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CHARACTERIZATION OF STREPTOMYCETES HAVING ANTIBIOSIS ACTIVITIES ISOLATED FROM SOIL IN WESTERN REGION OF KSA

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ABSTRACT

In this study we are focused on the color groups of actinomycetes, in particularly streptomyctes, in soil of western region of KSA. Therefore, we collected soil samples from different climate locations in KSA (Taif, Makah and Jeddah).The color groups of the isolated actinomycete isolates were determined. The antagonistic activities of the isolated actinomycetes were also tested against seven microorganisms including, bacteria and fungi. The highest active isolates were identified as strains of *S. polychromogenes* (isolate 08), *S. chattanoogensis* (isolate 14), *S. lucensis* (isolate 20), *S. violaceus* (isolate 21), *S. violans* (isolate 32), *S. griseorubiginosus* (isolate 34), and *S. antibioticus* (isolate 35). It was show that the 7 selected streptomycete isolates were able to grow in the presence of 7% NaCl in the starch nitrate agar medium. At concentration of 10.5% NaCl, four isolates grew with weak growth (+) and three isolates showed in-doubt growth (±).

Keywords: Actinomycetes, *Streptomyces*, Identification, Taif, KSA

INTRODUCTION

Streptomycetes are widely distributed in terrestrial and aquatic habitats. Soil, fodder and composts appear to be the primary reservoirs for streptomycetes. Indeed, it appears that streptomycetes exist in soil for long periods as resting arthrospores that germinate given the occasional presence of exogenous nutrients (Mayfield *et al.*, 1972). It is interesting that *Streptomyces* strains continue to provide a larger number and wider variety of new antibiotics than any other actinomycete genus, suggesting that substantial numbers of *Streptomyces* species or strains with novel antibiotic productivity exist in nature (El-Nasser *et al.*, 2010; Baskaran *et al.*, 2011 and Hozzein *et al.*, 2011).

Qiu *et al.* (2009) reported that the genus of *Streptomyces*, a saprophytic Gram-positive bacterium, has properties, which make them useful as pharmaceutical and biocontrol agents. These Gram-positive filamentous bacteria exhibit a broad spectrum of antimicrobial activity against fungi and bacteria (HongJian *et al.*, 2009 and Singh *et al.*, 2009). In several studies actinomycetes from cultivated fields were found to be antifungal agents antagonistic towards many different fungal pathogens (Mansour and Mohamed, 2006; El-Nasser *et al.*, 2010 and Atta *et al.*, 2011).

In Egypt, eighty five halotolerant actinomycete isolates were isolated by Saleh *et al.* (1990) from different marine and lakes ecosystems. These isolates were greatly varied in their salt tolerance range from 0.05 to 20%. Four out of the seven *Streptomyces* strains appeared high antagonistic activity against 12 test microorganisms used (Zaki *et al*., 1993). The test microorganisms were representative fungi, i.e., *Fusarium oxysporum F.* sp. Lycopersci-123, *Rhizoctonia solani, Alternaria solani* and *Helminthosporium gramenium*-133; yeast, i.e., *Candida albicans* CAIM-352 and *C. tropicalis* CAIM-2 and bacteria, i.e., *Bacillus cereus*-1283, *B. megaterium*-1066, *B. mycoides*-1084, *B. subtilis*-1007; *Escherichia coli*-1319 and *Staphylococcus aureus* coagulase+ve.

El-Abyad *et al*. (1993) designed an *in vitro* and *in vivo* investigation to explore the potential of microbial antagonism in the control of some tomato diseases including bacterial, *Fusarium* and *Verticillium* wilts; early blight; bacterial canker. They used *Streptomyces pulcher, S. canescens* and *S. citreofluorescens*. The *in vitro* studies showed that an 80% concentration of the culture filtrate of either *S. pulcher* or *S. canescens* significantly inhibited spore germination, mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici, V. alboatrum* and *Alternaria solani*.

The goal of this study could be summarized in paying an attention to the color groups of actinomycetes, in particularly streptomyctes, in soil of western region of KSA and their antagonistic activity.

MATERIALS AND METHODS

Collection of soil samples: To reach such aim, rhizosphere and non-rhizosphere soils were collected from east, north, middle, south and west regions of Jeddah, Makah and Taif area. At each location, soil samples were randomly collected from five sites in sterile bottles and thoroughly mixed together to form one representative sample. All samples were subjected to microbiological analyses.

Determination of microbial total count: Microbiological analyses include the determinations of total microbial counts (Jacobs and Gerstein, 1960), fungal counts (Abou-Zeid and El-Fattah, 2007) and actinomycete counts (Waksman and Lechevalier, 1961) were carried out.

Isolation and purification of actinomycetes: Starch nitrate agar medium (Waksman and Lechevalier, 1961) was used for the isolation, purification and maintenance of actinomycetes existing in all tested samples. Isolation of actinomycetes was carried out by plate technique. Inoculated plates were incubated at 30±2°C for 7 days.

Antimicrobial activities of actinomycetes: Antimicrobial activities of selected Streptomycetes against pathogenic bacteria (*Sallmonella* sp. and *Staphylococcus aureus*); non-pathogenic bacteria (*E. coli*, Sarcina sp. and *Micrococcus* sp.) and two fungi (*Aspergillus* sp. and *Alternaria* sp.) were carried out. These test organisms were kindly provided by Department of Biology (Girls Branch, Qarwah), Faculty of Science, Taif University.

