates were streaked across diameter on ISP 2 medium (g/l: Bacto yeast extract 4g, Malt extract 10g, Glucose 4g, Agar 20g). After incubation at 28°C for 7 days, 24 hrs cultures of pathogenic microorganisms were streaked perpendicular to the central strip of actinobacterial culture and the plates were again incubated at 37°C for 24 hrs and zone of inhibition was measured.

RESULTS AND DISCUSSION

Diversity of actinobacteria

In this study, totally two different types of media were used for enumeration of actinobacteria in desert soils. The average numbers of microbial colonies grown on the two different media are summarized in table 1. Actinobacteria counts ranged from a high of $21.8x10^3$ to a low of $1.6x10^3$ in SCA medium samples collected from Hazlullah and Al-Kharj respectively, whereas in MM medium the maximum actinobacterial counts recorded $8.7x10^3$ while the minimum $0.9x10^3$ recorded from Hazlullah and Janadriyah respectively (Table 1). For isolation of soil actinobacteria,

selection of media is important for understanding their ecological properties and for discovery of novel strains which can produce useful bioactive secondary metabolites. Hence, various media and techniques have been developed for selective isolation of actinobacteria in general, rare actinobacteria or certain genera [13-17]. Although, some work has been done previously on desert actinobacteria [18-20], and very limited studies have reported the effectiveness of special selective media for actinobacteria from desert habitats [21,22].

Hozzein et al. [21] reported that the minimal media was more selective for actinobacteria isolation compared than other media including soil extract and glucose yeast extract, and also the total actinobacteria showed its highest percentages in five out of the six studied sites on MM and it reached 60.91%. In this study, the maximum percentage of culturable actinobacterial colonies 26.20% in Hazlullah site on SCA medium, whereas 15.16% of actinobacterial colonies found in MM medium (Fig. 2). The results proved that starch casein agar was more suitable medium compared than minimal medium and also the results negatively correlated with previous study of Hozzein et al. [21].

Totally 134 morphologically distinguished culturable actinobacterial isolates were isolated from 10 different desert soil samples. The maximum 31 isolates were recorded from Ad Diriyah region when compared to other sampling sites. While the minimum number of isolates 4 was recorded from Janadriyah site (Fig. 3). The maximum actinobacteria percentage contribution 23.13 was also recorded in Ad Diriyah, while minimum 2.99% recorded in Janadriyah and remaining percentage contributed by the rest of the sites (Fig. 4).

Table 1. Population dynamics of Actinobacteria in desert soils

Medium	Dilution factors	[a] Sampling stations and number of colonies cfu/g in dw									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Starch Casein Agar	10^{-3}	8.0	20.5	8.8	21.8	10.3	2.2	8.9	2.5	1.9	1.6
	10^{-4}	2.5	9.8	6.2	12.0	6.6	2.0	3.4	1.2	1.2	0.5
Minimal medium	10^{-3}	2.0	7.5	2.3	8.7	4.5	1.0	2.5	1.5	0.9	1.2
	10^{-4}	1.0	3.0	1.5	3.5	2.8	0.8	1.8	0.8	0.6	0.2

[a] Sampling stations listed in table 1.

