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Antibacterial Activity of the Actinomycetes Isolated from the Plant Rhizosphere Soil in Riyadh Desert Region of Saudi Arabia

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Antibacterial activity of actinomycetes isolated from Riyadh desert area of Saudi Arabia was studied. A total of 130 actinomycetes were subjected to primary screening by perpendicular streak method against human pathogenic Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923) and Gram-negative (*Salmonella suis* ATCC 13076, *Pseudomonasaeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigella sonnei* ATCC 11060) bacteria and yeast like human pathogenic fungus *Candida albicans* ATCC 10231. Preliminary screening test showed that about 100 isolates were active against one or more examined test pathogens. All isolates were subjected to secondary screening by agar well diffusion method. Finally 28 isolates were selected for further study on the basis of (a) broad spectrum activity and (b) larger zone of inhibition (>20 mm) in comparison to others. The result of primary and secondary screening revealed that most of the potent isolates were active against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) compared with gram negative bacteria (*Salmonella suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigella sonnei* ATCC 11060). Our results indicated that there is a great probability of finding new compounds in unexplored region like desert area of Riyadh, Saudi Arabia because of its wide and very unique diversity of anbioactive compounds producing genus *Streptomyces* in the family Streptomycetaceae. Therefore, further research should be done to explore the new antibiotics from the new species of the producing organism.

Key words Actinomycetes, *Streptomyces*, Isolation ,Screening, Antibiotics, Active compounds, Antibacterial, Antifungal.

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil (Oskay *et al.*, 2004). They have providedmany important bioactive compounds of high commercial value and continued to be routinely screened for new bioactive compounds. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics, including many of medically important compounds have been isolated fromactinomycetes (Okami *et al.*, 1988). Almost 80% of the world'santibiotics are known to come fromactinomycetes, mostly from the genera*Streptomyces* and *Micromonospora*(Pandey *et al*., 2004; Baltz , 2005 ; Baltz , 2007). Majority of the actinomycetes in soil that arepotential drug sources remain uncultivable, and therefore inaccessible for novel antibiotic discovery. Although soils have been studied and screened by the pharmaceutical industry for about 50 years, only a small fraction of the surface of the globe has been sampled, and only a small fraction of actinomycetes taxa has been discovered. Goodfellowreviewed the literature on isolation of actinomycetes and suggested that only 10% of the actinomycetesare isolated from nature (Goodfellow *et al.*, 1984). Most of the antibiotics

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inuse today are derivatives of natural products of actinomycetes and fungi (Butler andBuss, 2006; Newman andCragg, 2007).

The Kingdom of Saudi Arabia is one of the biggest Arabian countries and recently, the attention of researchers have been directed towards this area in search for bioactive compound producing actinomycetes from desert and surrounding soil samples(Al-askar *et al* 2011; Al-kahtani *et al* 2011; Ara *et. al* . 2012).

According to the World Health Organization, over-prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens. Nowadays, the drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics. Because of this, many scientists and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from different untouched habitats, for the production of antibiotics (Oskay *et al.*, 2004). Serious infections caused by bacteria have become resistant to commonly used antibiotics and have also become a major global healthcare problem in the 21st century (Alanis, 2005). *Staphylococcus aureus*, for instance, a virulent pathogen that is responsible for a wide range of infections, has developed resistance to most classes of antibiotics (Enright, 2003). Clinicians and public health officials have faced hospital acquired drug resistant *S. aureus*, which also bears resistance to many antibiotics. Hence there is need to rediscover new drugs active against these drug resistance pathogens. Therefore, we were interested in screening the Riyadh desert actinomycetes as a new source for production of novel active compounds.

MATERIALS AND METHODS

Soil sample collection

Soil samples were collected from different parts of Riyadh desert area of Saudi Arabia. Each collection was made from 10-15 cm depth of the plant rhizosphere soil (Saadoun and Gharaibeh, 2003). These were air-dried for 1 week (Williams *et al*, 1972) crushed and sieved. The sieved soils were then used for actinomycete isolation.