Standard inoculums (Standard inoculums containing 1.5 mil spores $ml^{-1)}$ for each tested streptomycete strain was prepared by scraping the heavy spores from the surface of the growth of starch casein slant. in the presence of 5 ml sterilized distilled water. An aliquot of 2 ml of this standard inoculums was transferred aseptically to 50 ml starch nitrate medium (Waksman and Lechevalier, 1961) in 250 ml conical flask. Inoculated plates were incubated at 30±2°C for 6 days on rotary shaker (130 rpm^{-1}). Thereafter, growth was centrifuged under aseptic condition; 0.1 ml of the supernatant was used as a source containing antimicrobial substances.

Strains of bacteria were cultivated on nutrient agar medium (Jacobs and Gerstein, 1960) and fungi on potato glucose agar medium (Waksman and Lechevalier, 1961). Antagonistic activity was determined by measuring the inhibition zones (mm) using the diffusion methods as described by British Pharmacopoeia (1968). To serve as control, 0.1 ml of uninoculated starch nitrate broth was poured in other holes for each culture.

Identification of the selected streptomycetes having antibiosis activities: Of these selected isolates, streptomycete isolates having antibiosis activities were selected and further identified up to species according to keys proposed by Bergey's Manual of Determinative Bacteriology (1974). Media as well as methods used in these keys were described by Shirling and Gottlieb (1966). Identification was based on cultural, morphological and physiological characteristics as described by Mohamed *et al*. (2000).

The selected streptomyces isolates having antibiosis activities against the pathogenic as well as non-pathogenic microorganisms were tested for their abilities to grow at increasing salt concentrations of 0.05 (normal salt concentration of the medium), 3.5, 7.0, 10.5, and 14.0% salt, NaCl) using starch nitrate agar medium (Waksman and Lechevalier, 1961). Inoculated plates were incubated at 30±2°C up to 14 days to insure the growth of tested isolates. The growths of streptomycete isolates on media with and without NaCl were recorded as described by Mohamed *et al.* (2000).

RESULTS

Collection of soil samples: A number of 31 soil samples were collected from three locations (Table- 1). Five soil samples from each of rhizosphere and non-rhizosphere were collected from Taif and Makah areas. While 5 and 6 soil samples from rhizosphere and non-rhizosphere were collected from Jeddah, respectively.

Table -1: Source and types of soil samples used for isolation of actinomycetes.

Regions	Type of soil samples	
	Rhizosphere (R)	Non-rhizosphere (NR)
	Total number of soil samples	Total Number of soil samples
Jeddah	5	6
Makah	5	5
Taif	5	5
Subtotal	15	16
Total	31	

R: Rhizosphere. **NR: Non-rhizosphere.**

Determination of microbial total count: Data showed that the total counts of bacteria were the highest followed by actinomycetes and fungi in the majority of soil samples. The rhizosphere soil samples appeared total counts higher that non-rhizosphere soil samples.

Isolation and purification of soil-actinomycete isolates: A number of 40 actinomycete isolates were obtained (19, 14 and 7 from Jeddah, Makah and Taif respectively). As interestingly, 20 isolates were obtained from each of rhizosphere and non-rhizosphere soils. All actinomycete isolates were purified and maintained on starch nitrate agar medium containing 3.5% NaCl. The isolates were found to belong to genus Streptomyces; they were classified into groups according to the same aforementioned key.

Distribution of color groups of isolated streptomycetes: The streptomycete isolates were divided based on their serial color groups to 22, 8, 7, 2 and 1 isolates belonging to gray (55%), violet (20%), white (17.5%), red (5%) and blue (2.5%) color series groups, respectively (Table-2). Data also show that no red series isolates were obtained from Taif and Jeddah soil samples. The blue color series group was found only from Jeddah soil samples. The streptomycete isolates represented 47.5, 35.0 and 17.5% for the soil samples collected from Jeddah, Makah and Taif, respectively.

Table-2: Distribution of color groups of streptomycetes isolated from Jeddah, Makah and Taif at western region of KSA.

Color series groups		Western regions, KSA			Total	
		Jeddah	Makah	Taif	No.	%
Gray		11	9	2	22	55.0
Red		0	2	0	2	05.0
Violet		4	1	3	8	20.0
White		3	2	2	7	17.5
Blue		1	0	0	1	02.5
Total streptomycete isolates	No.	19	14	7	40	
	%	47.5	35.0	17.5	100	

Determination of antagonistic activities of the isolated streptomycetes: Data in **Table -3** showed that the actinomycete isolates were varied in their antagonistic activities, as 2, 9, 7, 8, 10, 3 and 1 Streptomycete isolates were active against 7 (100%), 6 (85.7%), 5 (71.4%), 4 (57.1%), 3 (42.9%), 2 (28.6%) and 1 (14.3%) test organisms. Only 7 isolates (8, 14, 20, 21, 32, 34 and 35) were selected for further studies. Results are illustrated in **Figures 1-3.**

Table -3: Antibacterial and antifungal activities of streptomycete isolates obtained from soils of western region, KSA after 48 h from incubation.

