JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2309-2314

Studies of Desert Actinomycetes on Water Agar Medium Isolated from Riyadh Desert Soil, Kingdom of Saudi Arabia

Ismet Ara

Department of Botany and Microbiology, College of Science,
King Saud University, P. O. Box 22452, Riyadh-11495, Kingdom of Saudi Arabia.

(Received: 22 May 2014; accepted: 29 June 2014)

In this study, 150 soil samples were collected from several locations of Riyadh desert areas in Saudi Arabia. Samples were prepared for isolation using the standard serial dilution method on tap water agar (TWA) medium. 150 isolates of actinomycetes, isolated from the samples were tentatively identified as streptomycetes based on their phenotypic and biochemical characteristics. All purified isolates exhibited a range of about 15 streptomycetes like color groups on yeast extract-starch soluble (YS) agar medium including grey series , white series, brown series and yellow series. Further, scanning electron microscopic studies of the strains showed the different morphology of the aerial mycelium on YS agar medium. These morphological structures and cell wall diaminopimelic acid test have tentatively proved that almost all the selected isolates were under the genus *Streptomyces* in the family *Streptomycetaceae*. This is the first intensive study and we confirmed that the desert soil of Saudi Arabia is good sources for the isolation of diverse streptomycetes. More intensive study will be conducted on the isolated streptomycetes strains to utilize as biocontrol agents and there is a probability of finding new streptomycetes species in unexplored soil samples in Saudi Arabia as indicated by its unique diversity.

Key words: Actinobacteria, Streptomycetes, SEM, Desert soil, Riyadh, Saudi Arabia.

Actinomycetes are one of the most attractive families of industrial bacteria on account of their superior potential for producing valuable secondary metabolites including antibiotics, anti-cancer drugs, immunosuppressors and enzyme inhibitors[1, 2]. Their population has been primarily identified as one of the major group of soil population. The species belonging to the genus *Streptomyces* are especially prolific and can produce many types of antibiotics and other class of biologically active secondary metabolites. *Streptomyces* constitute 50% of the total population of soil actinomycetes and 75-80% of the commercially and medicinally useful antibiotics have been derived from this genus[3]. The list of novel microorganisms and products derived from poorly explored areas of the world like China, Australia, Antarctica and Jordan suggests that a careful exploration of new habitats might continue to be useful[2]. The search for new antibiotics continues to be most important in research programs around the world because the increase of the resistant pathogens and toxicity of some used antibiotics[4]. Saudi Arabia is the largest country in the Arabian Peninsula, filled with sandy deserts, associated semi-desert and shrub land. Riyadh (24°38‘N46°43’E) is the capital, located in the central region and the largest city of Saudi Arabia. Summer temperatures are very hot, approaching 50 degrees Celsius. The average high temperature in July is 43.5°C. Winters are mild with

* To whom all correspondence should be addressed.
*Corresponding author: IsmetAra, E-mail: drmoonismet@gmail.com;
Phone (office): +966 1478 9585 Ext. 1639; Cell phone: +966534509242

cold, windy nights. The overall climate is arid, receiving very little rainfall, but the city receives a fair amount of rain in March and April. It is also known to have many dust storms. The dust is often so thick that visibility is under 10 meters. Hence the city is mostly covered with barren lands and desert areas which attracts the attention of researchers to search for different actinobacteria from desert and surrounding soil samples.

Materials and methods

Source of soil samples

Soil samples were collected in clean sterile plastic bags from various desert locations in Riyadh, Saudi Arabia (Figure 1a,b). Several diverse habitats in different areas were selected for the isolation of streptomycetes isolates. These habitats included the rhizosphere of plants and agricultural soil. The samples were taken from up to 20 cm depth, after removing approximately 3 cm of the soil surface[5,6,7].

Preparation of samples and actinomycetes isolation

The collected soil samples were sieved and air-dried at room temperature for seven days. The actinomycetes were isolated from the air dried desert soil samples by soildilution plate technique (Figure 2) on the modified TWA medium and incubated at 30°C for 3-4 weeks. The actinomycetes colonies were picked up and purified on yeast extract-soluble starch agar (YSA) medium without antifungal or antibacterial antibiotics[8].

