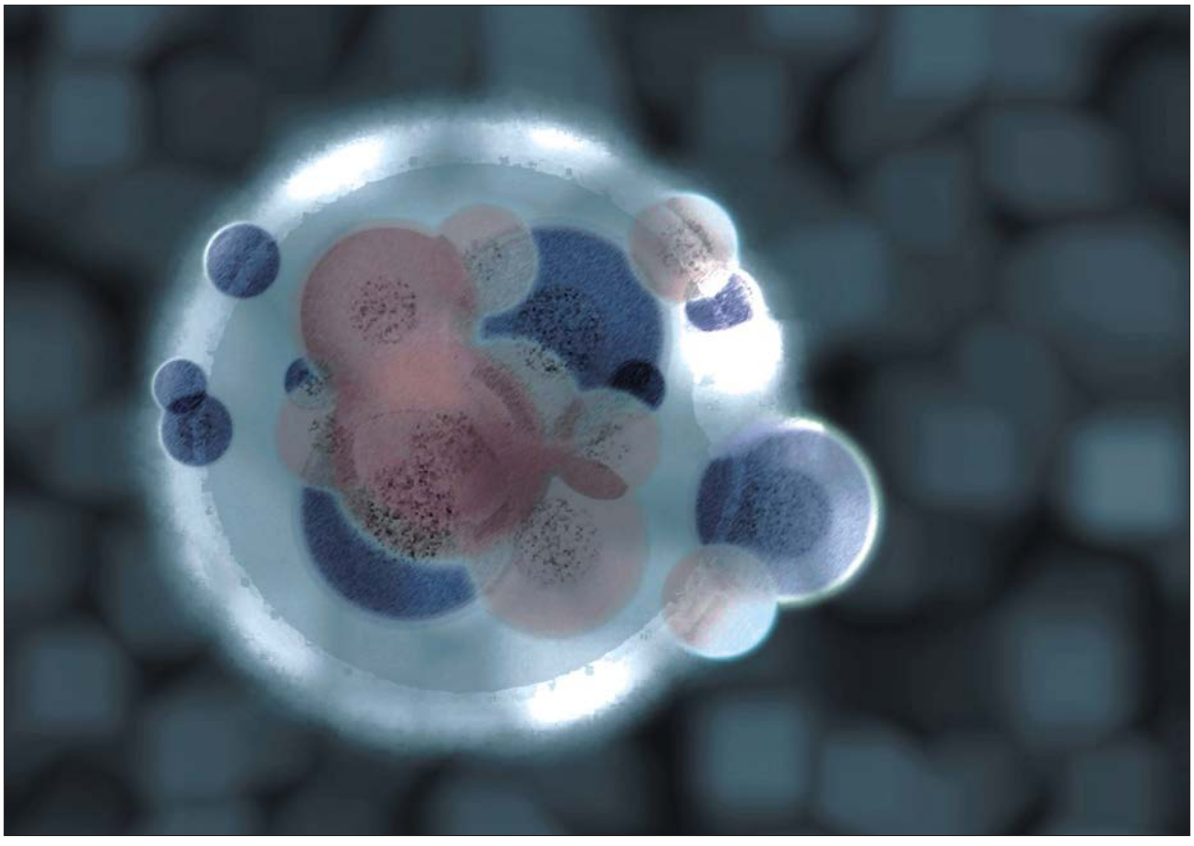

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Screening of Antagonistic Activity in Different *Streptomyces* Species Against Some Pathogenic Microorganisms

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Abstract: One hundred eighty eight *Streptomyces* species, isolated from Egyptian soils were screened for their antagonistic activity against nineteen fungal and bacterial species. Three red coloured isolates (No. 28, 152, 157) were the most active species, antagonizing all tested organisms, with variable sensitivity to *Streptomyces* antibiotics. *Candida albicans* was the most sensitive, inhibited by higher numbers of *Streptomyces* sp. Maximum degree of antagonistic activity was attained by five *Streptomyces* isolates against *C. albicans*. All isolates have been grouped according to the colour of aerial mycelium. Most of the antifungal isolates were found to be within the gray series, which were represented by 33 isolates out of 60 antifungal isolates.

Key words: *Streptomyces*, antagonistic activity, yeasts, fungi, bacteria

INTRODUCTION

Actinomycetes are world wide in their distribution and most of them are soil inhabitants a period. They are isolated from soil and they are numerically less dominant than the bacteria and more prominent than the fungi. They are present in the greatest number in the top few inches of the soil and decrease with depth (Moharram, 1991; Anderson and Wellingtone, 2001; Osman *et al*., 2005).