Isolates No.	Antibiosis activities							TAMs	
	Bacteria					Fungus (1)	Fungus (2)		
	Salmonella sp.	*Staph. aureus*	*Micrococcus* sp.	*Sarcina* sp.	*E. coli*			No.	%
Rhizosphere-soil isolates									
01	0.0*	1.1	1.3	1.2	1.4	1.2	1.2	6	85.7
02	0.0	1.1	1.2	1.9	1.3	1.2	1.2	6	85.7
03	2.4	2.0	1.2	1.4	0.0	1.0	0.0	5	71.4
09	0.0	0.0	0.0	1.2	1.2	0.0	1.0	3	42.9
10	1.4	0.0	0.0	4.0	1.6	0.0	2.4	4	57.1
11	1.2	0.0	1.6	1.6	1.4	1.0	1.8	5	71.4
15	0.0	0.0	0.0	1.6	1.6	1.2	1.1	4	57.1
16	0.0	0.0	0.0	1.2	2.0	0.0	2.4	4	57.1

17	**1.2**	**0.0**	**1.2**	**1.8**	**1.4**	**0.0**	**1.4**	**5**	**71.4**
18	**0.8**	**0.8**	**0.8**	**1.2**	**1.4**	**0.0**	**0.8**	**6**	**85.7**
21	**1.2**	**1.2**	**1.4**	**1.8**	**1.4**	**1.2**	**1.8**	**7**	**100**
22	**0.0**	**1.4**	**0.0**	**1.4**	**1.8**	**0.0**	**0.0**	**3**	**42.9**
23	**1.2**	**1.8**	**1.2**	**1.2**	**0.0**	**2.8**	**0.0**	**5**	**71.4**
24	**0.0**	**0.0**	**0.0**	**1.8**	**0.0**	**1.6**	**0.0**	**2**	**28.6**
26	**0.0**	**2.0**	**0.0**	**0.0**	**1.2**	**1.0**	**0.0**	**3**	**42.9**
27	**0.0**	**0.0**	**0.0**	**1.2**	**1.2**	**1.0**	**0.0**	**3**	**42.9**
29	**0.0**	**1.8**	**0.0**	**1.4**	**1.2**	**1.4**	**0.0**	**4**	**57.1**
30	**0.0**	**1.2**	**0.0**	**1.4**	**1.6**	**0.0**	**0.0**	**3**	**42.9**
34	**0.8**	**1.2**	**0.0**	**1.6**	**1.6**	**1.2**	**1.0**	**6**	**85.7**
35	**0.8**	**1.6**	**1.2**	**0.0**	**1.2**	**1.0**	**0.8**	**6**	**85.7**
Non-rhizosphere-soil isolates									
04	**0.0***	**0.0**	**0.0**	**0.0**	**1.2**	**0.0**	**1.0**	**2**	**28.6**
05	**0.0**	**1.8**	**1.4**	**0.0**	**1.1**	**0.0**	**1.2**	**4**	**57.1**
06	**0.0**	**0.0**	**0.0**	**1.8**	**1.2**	**1.0**	**1.0**	**4**	**57.1**
07	**0.0**	**2.0**	**0.0**	**1.4**	**1.6**	**0.0**	**1.2**	**4**	**57.1**
08	**0.0**	**1.4**	**1.4**	**1.2**	**1.2**	**1.0**	**1.6**	**6**	**85.7**
12	**1.4**	**0.0**	**1.2**	**1.4**	**1.4**	**1.2**	**2.4**	**5**	**71.4**
13	**0.8**	**0.8**	**1.2**	**0.8**	**1.0**	**0.8**	**0.0**	**6**	**85.7**
14	**0.8**	**1.2**	**1.4**	**0.8**	**0.8**	**0.8**	**0.0**	**6**	**85.7**
19	**0.0**	**0.0**	**0.0**	**1.2**	**1.6**	**0.0**	**1.8**	**3**	**42.9**
20	**0.8**	**0.8**	**0.8**	**1.4**	**1.4**	**0.0**	**1.0**	**6**	**85.7**
25	**0.0**	**1.8**	**0.0**	**0.0**	**1.4**	**1.2**	**0.0**	**3**	**42.9**
28	**0.0**	**1.4**	**0.0**	**1.4**	**0.0**	**1.6**	**0.0**	**3**	**42.9**
31	**0.0**	**2.0**	**0.0**	**1.4**	**0.0**	**0.0**	**0.0**	**2**	**28.6**
32	**0.8**	**1.4**	**0.8**	**1.4**	**1.0**	**1.4**	**0.8**	**7**	**100**
33	**0.0**	**1.8**	**0.0**	**1.2**	**1.4**	**0.0**	**0.0**	**3**	**42.9**
36	**0.0**	**1.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1**	**14.3**
37	**1.4**	**1.6**	**1.2**	**1.8**	**0.0**	**1.6**	**0.0**	**5**	**71.4**
38	**1.4**	**1.8**	**1.4**	**1.8**	**0.0**	**0.0**	**0.0**	**4**	**57.1**
39	**1.2**	**1.6**	**0.0**	**1.2**	**1.2**	**0.0**	**1.0**	**5**	**71.4**
40	**1.2**	**1.8**	**0.0**	**1.6**	**0.0**	**0.0**	**0.0**	**3**	**42.9**
TESI	**18**	**28**	**18**	**34**	**31**	**22**	**22**	**100%**	
%	**45**	**70**	**45**	**85**	**77.5**	**55**	**55**		

TESI: Total effective streptomycete isolates. Fungus 1: *Aspergillus* sp. Fungus 2: *Alternaria* sp. *: Zone of inhibition (mm). TAMs: Total affected microorganisms.