Physiological and morphological characteristics

All selected isolates were examined for a range of standard physiological tests following established methods[9]. The cultural characteristics of the isolates were recorded as modified by Ara *et al*. (2011). The morphological features were observed after examining the cover slip cultures by the light microscope and later for fresh agar block using scanning electron microscope (SEM) (JEOL JSM-6060LV) (Figure 6) and the procedure modified by Ara *et al*.(2011).

Cell wall diaminopimelic acid (DAP) test

The isomers of diaminopimelic acid (DAP) in the cell wall peptidoglycan were determined by using TLC as described by Staneck and Roberts (1974).

RESULTS AND DISCUSSION

Isolation of actinomycetes

Gram-positive, filamentous, bioactive compound producing tentative streptomycetes group was studied in Riyadh and our data indicated that it is an eminently suitable ecosystem for diverse streptomycetes group. Actinomycetes counts ranged from 50×10^2cfu/g to 700×10^2cfu/g in the soil samples collected in Riyadh area (Figure 1 and 2). A total of 150 actinomycetes were isolated using simple dilution technique with normal saline (0.9% NaCl) on TWA medium (Figure 2).

Physiological, morphological and biochemical characteristics

All purified isolates were characterized physiologically and morphologically. Physiological characteristics were done by biochemical tests such as, fermentation of citrate, starch hydrolysis, gelatin hydrolysis, casein hydrolysis, milk coagulation, triple sugar iron test etc. (Table 1).

The streptomycetes isolates exhibited a range of diverse aerial mycelium colors (such as: grey, dark grey, pale grey, white grey, grey white, white, off white, chalk white, blackish grey, pink white, red white, brown red, dark brown, brown white, cream white, yellow and yellow white) on yeast extract-starch soluble agar medium (Figure 3).

The highest number of streptomycetes group was found in soil samples collected from Al-Thumama desert area, Riyadh. In all the 150 soil samples about 15 streptomycetes color series were observed and among them, grey and off white series represented the most dominant color group in our study. These diverse color groups showed high possibility of the different streptomycetes in the desert soil samples. Our finding is in concordance with Dhanasekaran *et al*. (2008) and they reported high diversity of *Streptomyces* population isolated from agricultural soils.

Morphological observations were made using scanning electron microscopy and light microscopy and the cultural, physiological data tentatively confirmed that almost all the selected isolates belonged to the dominant genus *Streptomyces* in the family *Streptomycetaceae* (Figure 4). The thin layer chromatography results (LL-DAP) of the cell wall diaminopimelic acid for

Table 1. List ofdifferentstreptomycetes-like actinomycetesbased on physiological, biochemical characteristics and colony coloras cultural characteristics

*Isolate code no.	Citrate utilization	Urease production	Casein hydrolysis	[a]Milkpe-ptoni zation	Gelatin lique faction	[b]Lactose ferme ntation Kligler Iron in KIA	[b]Glucose fermen tation in KIA	H_2S prod-uction in KIA	Growth on 6% NaCl	Growth on 7% NaCl	Colony color (aerial mycelium color)
**DS-1(20)	+	+	+	+	-	-	-	-	+	+	Pale grey white (cream brown)
DS-1(21)	-	-	+	+	+	-	-	-	+	±	Grey white (red brown)
DS-1(22)	+	+	+	+	+	-	-	-	+	+	Pale grey white (yellow)
DS-2(7)	+	-	+	+	+	+	-	-	+	+	Off white (cream brown)
DS-6(5)	+	+	+	+	-	-	-	-	+	+	Pale grey (pale brown)
DS-6(32)	±	+	+	+	+	-	-	-	+	+	Dark grey (dark brown)
DS-6(33)	+	+	+	+	+	+	+	-	+	+	Dark grey white (dark brown)
DS-6(34)	-	+	+	+	+	+	+	-	+	+	Off white (yellow)
DS-6(48)	-	+	+	+	+	-	-	-	+	+	Dark grey white (dark brown)
DS-6(51)	+	+	+	+	+	-	-	-	+	+	Pale grey (red brown)
DS-8(6)	-	±	+	+	+	-	-	-	+	+	Off white (grey brown)
DS-8(7)	+	-	+	-	+	-	-	-	+	+	Off white (cream)
DS-8(14)	-	+	+	+	+	-	-	-	+	+	Off white (cream)
DS-8(18)	+	+	±	±	-	-	-	-	+	+	Pale grey (grey brown)
DS-8(23)	+	+	+	±	+	-	-	-	+	+	Grey (cream)
DS-8(24)	+	+	+	+	+	+	+	-	+	+	Grey white (grey brown)
DS-8(32)	+	+	+	±	+	-	-	-	+	+	Dark brown (grey)
KSU-2(1)	-	-	+	+	+	-	-	+	-	-	Off white (pink red)
KSU-2(3)	-	-	+	±	-	-	-	+	+	±	Pale grey (yellow)
KSU-2(12)	+	-	+	+	+	-	-	+	±	-	Pale grey white (pale orange brown)
KSU-2(14)	-	-	-	+	-	-	-	+	±	-	Grey (yellow)
KSU-2(16)	+	+	+	+	+	+	+	+	±	-	Grey white (orange brown)
KSU-2(18)	+	±	+	+	+	-	-	+	±	-	Grey (brown)
KSU-2(20)	+	+	+	+	-	+	+	-	+	+	Pale grey (yellow)
KSU-2(29)	-	+	+	±	+	+	+	-	+	+	Pale grey white (yellow)

