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TAXONOMICAL STUDIES ON ACTIVE *Streptomyces* ISOLATES FROM EGYPTIAN SOIL

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ABSTRACT

Two hundred putative streptomycete isolates were recovered from agricultural soil samples collected from Assiut (Kossia), Dakahlia (Belqas), and Giza Governorates of Egypt. Only 25 of these isolates showed incongruous antimicrobial activities against reference Gram-positive, Gram–negative species of bacteria, yeast and filamentous fungi. These active isolates were characterized morphologically, culturally, physiologically, biochemically and chemotaxonomically to species level. The comparative analysis of the different characteristics using SPSS statistical software divided the 25 bioactive streptomycetes into 6 clusters. The dominant clusters were *Streptomyces lydicus* followed by *S. atroolivaceus*.

Key words: *antimicrobial activity - cell wall analysis - numerical taxonomy.- Streptomyces* sp.

1. INTRODUCTION

Actinomycetes comprise an extensive and diverse group of mycelial bacteria and have substantial practical significance. They are primarily soil inhabitants and considered as one of the major communities of soil microbial population, and their occurrence is greatly influenced by the environmental conditions (Basilio *et al.*, 2003). *Streptomyces* is the most commonly isolated genus of actinomycetes. It is very important, both ecologically and medically as one of the major prolific producers of economically important bioactive secondary metabolites such as antibiotics, vitamins, herbicides, pesticides, anti-parasitic, and enzyme like cellulase and xylanase used in waste treatment (McCarthy and Williams, 1992; Sanglier *et al*., 1996; Horan, 1999; Lazzarini *et al.*, 2000 and Takahashi and Omura, 2003). They can be distinguished from all other actinomycetes in morphological, cultural, physiological and chemotaxonomical characteristics (Shirling and Gottlieb, 1966; Soput *et al*., 1967; Lechevalier, and Uechevalier, 1970 & 1980; Minnikin *et al*., 1980; Alderson *et al*., 1985 and Christova *et al*., 1995).

A considerable step in advance of the taxonomy of the streptomycetes is the numerical classification of Williams *et al*. (1983). This study was formed on the basis of the classification of the species of genus *Streptomyces* in Bergey's manual (Williams *et al*., 1989). Isolation and screening of *Streptomyces* for the production of novel bioactive products has been intensively pursued along by scientists.

In the present research, isolation and biological activities as well as characterization of active streptomycetes isolates have been studied.

2. MATERIALS AND METHODS

2.1. Sampling

Ten cultivated soil samples were collected in sterile plastic bags at a depth of 15-20 cm, from Assiut (Kossia), Dakahlia (Belqas) and Giza Governorates and air dried at room temperature.

2.1.1. Isolation of streptomycetes colonies

Ten grams of soil sample were homogenized in 90 ml buffered phosphate solution (pH 7.0). Soil suspension was serially diluted and plated onto starch nitrate agar plates (Waksman,1962) following pour plating technique; the plates were incubated at 28 °C for 7-14 days. Firm cartilaginous rough chalky colonies of streptomycetes were selected and purified.

2.1.2. Determination of antimicrobial activity

The purified isolates of streptomycetes were cultivated for 5 days on starch nitrate agar plates at 28 °C. A disk of 0.8 mm diameter of the resulted culture was cut by sterile cork borer and aseptically transferred to inoculate 250 ml Erlenmeyer flasks containing 50 ml of sterile starch nitrate broth medium and allowed to grow at 28-30 °C for 5 days at 180 rpm on a rotary shaker (New Brunswick Scientific, Edison, N. J.,

USA). The broth culture was aseptically filtrated through Whatman filter paper No. 1 (Shirling and Gottlieb, 1968). A sterile cork borer was used to make holes (0.8 mm in diameter) in the plates seeded with the test organism, then only 0.1 ml of each filtrate was aseptically transferred into each hole and incubated at 28 °C for 1 and 3 days for bacteria and fungi, respectively. Antagonism was determined by measuring the size of inhibition zone around holes in millimeter.