Figure -1: Antibiosis activities of two *Streptomyces* isolates against *Staphylococcus aureus* 48 h post incubation.

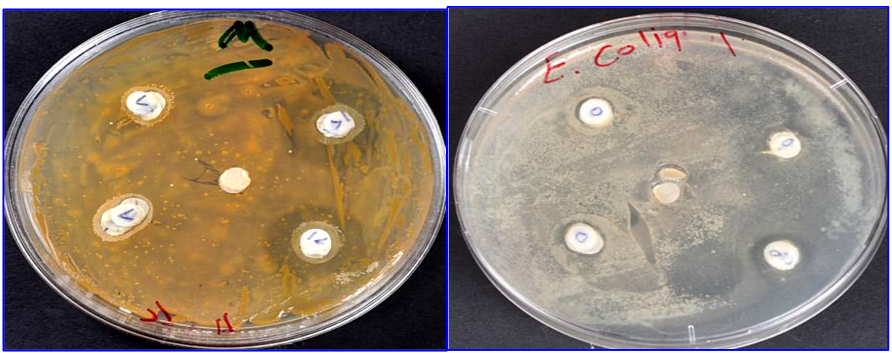

Figure -2: Antibiosis activities of two *Streptomyces* isolates against *Micrococcus sp.* (left) and *E. coli* h (Right) 48 h post incubation.

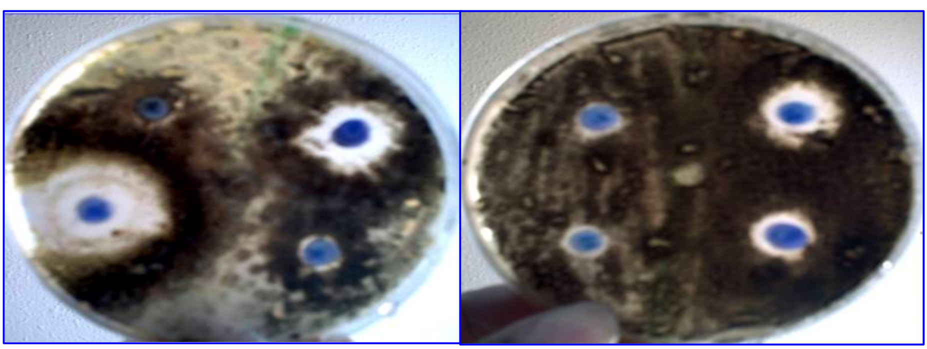

Figure -3: Antibiosis activities of two *Streptomyces* isolates against *Alternaria sp.* (Left) and *Aspergillus sp.* (Right) 72 h post incubation.

Salt tolerance range of selected strepto-mycete isolates: Data in **Table - 4** shows that the 7 selected streptomycete isolates were able to grow in the presence of 3.5% NaCl in the starch nitrate agar medium. At concentration of 7% NaCl, only 3 out of the 7 isolates showed moderate growth (++), while 4 isolates showed weak growth (+). At concentration of 10.5% NaCl, four isolates grew with weak growth (+) and three isolates showed in-doubt growth (±). No growth was found in the case of 14% NaCl.

Table -4: Salt tolerance range of selected streptomycete isolates showing high antibiosis activities post 14 days from inoculation on starch nitrate agar medium contains different concentrations of NaCl (%).

Isolates No.	Growth of streptomycete isolates on different concentrations of NaCl (%)				
	Control	3.5	7.0	10.5	14.0
08	++++	+++	+	+	-
14	++++	++	++	±	-
20	++++	+++	++	+	-
21	++++	+++	++	±	-
32	++++	++	+	+	-
34	++++	+++	+	+	-
35	++++	++	+	±	-

-: No growth. ±:In doubt. +:Weak growth. ++: Moderate growth. +++: Good growth. ++++:Abundant.

Identification of selected strepto-mycetes isolates: Results in **Tables (5, 6, 7, 8, 9, 10 and 11)** and illustrated by **Figures (4, 5, 6, 7, 8, 9 and 10)** showed that the seven streptomycete isolates (8, 14, 20, 21, 32, 34 and 35) could be strains of *S. polychromogenes*, *S. chattanoogensis*, *S. lucensis*, *S. violaceus*, *S. violans*, *S. griseorubiginosus* and *S. antibioticus*, with slight differences.

Table -5: Cultural, morphological and physiological characteristics of streptomycete isolate 8 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).

Characters	Isolate 8	*S. polychromogenes*
Color of aerial mycelium	Light Blue	Blue
Spore-chain	RF	RF
Melanoid pigment	C+	C+
Spore surface	Smooth	Smooth
Growth on Czapek's medium	Excellent	Excellent
Color of substrate mycelium	Green olive - white	Green/ yellow on some media
Diffusable pigments	Light brown	ND
Utilization of Carbon:		
No carbon	-	-
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	-
D-Fructose	+	+

D-Mannitol	+	-
i-Inositol	±	-
Sucrose	+	ND
Antagonistic activity	Antibacterial and antifungal	Antifungal
Sensitivity to streptomycin	Sensitive	Sensitive
NaCl tolerance	7-10.5%	4-7%

RF: Rectus-Flexibilis (spores in straight (R) or flexuous (F) chains). C+: Produces melanoid pigment. +: Growth. -: No growth. ND: No data.