*Growth of all the 26 strains is positive with 0-5% NaCl, pH 4.8, 5.8, 6.3, 7.5, 8.0; 25oC, 28oC, and 37oC; carbon sources galactose, xylose, starch, fructose, glycerol, glucose, sucrose, mannitol; **, Growth of all the 28 strains is negative with 4oC, 10oC, 50oC temperature ** DS, Al-Thumama desert soil; KSU, King Saud University, Malaz

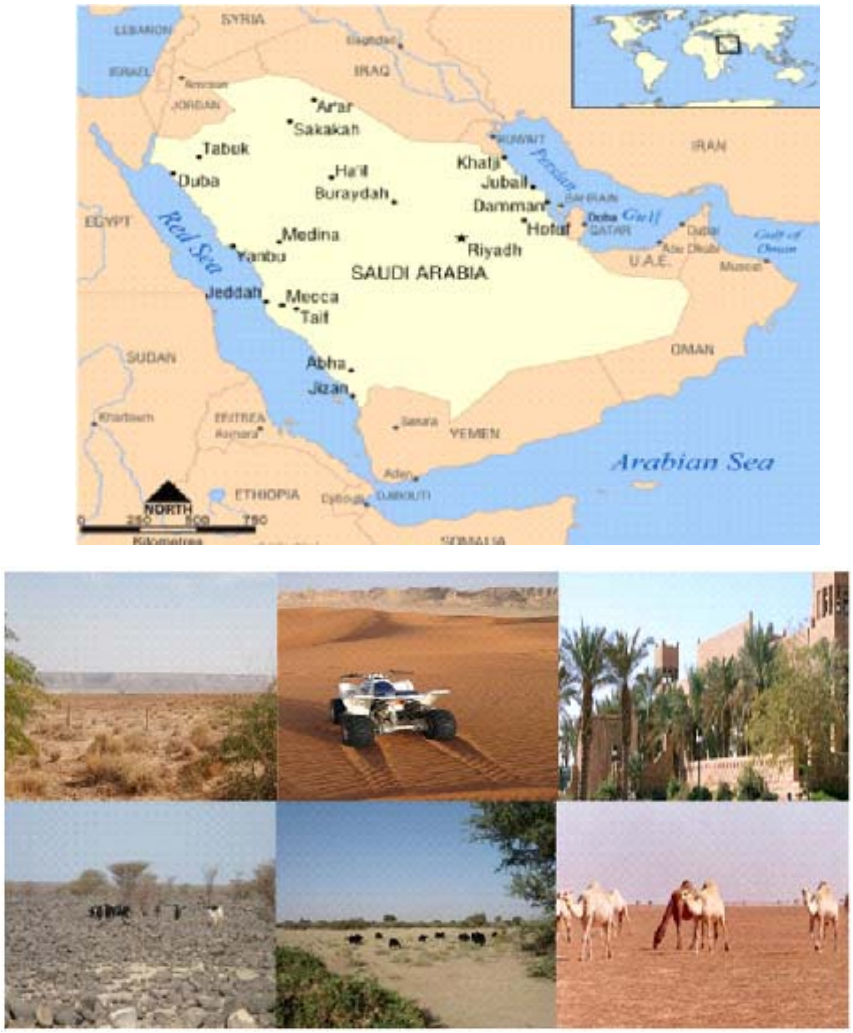

Fig. 1. (a)Map of Kingdom of Saudi Arabia and (b) different sampling sites in Riyadh areas and surroundings.