Soil preparation and the isolation of desert actinomycetes

The desert actinomycetes were isolated from the air dried desert soil samples by soil dilution plate technique on the minimal medium (MM) recommended by Hozzein *et al*. (2008) for isolation of actinomycete strains from the desert environments and incubated at 28C° for 3-4 weeks. Then the colonies were purified on yeast extract-starch agar without antifungal or antibacterial antibiotics. The selected strains were examined for a range of standard established methods tests (Williams *et al*., 1983). The cultural characteristics of the strains were recorded as modified by Ara *et al*(2007; 2008; 2010). The morphological features were observed by the light and scanning electron microscopes (JEOL JSM-6060LV) (Fig. 6) and the procedure described by Ara *et al* (2008; 2010; 2011).

Antibacterial compound extraction

The 13 strains of actinomycetes producing antibacterial compound were inoculated into flasks containing SGY broth (10g starch soluble,10g glucose, 10g glycerol , 2.5g corn flower, 5g peptone, 2g yeast extract, 3g $CaCo_3$ and lL distilled water (Ara *et al*.2002,2003) and incubated at 37°C in a rotary shaker for 10 days.The antibacterial compound was recovered from the broth by solvent extraction method following the process described by (Westley *et al*., 1979; Liu *et al*., 1986)in which methanol was added to the flasks containing the mixture ofstrains and broth in the ratio of1:1 (v/v),after which were returned to the shaker for 24 hours then filtered to separate the mycelium from the liquid.

The methanol phase that contained the antibiotic was separated from the aqueous phase by evaporation to dryness in hot air oven and two drops of distilled water was added to the residue obtained. Thus obtained crudeextract was used to determine antimicrobial activity.

Screening for antimicrobial activities

The screening method consisted of two steps; Primary and secondary screening.

All130 selected pure actinomycetes were subjected to primary screening by perpendicular streak method (Egorov, 1985) (Fig. 1) against human pathogenic Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923) and Gram-negative (*Salmonella suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigella sonnei* ATCC 11060) bacteria and human pathogenic

fungus *Candida albicans* ATCC 10231. Secondary screening was performed by agar well method for which the antimicrobial activity of the crude extract was tested by agar well diffusion method assay on the same test pathogens. The presence of inhibition zones around the active compound(s) was determined.

RESULTS AND DISCUSSION

In preliminary screening (Fig. 1), out of the isolated 130 organisms, 100 (77%) showed activity against the used test organisms. In the secondary screening (Fig.2) the antimicrobial activities of the selected 28 active desert actinomycete strains were classified into 7 groups according to their spectrum of activity on different test organisms and it was found that allof the active isolates have activity against *B. subtilis*,75% have activity against *S. aureus*, 71% have activity against *C. albicans*, 53% against *S. sonnei*, 46% against *P. aeruginosa*, 39% against *S. suis* and 25% against *E. coli* (Fig. 3). The result of primary and secondary screening reveals that most of the potent isolates were active against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) compared with gram negative bacteria (*Salmonella suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigella sonnei* ATCC 11060). All the strains were identified by studying their morphology, physiological and biochemical characteristics and were consistent with the classification to genus *Streptomyces* (Fig. 4; Table 1).

Fig.1. Primary screening by perpendicular streak method (a) control plate, (b) and (c) test plates

Fig. 2. Secondary screening of the potent bioactive actinomycetes by agarwell diffusion method