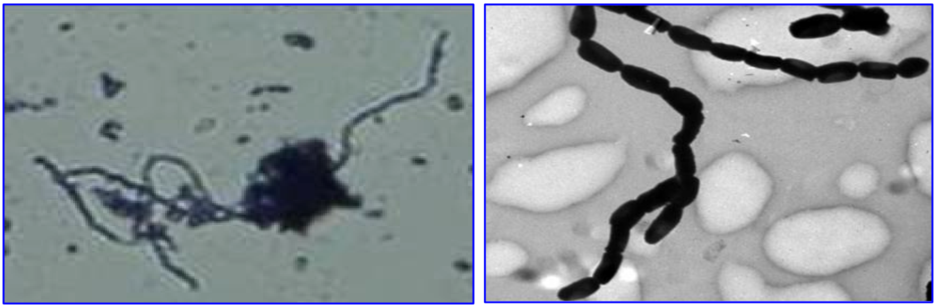

Figure -4: Microphotograph and electron micrograph of streptomycete isolate No. 8 shows RF chain (X-1000) and smooth spore surphace (X-10000).

Table -6: Cultural, morphological and physiological characteristics of streptomycete isolate 14 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).

Characters	**Isolate 14**	***S. chattanoogensis***
Color of aerial mycelium	Gray	Gray
Spore-chain	Spiral	Spiral
Melanoid pigment	C-	C-
Spore surface	Spiny	Spiny
Growth on Czapek's medium	Excellent	Excellent
Color of substrate mycelium	White-grayish	
Diffusable pigments	Rose	
Utilization of Carbon:		
No carbon	-	-
D-Glucose	±	+
D-Xylose	±	-
L-Arabinose	-	-
L-Rhamnose	±	-
D-Fructose	±	+
D-Mannitol	-	+
i-Inositol	+	+
Sucrose	-	+
Antagonistic activity	Antibacterial and antifungal	Slight antibacterial and antifungal
Sensitivity to streptomycin	Sensitive	Sensitive
NaCl tolerance	3.5 – 7%	≥ 7-10%

C-: Not produce melanoid pigment. +: Growth. -: No growth. ND: No data.

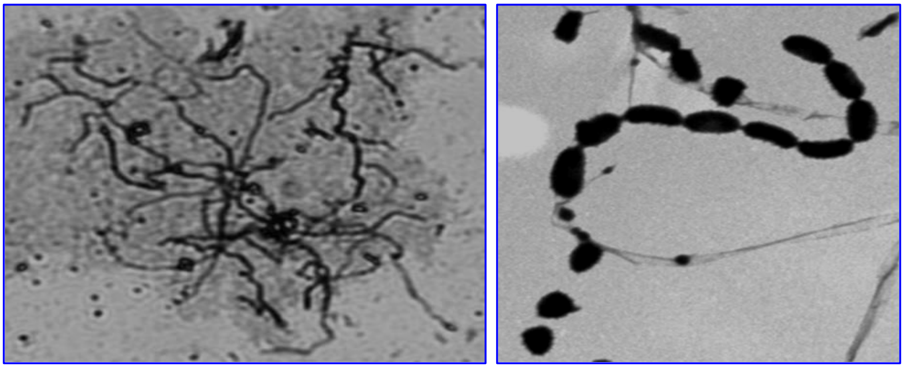

Figure -5: Microphotograph and electron micrograph of streptomycete isolate No. 14 shows S chain (X-1000) and spiny spore surphace (X-10000).

T**able -7: Cultural, morphological and physiological characteristics of streptomycete isolate 20 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).**

Characters	**Isolate 20**	***S. lucensis***
Color of aerialmycelium	Gray	Gray
Spore-chain	Spiral	Spiral
Melanoid pigment	C+	C+
Spore surface	Spiny	Spiny
Growth on Czapek's medium	Excellent	ND
Color of substrate mycelium	Green olive - Light brown	ND
Diffusable pigments	Rose	ND
Utilization of Carbon:		
No carbon	-	ND
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	-
D-Fructose	+	+
D-Mannitol	+	+
i-Inositol	-	-
Sucrose	+	+
Antagonistic activity	Antibacterial and antifungal	Antifungal
Sensitivity to streptomycin	Not sensitive	ND
NaCl tolerance	7-10.5%	7 – 10%

C+: Produces melanoid pigment. +: Growth. -: No growth. ND: No data.