Soil actinomycetes particularly *Streptomyces* sp. enhance soil fertility and have antagonistic activity against wide range of soil-borne plant pathogen (Song *et al*., 1998; Aghighi *et al*., 2004). Many investigators reported that different streptomycetes have either inhabitiory or stimulatory effects on other different microorganisms (Abdel-Gawad, 2002; Kim *et al*., 2003; Zuniga *et al*., 2004).

Streptomyces sp. Uk10 isolated from a soil sample collected from Kiev, Ukranie produced a tetraene polyene macrolide designated HA-2-91, which exhibited an antifungal activity against *Candida albicans*, *Saccharomyces cerevisiae* and *Paecilomyces variotii* (Gupte and Naik, 1991).

In Egyptian soils, distribution of actinomycetes was studied by El-Abyad *et al*. (1993), El-Shanshoury (1994, 1995), El-Shanshoury *et al*. (1996) and Moussa (1999).

Yan Min *et al*. (2000) tested the antagonistic activity of 26 strains of *Streptomyces* sp. against several important pathogens, including *Alternaria solani*, *Botrytis cinerea* of tomato, *Xanthomonas campestris* of cabbage and *Erwinia carotovora* of Chinese cabbage.

Streptomyces are considered as antibiotic producers, making three quarters of all known products (Saadoun and Gharaibeh, 2003). *S. reticuli*, *S. hygroscopicus* and *Micromonospora* sp. isolated from composted soil, antagonized some fungal species (Adeleye *et al*., 2004). *S. griseus* exhibited an antifungal activity against some phytopathogenic fungi (Hoster *et al*., 2005). Kamel *et al*. (2007) tested the antagonistic activity of eleven *Streptomyces* species against the tomato pathogens, *Fusarium oxysporum* and *Alternaria solani*. They found that 5 *Streptomyces* isolates were able to antagonize *F. oxysporum*, while 6 isolates of actinomycetes inhibited *A. solani*.

The aim of this study was to isolate *Streptomyces* sp. from different Egyptian soils and to screen for their antagonistic activity against some microorganisms.

MATERIALS AND METHODS

The soil used in this experiment was clayey in texture and alkaline in reaction, collected from different areas around applied Research Center for medicinal Plants of Natural Organization for Drug Control and Research, Egypt. The soil allowed to be air-dried for 7 days and then was sieved through a 4 mm sieve for the experiment.

Different microorganisms including Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi were kindly provided by the Microbiological Center, Ain-Shams University, Faculty of Agriculture and by Bacteriological Department, Laboratory of National Organization for Drug and Research, Egypt. These were

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Bacillis subtilis, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosae*, *Shigella* sp., *Salmonella typhimurium*, *Klebsiella pneumonia*, *Candida albicans*, *C. glabrata*, *C. nonalbicans*, *C. parapsilosis*, *C. tropicalis*, *Saccharomyces cerevisiae*, *Alternaria solani*, *Fusarium oxysporum*, *F. solani*, *Aspergillus flavus* and *Aspergillus niger*.

The cultures were maintained on slants of appropriate medium where the bacteria were kept on slants of nutrient agar medium, yeasts were kept on sabouraud's dextrose agar medium and the fungi were kept on slants of Czapek´s-Dox agar medium.

The soil dilution plate technique was used for the isolation of *Streptomyces* sp. (Johnson and Curl, 1972). The samples were pretreated by mixing with $CaCO_3$ and incubated for 10 days at 28°C in a water saturated atmosphere to increase the number of actinomycetes in the soil samples. A known weight of soil was shaken in sterile distilled water and then subjected to a progressive series of dilution. A one mL sample of the proper dilution was spread onto the surface of starch nitrate medium and the plates were incubated for 8 days at 28°C. The developed colonies of actinomycetes were isolated and the pure cultures were preserved on agar slants of starch nitrate medium.

The identification of selected *Streptomyces* isolates was carried out according to Shirling and Gottlieb (1966), Williams *et al*. (1983) and Bergey's Manual Systematic Bacteriology (Williams, 1989).