Fig. 2. Flow chart for the isolation of actinomyceteson tap water agar (TWA) from Riyadh soil samples

Fig. 3. Desert streptomycetes isolates based on the cultural characteristics (by the production of whitish, grey, blackish, chalky white and red white powdery aerial mycelium) purified on yeast extract-starch soluble agar medium at 30°C for 3 weeks.

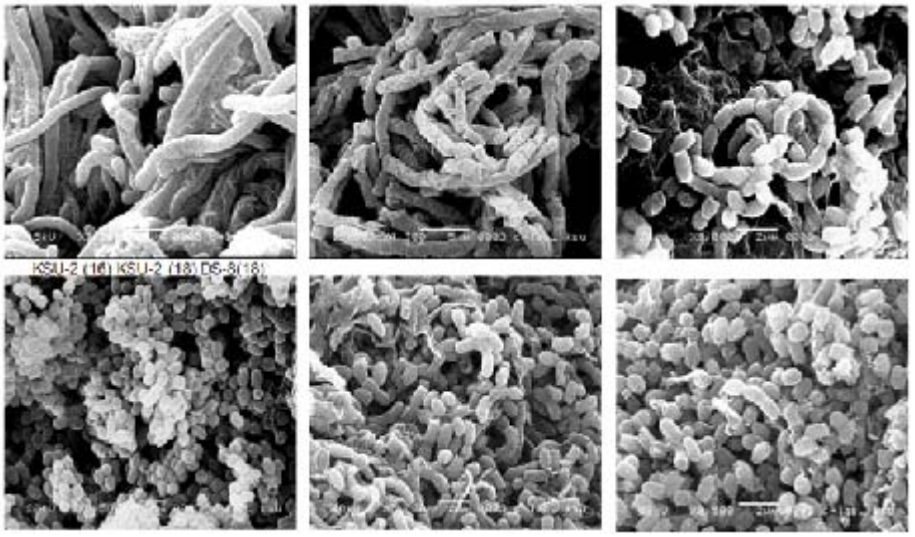

Fig. 4. Scanning electron microscopic (SEM) photographs of characteristics spore chain formation of selected diversestreptomycetesisolates grown on yeast extract-starch agar medium at 28° C for 3 weeks

the selected strains showed that almost all the strains belong to the group streptomycetes.

Isolation of streptomycetes group from plant rhizosphere and agricultural soils have been reported elsewhere. Many of those isolates showed bioactive compound activity also enzyme and hormone secretions[10-18]. Nonetheless, our isolates need to be studied intensively for the potential of bioactive compound production against pathogenic microorganisms.

CONCLUSION

The desert habitats in Saudi Arabia can be considered as an inexhaustible resource for biotechnology that has not been well exploited. Although, previous studies on actinomycetes isolated from the Riyadh deserts are very few, their antimicrobial potential was encouraging. The streptomycetes group from our isolates showed high color diversity on tap water agar medium. More

intensive study need to be conducted on the streptomycetes isolates to utilize as biocontrol agents and there might a new probability of finding new species in unexplored Riyadh desert soil samples in the Kingdom of Saudi Arabia as indicated by its unique diversity.