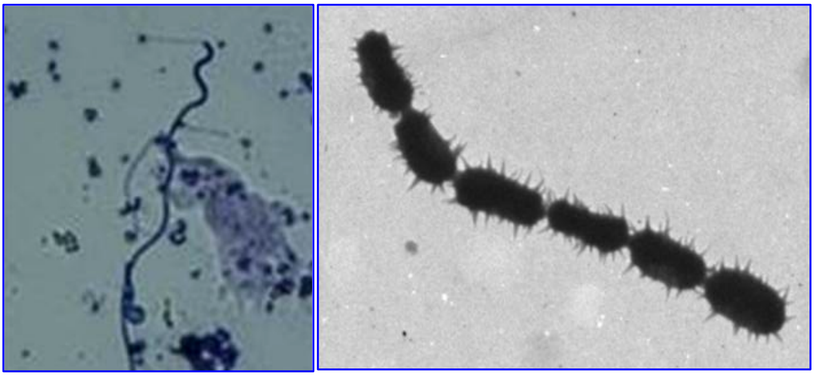

Figure -6: Microphotograph and electron micrograph of streptomycete isolate No. 20 shows S chain (X-1000) and spiny spore surphace (X-10000).

Table -8: Cultural, morphological and physiological characteristics of streptomycete isolate 21 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).

Characters	**Isolate 21**	***S. violaceus***
Color of aerial mycelium	Red	Red
Spore-chain	RA-Spiral	Spiral
Melanoid pigment	C+	C+
Spore surface	Spiny	Spiny
Growth on Czapek's medium	Excellent	Good
Color of substrate mycelium	Yellow	violet
Diffusable pigments	Creamy-brown	+ve on some media
Utilization of Carbon:		
No carbon	-	-
D-Glucose	+	+
D-Xylose	±	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
D-Mannitol	-	ND
i-Inositol	-	+
Sucrose	±	+
Antagonistic activity	Antibacterial and antifungal	Antibacterial, antifungal and antiviral
Sensitivity to streptomycin	Not sensitive	ND
NaCl tolerance	3.5- 7%	ND

RA: Retinaculum Apertum (Spore chains in the form of open loops, hooks or greatly extended coils of wide diameter). C+: Produces melanoid pigment. +: Growth. -: No growth. ND: No data.

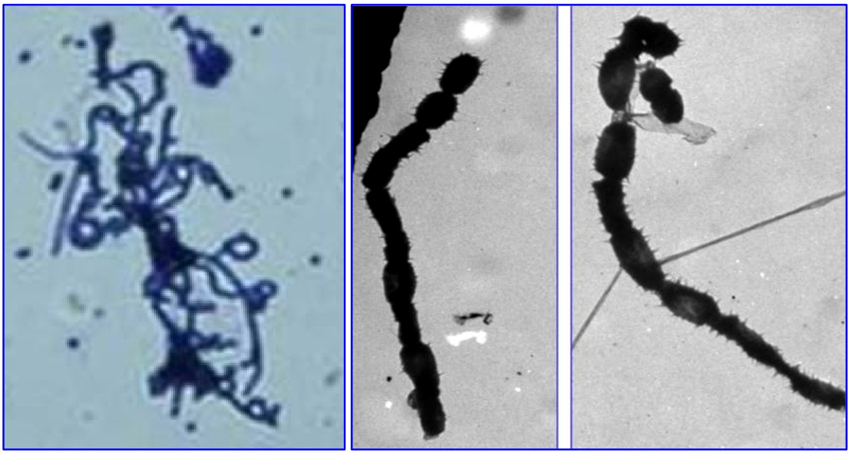

Figure -7: Microphotograph and electron micrograph of streptomycete isolate No. 21 shows RA-S chain (X-1000) and spiny spore surphace (X-10000).

Table -9: Cultural, morphological and physiological characteristics of streptomycete isolate 32 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).

Characters	**Isolate 32**	***S. violans***
Color of aerial mycelium	Violet	Violet
Spore-chain	RA-Spiral	Spiral
Melanoid pigment	C+	C+
Spore surface	Spiny	Spiny
Growth on Czapek's medium	Excellent	Good
Color of substrate mycelium	Dark brown	Pink to violet color
Diffusable pigments	Rose	+ve on some media
Utilization of Carbon:		
No carbon	-	-
D-Glucose	+	+
D-Xylose	+	ND
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
D-Mannitol	+	ND
i-Inositol	+	+
Sucrose	+	+
Antagonistic activity	Antibacterial and antifungal	Antibacterial and antifungal
Sensitivity to streptomycin	Not sensitive	ND
NaCl tolerance	7-10.5%	ND

RA: Retinaculum Apertum (Spore chains in the form of open loops, hooks or greatly extended coils of wide diameter). C+: Produces melanoid pigment.. +: Growth. -: No growth. ND: No data.

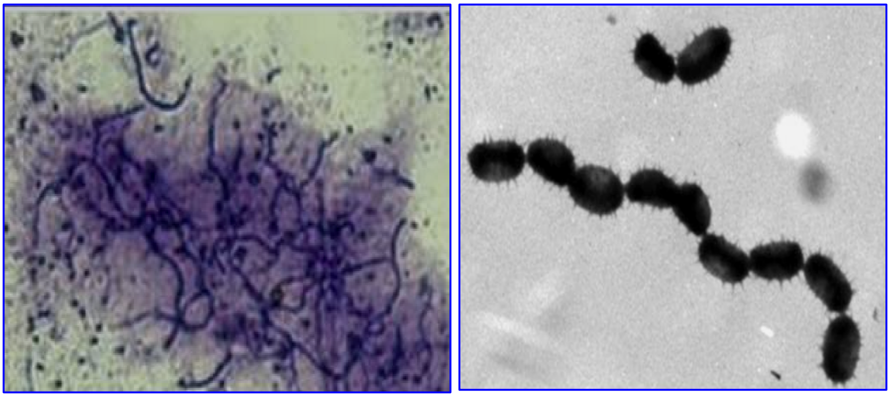

Figure -8: Microphotograph and electron micrograph of streptomycete isolate No. 32 shows RA-S chain (X-1000) and spiny spore surphace (X-10000).