Streptomyces isolates were tested for their antagonistic activity against the selected microorganisms by the diffusion agar method according to Hasegawa *et al*. (1990). The isolates were cultured on starch nitrate medium for 10 days at 28°C. Agar discs (5 mm) were cut off by a cork borer and transferred to the surface of agar plates, previously inoculated with the test organism. The petri dishes were kept in a refrigerator for 1 h before incubation to permit the diffusion of antimicrobial substances. The diameter of inhibition zones was measured after incubation for 48 and 72 h at 28°C for yeast and fungi and 24 h at 37°C for bacteria.

RESULTS

One hundred eighty eight *Streptomyces* isolates were screened from the Egyptian soil and assayed for their antagonistic activity against some pathogenic microorganisms. Present results in this study concentrated only on the most potent strains. Table 1 showed that the red coloured isolates (No. 28, 152, 157) were the most active species and have a wide range antifungal activity, so they antagonized all tested pathogenic fungi, *Candida albicans*, *C. glabrata*, *C. nonalbicans*, *C. parapsilosis*, *C. tropicalis*, *Saccharomyces cerevisiae*, *Alternaria solani*, *Fusarium oxysporum*, *F. solani*, *Aspergillus flavus* and *Aspergillus niger*. The gray isolates No. 10, 21 and 49 inhibited the growth of (9-10) tested fungi. Similar results were obtained by the green isolate No. 153 and red isolate No. 138. The white isolate No. 42 was least affective, so it antagonized only seven fungal species and the rest of organisms *C. glabrata*, *C. nonalbicans*, *C. parapsilosis*, *C. tropicalis* were insensitive to antibiotics produced by the *Streptomyces* isolate No. 42.

Table 2 shows screening of the most potent *Streptomyces* isolates for their antagonistic activity against some bacterial species. Ten isolates No. 10, 12, 21, 164 (grey); 22 (yellow); 28, 44, 138, 152, 157 (red isolates) and 164 (G) were the most active against all tested Gram -ve and Gram +ve bacteria (*B. subtilis*, *S. aureus*, *M. luteus*, *E. coli*, *K. pneuomiae*, *Ps. aeruginosae*, *S. typhimurium* and *Shigella* sp.) an indication of their sensitivity to *Streptomyces* metabolites. Five *Streptomyces* isolates No. 49, 65, 115, 170 (gray) and 106 (yellow) antagonized seven bacterial species, whereas the rest of *Streptomyces* inhibited the growth of (5-6) tested pathogenic bacteria only.

Table 1: Screening of *Streptomyces* isolates for their antifungal activities against some fungal pathogens (Inhibition zones, mm)

Streptomyces isolate No.	Test organisms used: Inhibition zone (mm)										
	Alternaria solani	*Aspergillus flavus*	*A. niger*	*Candida albicans*	*C. glabrata*	*C. nonalbicans*	*C. parapsilosis*	*C. tropicalis*	*Fusarium oxysporum*	*F. solani*	*Saccharomyces cerevisiae*
10 (G)	20	15	19	26	22	18	20	19	22	0	24
21 (G)	18	0	16	28	17	18	16	0	24	18	25
28 (R)	18	19	22	28	18	20	28	20	24	15	24
42 (W)	16	16	22	30	0	0	0	0	17	17	18
49 (G)	0	17	15	25	17	17	21	17	23	16	20
138 (R)	17	18	18	21	18	19	0	0	18	0	17
152 (R)	18	20	23	32	25	20	28	23	27	16	28
153 (Gr)	0	18	20	30	25	19	23	23	22	0	25
157 (R)	16	16	19	28	22	19	28	23	20	16	26

G = Gray, R = Red, W = White, Gr = Green

Table 2: Screening of *Streptomyces* isolates for their antimicrobial activities against Gram +ve and Gram -ve pathogenic bacteria

	Test organisms used							
	Inhibition zone (mm)							
Streptomyces isolates	Gram(+ve)			(Gram-ve)				
No.	*B. subtilis*	*M. luteus*	*S. aureus*	*E. coli*	*K. pneumoniae*	*Ps. aeruginosa*	*S. typhimurium*	*Shigella* sp.
10 (G)	22	28	28	25	22	20	20	22
12 (G)	30	22	32	34	31	22	28	23
21 (G)	25	21	27	40	18	18	13	18
22 (Y)	34	30	23	27	20	17	18	22
28 (R)	23	28	20	30	22	22	24	17
44 (R)	21	26	20	30	20	33	19	23
49 (G)	16	25	24	23	23	0	18	25
50 (W)	20	40	29	0	20	22	0	0
65 (G)	18	18	24	31	25	0	32	23
72 (G)	15	30	18	18	0	0	28	23
96 (W)	19	43	37	35	0	0	0	29
97 (G)	17	38	37	35	0	0	25	30
98 (G)	17	36	35	30	0	0	0	26
106 (Y)	18	21	16	22	0	19	17	23
115 (G)	0	19	30	32	19	22	17	22
122 (G)	26	20	19	0	15	0	0	22
135 (W)	0	0	15	23	19	15	16	20
138 (R)	19	34	19	24	25	29	33	40
152 (R)	45	39	30	20	19	23	20	35
157 (R)	21	40	38	23	17	22	18	23
164 (G)	25	30	36	23	17	20	38	22
170 (G)	23	25	30	17	0	22	22	28
180 (G)	20	18	20	19	0	0	18	16