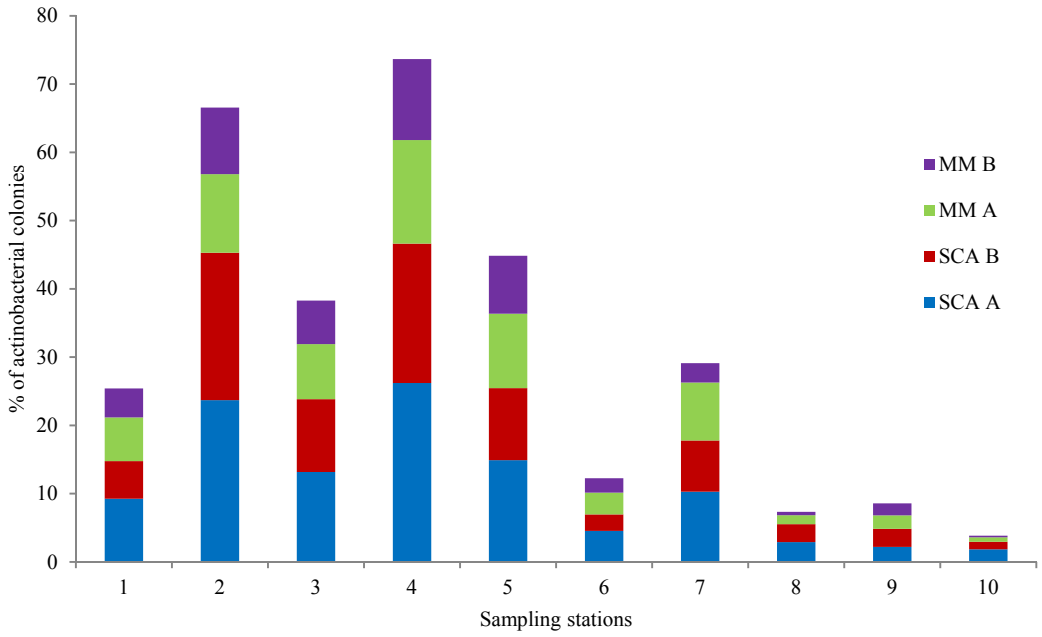

Fig. 2. Percentage of culturable actinobacterial count appeared in different media
SCA A – Starch Casein Agar 10^{-3}dilution, SCA B – Starch Casein Agar 10^{-4} dilution, MM A – Minimal medium 10^{-3}dilution, MM B – Minimal medium10^{-4} dilution.

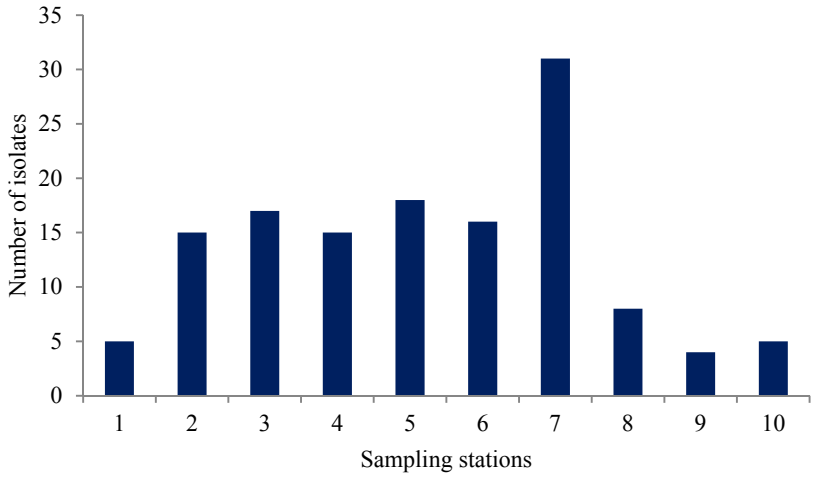

Fig. 3. Total number of morphologically distinguished culturable actinobacterial isolates from desert soil samples

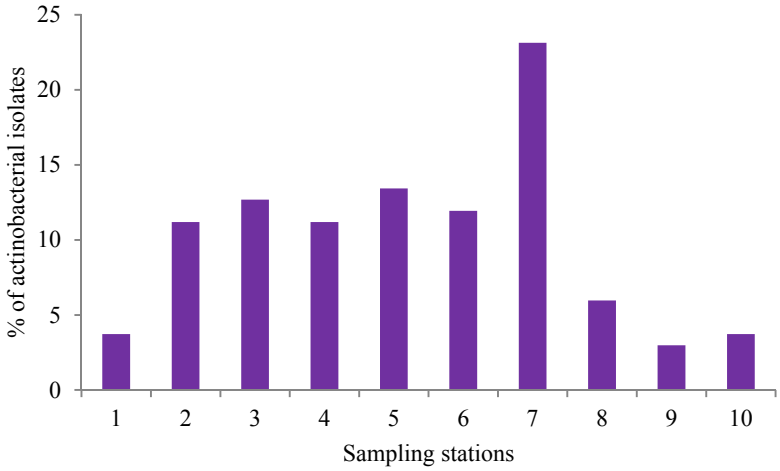

Fig. 4. Percentage of actinobacterial isolates in each station of desert soil samples

Table 2. Antimicrobial potential of actinobacterial isolates from desert soils of Saudi Arabia

Actinobacterial isolate	**[a]Test organisms and zone of inhibition (mm)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DA3-2	-	-	-	15±0.95	-	-	-	12±0.83	-	-
DA3-7	17±1.56	27±2.25	19±1.32	30±2.23	20±1.68	16±1.01	17±1.32	30±2.2	15±1.10	30±2.13
DA3-9	-	-	-	15±1.14	-	13±0.98	-	16±1.24	5±0.32	5±0.21
DA3-10	-	-	-	-	-	-	-	-	-	9±1.0
DA 3-12	15±1.67	28±2.01	15±1.18	19±0.78	12±0.59	12±0.34	15±1.22	20±1.92	10±0.12	30±1.98
DA3-16	-	-	-	-	-	-	-	20±1.21	-	-
DA5-1	-	-	-	-	-	-	8±0.57	-	-	-
DA5-4	-	-	-	-	-	-	-	10±0.34	-	-
DA5-5	-	-	-	25±1.85	-	-	-	12±0.59	-	-
DA5-6	-	-	-	-	-	-	-	13±0.54	-	-
DA5-7	-	-	-	25±1.45	-	-	-	17±1.23	-	-
DA6-1	-	-	-	30±2.34	-	-	-	8±0.57	-	-
DA7-1	-	-	-	24±1.79	-	-	-	10±1.0	-	-
DA7-2	17±1.21	10±1.0	16±1.23	25±1.76	15±1.05	-	17±1.43	18±1.90	10±0.32	14±1.01
DA7-4	-	25±1.89	20±1.53	30±2.35	-	-	-	30±2.12	-	-
DA7-12	-	-	-	-	-	-	-	9±0.80	-	-