Table - 10: Cultural, morphological and physiological characteristics of streptomycete isolate 34 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).

Characters	**Isolate 34**	***S. griseorubiginosus***
Color of aerial mycelium	Gray	Gray
Spore-chain	RA	RF (RA)
Melanoid pigment	C+	C+
Spore surface	Smooth	Smooth
Growth on Czapek's medium	Excellent	ND
Color of substrate mycelium	Light gray	Rosy reddish
Diffusable pigments	Gray	Red–brownish on some media
Utilization of Carbon:		
No carbon	-	ND
D-Glucose	±	+
+D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
D-Mannitol	+	+
i-Inositol	+	+
Sucrose	+	+
Antagonistic activity	Antibacterial and antifungal	Antibacterial and antiyeast
Sensitivity to streptomycin	Not sensitive	ND
NaCl tolerance	7-10.5%	ND

RA: Retinaculum Apertum (Spore chains in the form of open loops, hooks or greatly extended coils of wide diameter). RF: Rectus-Flexibilis (spores in straight (R) or flexuous (F) chains). C+: Produces melanoid pigment.. +: Growth. -: No growth. ND: No data.

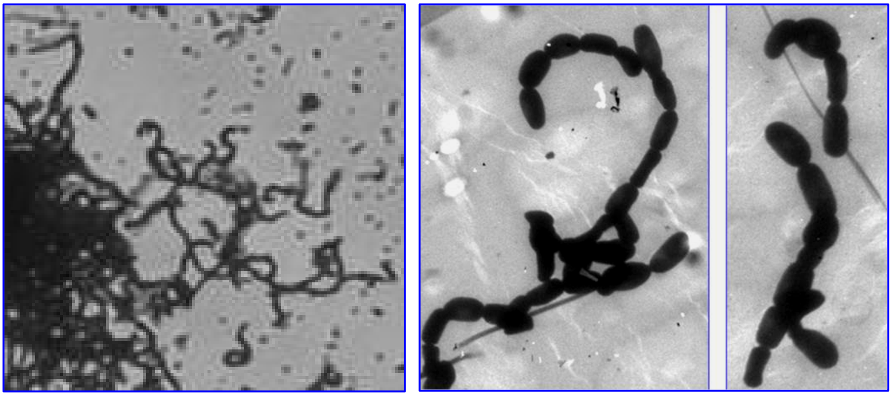

Figure -9: Microphotograph and electron micrograph of streptomycete isolate No. 34 shows RA chain (X-1000) and smooth spore surphace (X-10000).

Table -11: Cultural, morphological and physiological characteristics of streptomycete isolate 35 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974).

Characters	**Isolate 35**	***S. antibioticus***
Color of aerial mycelium	Gray	Gray
Spore-chain	RA	RF
Melanoid pigment	C+	C+
Spore surface	Smooth	Smooth
Growth on Czapek's medium	Excellent	Poor
Color of substrate mycelium	Dark Creamy-brown	ND
Diffusable pigments	Rose	ND
Utilization of Carbon:		
No carbon	-	-
D-Glucose	±	+
+D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
D-Mannitol	+	+
i-Inositol	+	+
Sucrose	+	-
Antagonistic activity	Antibacterial and antifungal	Produces the actinomycin X(B) complex
Sensitivity to streptomycin	Not sensitive	Sensitive
NaCl tolerance	3.5 – 7%	≥7-10%

RA: Retinaculum Apertum (Spore chains in the form of open loops, hooks or greatly extended coils of wide diameter). RF: Rectus-Flexibilis (spores in straight (R) or flexuous (F) chains).+: Growth. -: No growth. ND: No data.

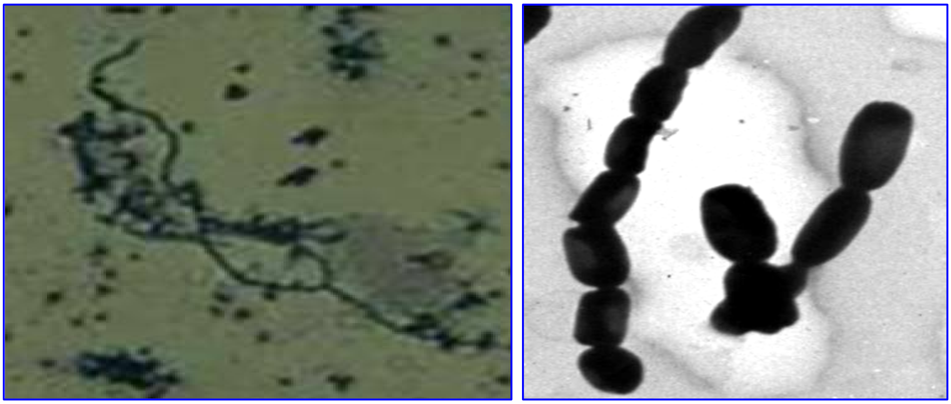

Figure -10: Microphotograph and electron micrograph of streptomycete isolate No. 35 shows RA chain (X-1000) and smooth spore surphace (X-10000).