G = Gray, Y = Yellow, W = White, R = Red

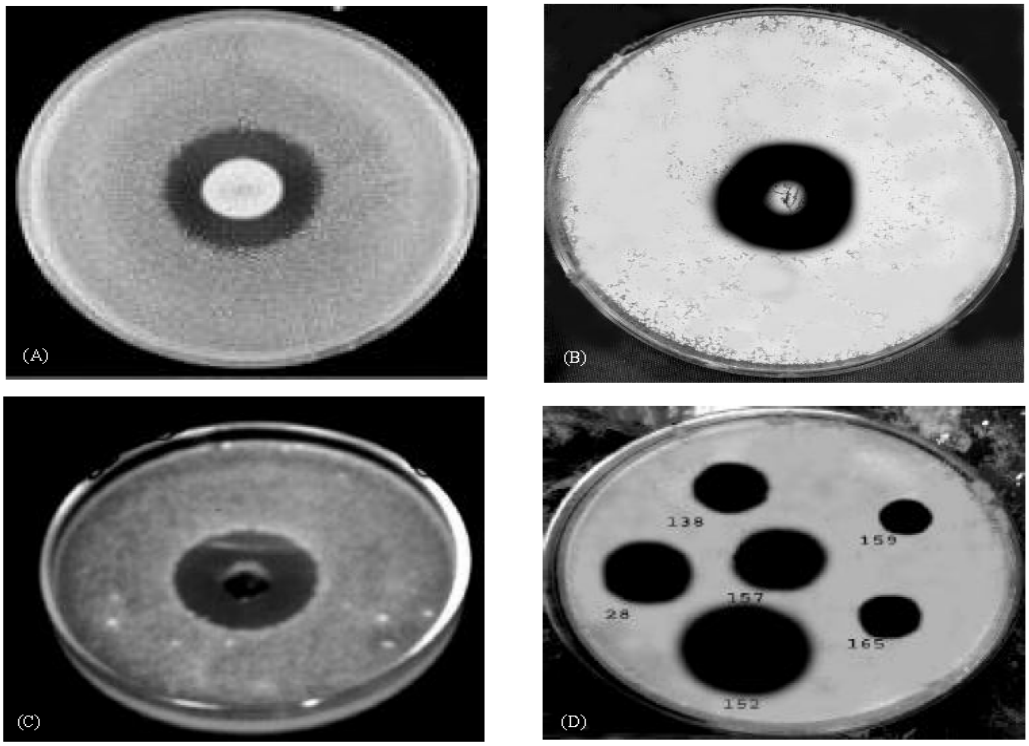

Fig. 1: Antagonistic activity of some *Streptomyces* isolates against *C. albicans*. (A) No. 28, (B) No. 157, (C) No. 152 and (D) 28, 138, 152, 157, 169 and 165

Table 3: No. of *Streptomyces* isolates showing different degrees of antifungal activity against the tested pathogenic fungi

	Degree of activity				
	No. of isolates				
Fungi	Very weak	Weak	Moderate	Good	Total No. of active isolates
Saccharomyces cerevisiae	2	12	11	0	25
Candida albicans	2	5	20	5	33
C. tropicalis	2	9	5	0	16
C. glabrata	3	9	4	0	16
C. nonalbicans	2	15	2	0	19
C. parapsilosis	1	14	9	0	24
Alternaria solani	0	14	0	0	14
Fusarium oxysporum	2	15	12	0	29
F. solani	2	8	1	0	11
Aspergillus niger	3	15	9	0	27
A. flavus	0	19	1	0	20