[a] Test organisms :1- *Klebsiella pneumonia* ATCC 13882, 2- *Enterococcus faecalis* ATCC 49532, 3- *Candida albicans* ATCC 2091, 4- *Saccharomyces cerevisiae* ATCC 9763, 5- *Escherichia coli* ATCC 10536, 6- *Proteus vulgaris* ATCC 33420, 7- *Streptococcus typhimurium* ATCC 13311, 8- *Cryptococcus neoformans* ATCC 90113, 9- *Pseudomonas aeruginosa* ATCC 27883, 10- *Staphylococcus aureus* ATCC 6538P ; - no activity

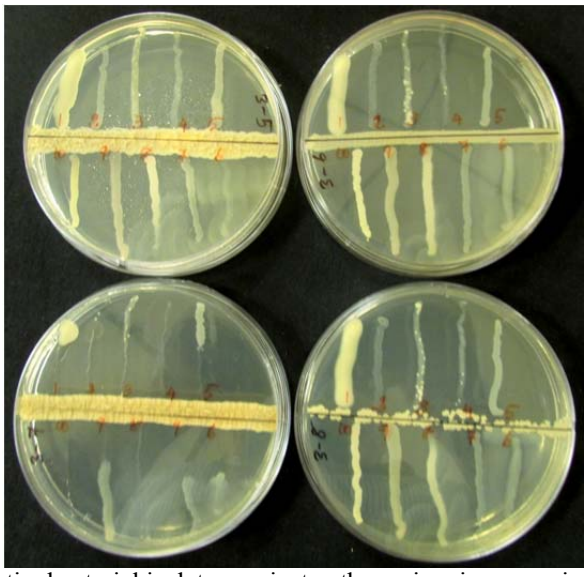

Fig. 5. Antimicrobial activity of actinobacterial isolates against pathogenic microorganisms by cross streak method
3-5, 3-6, 3-7, 3-8 actinobacterial isolates; 1-10 pathogenic microorganisms: 1- *Klebsiella pneumonia* ATCC 13882, 2- *Enterococcus faecalis* ATCC 49532, 3- *Candida albicans* ATCC 2091, 4- *Saccharomyces cerevisiae* ATCC 9763, 5- *Escherichia coli* ATCC 10536, 6- *Proteus vulgaris* ATCC 33420, 7- *Streptococcus typhimurium* ATCC 13311, 8- *Cryptococcus neoformans* ATCC 90113, 9- *Pseudomonas aeruginosa* ATCC 27883, 10- *Staphylococcus aureus* ATCC 6538P

Antimicrobial screening

Desert habitats are eminently suitable ecosystems for isolation of many novel actinobacteria which could be good source for potentially useful active metabolites and biotechnological applications [21]. In this study, totally 134 culturable actinobacteria were isolated and screened their antimicrobial potential by cross streak method. Altogether, only 16 isolates were exhibited the antimicrobial potential, while the remaining 118 isolates were not showed activity against pathogenic bacteria and yeast like fungi (Table 2).

The desert habitats in Saudi Arabia are one of the unexplored and inexhaustible resources for biotechnological applications. Although, very few studies on actinobacteria from Saudi Arabian deserts and their antimicrobial potential. Atta et al. [7] studied antimicrobial potential of 13 actinobacteria isolated from desert habitat of Al-khurmah Governorate, Saudi Arabia. Among them KH-4 isolate showed good antibacterial both Gram negative and Gram positive pathogenic bacteria and also unicellular and filamentous fungi. In the present study revealed that 16 actinobacteria isolates exhibited the antimicrobial activity, among them 6 isolates were potential against Gram-positive, 5 were Gram-negative and 14 were potential against yeast like fungi. However, only three isolates namely DA3-7, DA3-12 and DA7-2 showed broad spectrum antimicrobial activity including both Gram-positive and Gram-negative and also yeast like fungi (Table 2; Fig. 5). These results are very inspiring to continue screening more actinomycete isolates from the Saudi Arabian desert habitats and strongly support the idea especially actinobacteria from unexplored environments could be a very fruitful source of novel bioactive secondary metabolites.

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