DISCUSSION

Actinomycetes are microscopic soil microorganisms known to play a very supporting role in the degradation of organic matter in coffee habitats (Mythili and Ayyappa Das, 2011**)**. Some investigators throughout the world isolated streptomycetes from soils (Mohamed *et al*., 2000; Abdel-Fattah, 2005 and EL-Sayed *et al*., 2011).

We are here isolating actinomycetes from different soil samples collected from Makkah, Jeddah and Taif regions. Results of total counts showed that bacteria were the highest microorganisms followed by actinomycetes and fungi. A number of 20 actinomycete isolates were obtained from each of the rhizosphere soil and non-rhizosphere soil samples.

The isolates of this study were divided based on their serial color groups to belonging to violet, gray, white, red and blue color series groups. These results could be supported by Mohamed *et al.* (2000) and Saleh *et al.* (2011).

El-Sherbiny (2006) isolated an actinomycete from a sandy soil collected from Gabal Mokattam, Cairo, Egypt. The isolate was characterized by white, brown to light gray aerial mycelia and brown, yellow and red substrate mycelia on different ISP media.

The Gram-positive filamentous bacteria, *i.e.*, actinomycetes exhibit a broad spectrum of antimicrobial activity against fungi and bacteria (HongJian *et al*., 2009 and Singh *et al*., 2009**)**. In this work, the actinomycete isolates were tested for their antagonistic activities against some bacteria (*Salmonella* sp., *Staph. aureus*; *Micrococcus* sp. & *E. coli*) and fungi (*Aspergillus* sp. and *Alternaria* sp.). The isolates were varied in their activities as some of them appeared activities against 7, 6, 5, 4, 3, 2 and 1 microorganism out of seven test organisms that used in this study. Also, they showed different zone of inhibition against the tested microorganisms. These results agree with that found by Oskay (2009), El-Nasser *et al*. (2010), Raja *et al*. (2010), Baskaran *et al*. (2011) and Hozzein *et al*. (2011). Saadoun *et al*. (1999) showed that the isolated streptomycetes were grouped into six colour series, namely grey, white, yellow, green, red and polymorphic colours (pink, orange or violet) with total numbers of 29, 18, 14, 8, 3 and 9, respectively. The isolates (68%) showed reverse side culture pigmentation, 30% produced melanin and 25% produced other soluble pigments. Isolates (48%) were characterized by flexuous spore chains, 21% with spiral and 10% for each of the rectus and retinaculum apertum arrangement. Only seven out of the forty actinomycetes isolates that showed antagonistic activities against 87.5 to 100% of the tested microorganisms were subjected to determination of salt tolerant range.

Results showed that all of them were able to grow in the presence of 3.5% NaCl in the starch nitrate agar medium. At concentration of 7% NaCl, they were able to tolerated such concentration, as four of them appeared moderate growth (++) and the other three isolates showed weak growth when cultivated on starch nitrate agar medium supplemented with 7% NaCl. By increasing the NaCl concentration up to 10.5%, only three isolates showed a weak growth (+). No growth was found at 14% NaCl in the growth medium.

This result agrees with that found by Mohamed *et al.* (2000), Mohamed and Chaudrhy (2005), Singh *et al.* (2009), Balagurunathan *et al.* (2010), and Gulve and Deshmukh (2011). Mohamed and Chaudrhy (2005) showed that sixteen halotolerant *Streptomyces* strains varied in their salt tolerance range, in particular, with increasing NaCl concentration in the growth medium up to 140 g/L. It was also noted that all the applied *Streptomyces* strains tolerated NaCl concentrations up to 70 g/L. When NaCl concentration was raised to 105 g/L, strains except *S. melanogenes* Si-11, gave moderate growth. On the contrary, NaCl concentration of 140 g/L inhibited the growth of 50% of strains under investigation, but the other 50% of these strains gave moderate growth. In Egypt, a number of studies were achieved corresponding to the halotolerant streptomycetes isolated from soils (Mohamed 1998).

Zarandi *et al.* (2009) reported that the soil actinomycetes were having antagonistic activity against a wide range of plant pathogens. The results paid an attention to the possibilities of extraction, purification and identification of such substances. This idea could be supported by Mahfouz and Mohamed (2002). Using the proposed key of Pridham and Tresner (1974) the seven selected antagonistic *Streptomyces* isolates were found to be strains of strains of *S. polychromogenes* (isolate 08), *S. chattanoogensis* (isolate 14), *S.lucensis* (isolate 20), *S.violaceus* (isolate 21), *S.violans* (isolate 32), *S. griseorubiginosus* (isolate 34) and *S. antibioticus* (isolate 35), with slight differences based on their cultural, morphological and physiological characteristics.

The use of the proposed key of Pridham and Tresner (1974) was effective in taxonomy of the *Streptomyces* isolates of this study as mentioned above. These results are in the same trend with that of Abdel-Fattah (2005), Mohamed *et al.* (2005), El-Sherbiny (2006), HongHui *et al.* (2007) and Lin *et al.* (2011).

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