Table 4: Percentage of antifungal *Streptomyces* isolates for each colour series

Colour series of	Antifungal *S.* isolates		*Streptomyces* isolates	
S. isolates	(%)	Total No.	(%)	Total No.
Gray	17.55	33	48.4	91
Yellow	2.66	5	12.8	24
White	3.19	6	19.1	36
Violet	1.06	2	7.5	14
Red	5.32	10	9.5	18
Green	2.13	4	2.7	5
Total	31.91	60	100.0	188

Calculated as a percentage of total *Streptomyces*(*S*) isolates (188 isolates)

Table 3 showed that *C. albicans* was the most sensitive organism, where it was inhibited by higher number of *Streptomyces* isolates as compared with the other tested fungi, (Photos a, b, c and d). Also, the results revealed that, a good activity degree was attained by five *Streptomyces* isolates against *C. albicans* only.

All *Streptomyces* isolates have been grouped according to the colour of aerial mycelium (Moharram, 1991). Table 4 showed that the most isolates fell into the gray series representing (48.4%) of the total isolates. This is followed in descending order by white series (19.15%), yellow series (12.77%), red series (9.57%), violet series (7.45%) and green series (2.66%). Moreover, data revealed that most of antifungal isolates were found to be within the gray series, which represented by 33 isolates out of 60 antifungal *Streptomyces* isolates.

DISCUSSION

Streptomyces represent a major portion of the actinomycetes in Egyptian soil, with biological activities in producing bioactive compounds such as antibiotics, vitamins and enzymes.

Results of the present investigation showed that the most potent *Streptomyces* isolates have a wide range antifungal activity against all tested pathogenic fungi. *Candida albicans* was the most sensitive organism.

Present results are in agreement with those obtained by Kamel *et al.* (1993, 2007b), Mohamed (1995), Aghighi *et al.* (2004) and Osman *et al.* (2005) who reported that soil actinomycetes particularly *Streptomyces* spp. antagonized soil-borne plant pathogens. The degree of antagonistic activity greatly varied according to the strain and tested orgamism (Ahmed, 2003).

Kokare *et al.* (2004) studied the cultural properties of *actinopolyspora* on starch casein agar and other media and found a good antifungal activity against *Aspergillus niger*, *A. fumigatus*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium* sp. and *Trichoderma* sp.

One hundred sixty isolates of marine actinomycetes were tested against 4 phytopathogenic fungi (*Rhizoctonia solani*, *Pyricularia oryzae*, *Helminthosporium oryzae* and *Colletotrichum falcatum*). About 51% of isolates were found effective against *H. oryzae* and *P. oryzae*, 31% against *R. solani* and 12.5% against *C. albicans* (Kathiresan *et al.*, 2005).

Conversely, Sasaki *et al.* (2001) reported that the antibiotics (TPU-0031-A and B) of *Streptomyces* sp. isolated from the plant *Aucnba jabanica* exhibited no activity against fungi.

The results also, revealed that the bacterial growth is decreased by almost all isolates of *Streptomyces*. These results agree with those obtained by El-Abyad *et al.* (1996), Annie *et al.* (1997) and Sasaki *et al.* (2001). They reported that some *Streptomyces* species showed antagonistic activity against Gram +ve and Gram –ve bacteria.

The degree of antimicrobial activity of the antagonistic isolates was evaluated according to Abdel-Fattah (2001), who classified the antagonistic activity depending on mean diameters of inhibition zones (mm), to the following groups: Very weak activity (<16 mm), weak activity (16-19 mm), moderate activity (20-29 mm) and good activity (30 mm or more).

According to the colour of aerial mycelium, the most of antifungal isolates were found to be within the gray series. Mohamed (1995) isolated fifty pigmented *Streptomyces* isolates and classified them into 5 groups: blue, violet, yellow, red and brown. Some actinomycetes possess distinctive colours and some strains also produce soluble pigments, enzymes and other bioactive compounds (Hobbs *et al.*, 1990; Dieter *et al.*, 2003).

The most active isolates (No. 28, 152 and 157) were identified as *Streptomyces fradiae*, *S. lavendulae* and *S. fulvissimus*, respectively, for the future studies.

CONCLUSION

It was concluded that the bacterial growth is decreased by antibiotics produced by almost all isolates

of *Streptomyces*, except for a few Gram -ve bacteria such as *Ps. aeruginosae*, *K. pneumoniae* and *S. typhimurium* which were insensitive to some isolates.

Streptomyces fradiae, *S. lavendulae* and *S. fulvissimus* antagonized all tested pathogenic fungi and *Candida albicans* was the most sensitive organism.

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