ACKNOWLEDGMENTS

The research project was supported by a grant from the "Research Center of Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

REFERENCES

1. Olano, C., Me´ndez, C., Salas, J.A.Antitumor compounds from marine actinomycetes. *Marine Drugs* 2009; **7**: 210-248.
2. Zitouni, A., Boudjella, H., Mathieu, F., Sabaou, N., Lebrihi, A.,Mutactimycin, P.R.A new anthracycline antibiotic from *Saccharothrix* sp. SA 103. I. Taxonomy, fermentation, isolation and biological activities. *J Antibiot*. 2004; **57**:367-372.
3. Lazzarini, A., Cavaletti, L., Toppo, G.,Marinelli, F.Rare genera of actinomycetes as potential producers of new antibiotics. *Anton. Van. Leeuwen.*, **78**:399-405.
4. Takahashi, Y. Exploitation of new microbial resources for bioactive compounds and discovery of new actinomycetes. *Actinomycetologica*, 2004; **18**: 54-61.
5. Ara, I., Rizwana, H., Al-Othman M.R., Bakir, M.A.Studies of actinomycetes for biological control of *Colletotrichum musae* pathogen during post-harvest anthracnose of banana.;*Afr. J.Microbiol*. 2012a; **6**(17): 3879-3886.
6. Ara, I., Bukhari, N.A., Aref, N.M., Shinwari, M.M.A., Bakir, M.A. Antiviral activities of streptomycetes against tobacco mosaic virus (TMV) in Datura plant: Evaluation of different organic compounds in their metabolites. *Afr. J.Biotechnol*., 2012b; **11**(8): 2130-2138.
7. Ara, I., Rizwana, H., Al-Othman M.R., Bakir, M.A.Antagonism of actinomycetes against *Pestalotiopsis mangiferae*, causal agent of mango brown rot in post-harvest storage. *Afr. J.Microbiol. Res.*, 2012c; **6**(8):1782-1789.
8. Luedemann, G.M. *Micromonospora purpureo chromogenes* (Waksman and Curtis 1916) comb. nov. (Subjective synonym *Micromonospora fusca* Jensen 1932). *Int. Assoc.Microbiol. Soc*., 1971; **21**(3):240-247.
9. Williams, St., Goodfellow, M., Wellington,Emh., Vickers, J., Aldersong, C., Sneath, P.H.A., Sackinm, J., Mortimera, M. A probability matrix for identification of some streptomycetes. *J. Gen.Microbiol*., 1983; **129**: 1815-1830.
10. Ara, I., Paramaswari, S.,Vikineswary, S. Diversity of *Micromonospora* in Malaysian mangrove rhizosphere soil.; *Malaysian J. Sci*., 2002; **21**:51-59.
11. Ara I, Vikineswary S, Paramaswari S, Wong WH, Ward A, Seki T, Fiedler HP,Goodfellow M. Production and chemical characterization of antifungal metabolites from *Micromonospora* sp. M39 isolated from mangrove rhizosphere soil. *World J Microbiol Biotechnol*, 2002; **20**:523-528
12. Ara I, Tsetseg B, Daram D, Ando K. *Actinophytocola burenkhanensis* sp. nov isolated from soil of Mongolia. *Int J Syst Evol Microbiol;* 2011; **6**: 1033-1038
13. Ara I, Bukhari NA, Wijayanti DR, Bakir MA (2012d) Proteolytic activity of alkaliphilic, salt-tolerant actinomycetes from various regions in Saudi Arabia. *Afr J Biotechnol*; **11**(16):3849-3857
14. Dhanasekaran D, Thajuddin N, Panneerselvam A. Distribution and ecobiology of antagonistic streptomycetes from agriculture and coastal soil in Tamil Nadu India. *J Culture Collections*,2008; **6**:10-20
15. Jeffrey LSH. Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok Sarawak. *Afr JBiotechnol*, 2008; **7**(20): 3697-3702
16. Loqman S, Bouizgarne B, Barka EA, Clément C, Von Jan M, SpröerKlenk HP, Ouchdouch Y. *Streptomyces thinghirensis* sp. nov., isolated from rhizosphere soil of *Vitis vinifera*. *Int J Syst Evol Microbiol*, 2009; **59**: 3063-3067
17. Khamna S, Yokota A, PeberdyJF, Lumyong S. Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. PMID 20511457 2010.
18. Intra S, Mungsuntisuk I, Nihira T, Igarashi Y, Panbangred W., Identification of actinomycetes from plant rhizospheric soils with inhibitory activity against *Colletotrichum* spp. the causative agent of anthracnose disease. *BMC Research Notes* 2011; **4**: 98.