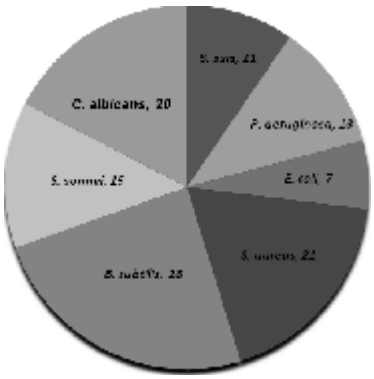


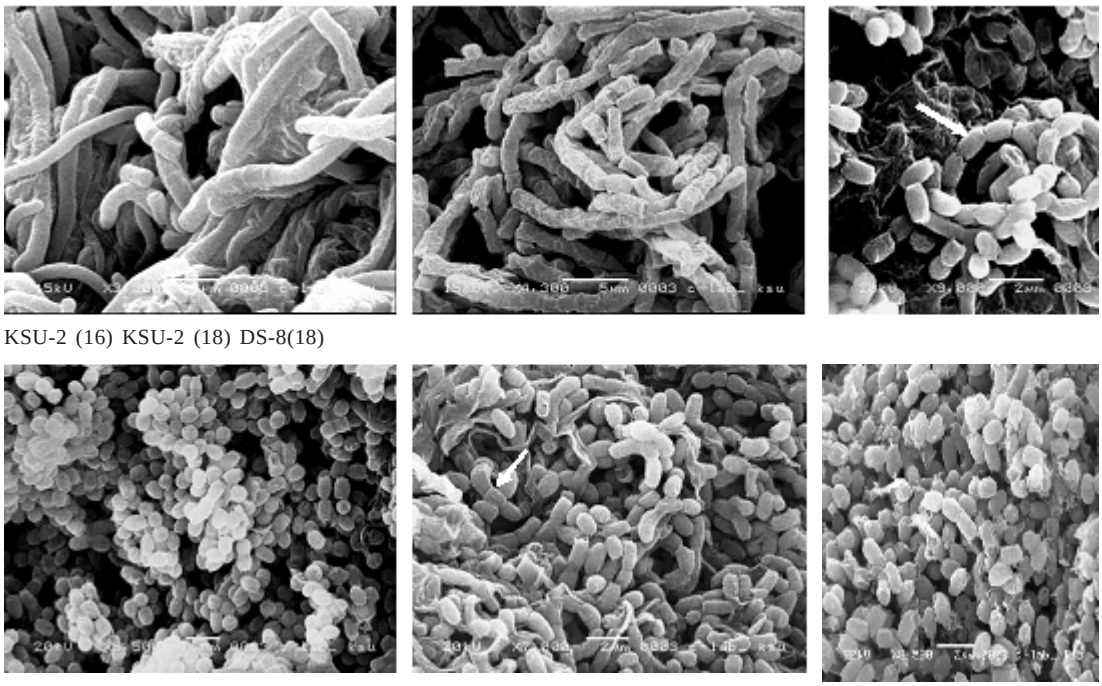
Fig. 3. Number of the active desert actinomycetes isolates according to their activity against test pathogens using crude extract agar well diffusion method

Isolation of *Streptomyces* species from Riyadh and its antimicrobial activity had been reported. Al-askar *et al* (2011) reported *Streptomyces spororaveus* RDS28, isolated from Riyadh Region. The strainshowed antifungal activities against *Rhizochtonia solani, Fusarium solani, Fusarium verticillioides, Alternaria*

Table 1. List of different potent desert actinomycetes based on physiological, biochemical characteristics and colony color

*Strain code no.	Citrate utilization	Urease production	Casein hydrolysis	[a]Milk peptonization and coagulation	Gelatin liquefaction	[b]Lactose fermentation in Kligler Iron	[b]Glucose fermentation in KIA Agar (KIA)	H_2S production in KIA	Growth on 6% NaCl	Growth on 7% NaCl	Colony color (substrate 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 28 strains is positive with 0-5% NaCl, pH 4.8, 5.8, 6.3, 7.5, 8.0; 25°C, 28°C, and 37°C; carbon sources galactose, xylose, starch, fructose, glycerol, glucose, sucrose, mannitol; **, Growth of all the 28 strains is negative with 4°C, 10°C, 50°C temperature ** DS, Al-Thumama desert soil; KSU, King Saud University, Malaz



KSU-2 (16) KSU-2 (18) DS-8(18)

KSU-2 (29) DS-8 (32) DS-8(24)

Fig. 4. Scanning Electron Microscopic photographs of selected potent strains grown on Yeast extract-Starch agar at 28 C° for 3 weeks (arrows indicate spore formation)

*alternate and Botrytis cinerea.*Other report from Saudi Arabia (Al-kahtani *et al*, 2011) noted that *Streptomyces*spp. isolated from Riyadh Region had antimicrobial and inhibitory activity against pathogenic and toxic fungi.

CONCLUSION

The desert habitats in Riyadh, 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 Saudi Arabian deserts are very few, their antimicrobial potential was encouraging. In the present study, the results showed that 77% of the isolated desert actinomycetes are biotechnologically active. These results are very encouraging to continue screening more actinomycete strains from the desert habitats and strongly support the idea that species of actinomycetes from underexploited environments could be a very fruitful source of novel bioactive secondary metabolites.

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