2.1.3. Organism test

Antibacterial activities of streptomecete isolates were tested *in vitro* against the following microorganisms : Gram negative bacteria (*Escherichia coli* NRRL B-3704), Gram positive bacteria (*Bacillus cereus*, B.subtilis* NRRL B-941, *Staphylococcus aureus*, Streptococcus pyogenes**), yeasts (*Candida albicans** and *Saccharomyces cerivesiae**) and fungi (*Asperagillus niger* NRRLA-326, *A. flavus* NRRL A-1957, *Macrophomina phaseoli* NRRL A-62743, *Botrytis allii* NRRL A-2502, *Deplodia-oryzae* ATCC-10936, *Fusarium oxysporium* NRRL A- 2018, *Trichoderma viride* NRRL A-63065).

* These microorganisms were kindly provided by the Department of Microbiology, Ain Shams University.

2.1.4. Characterization of the active isolates

Pure colonies of active streptomycetes were individuated by morphological, cultural, physiological and chemotaxonomical characters in accordance with the guidelines established by the International *Streptomyces* Project (Shirling and Gottlieb, 1966) and Bergey's Manual of Systematic Bacteriology (Locci, 1989). The characteristics of pure isolates in various media were recorded after incubation for 7 to 14 days at 28 °C (Oskay *et al*., 2004). The morphological observations (Spore chain morphology and spore surface ornamentation) were made with a light and transmission electron- microscopy (Zeiss EM-10 West Germany) using the methodology of Tresner *et al.* (1961). A range of phenotypic properties was examined using the standard procedures of Williams *et al*. (1983). Cultural characters including color of the spore mass, pigmentation of substrate mycelium and diffusible pigments were visually estimated by using Methuen Hand Book Color of Kenneth (1958). Physiological and chemotaxonomical features include utilization of different carbon sources, activities of lipolytic, proteolytic and lecithinase enzymes; pectin, chitin, xanthine and arbutin decomposition; melanin synthesis, nitrate reduction, hydrogen sulphide production, cell wall analysis and whole-cell sugars (Shirling and Gottlieb 1966; Szabo, *et al*., 1978).

2.1.5. Statistical analysis

The SPSS for windows release 6.0 statistical software group has been used to generate phenograms. Data were examined using the simple matching (S_{Sm} ; Sokal and Michener,1958) coefficient. Tree was generated by the UPGMA algorithm. The phenogram was printed and further evaluated using different systematic and determinative bacteriological manuals (Bergey's Manual of Systematic Bacteriology, Locci, 1989)

3. RESULTS

3.1. Screening of isolated streptomycetes for antimicrobial activities

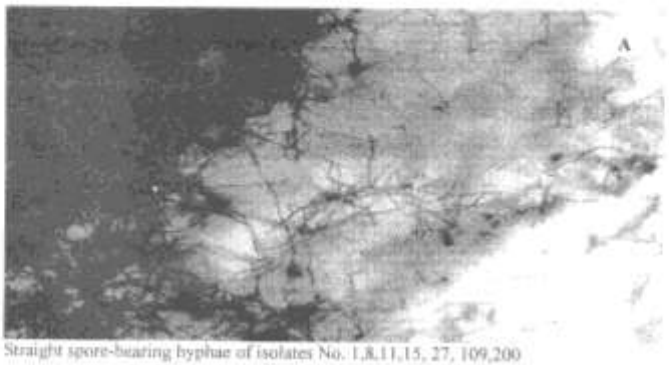
A total of 200 streptomycetes isolates was recovered from soil samples collected from different areas in Egypt. The antimicrobial activity of the isolates revealed that only 25 (12.5%) have a relatively inconsistent potency against the test organisms as shown in Table (1). Of the 25 isolates, 2 were active against both *E. coli* and *B. cereus, B. subtilis, Staphylococcus aureus* and *Streptococcus pyogenes;* 6 against Gram positive bacteria. Among them 3 of the isolates were very weak and/or weak active (Ø 14 - 18 mm) against *E. coli*, 9 with variable activity (Ø 12 – 20 mm) against *B. cereus*, 18 against *B. subtilis* (Ø 14- 26 mm), 21 against *Staphylococcus aureus* (Ø 14 – 30 mm) and 18 against *Streptococcus pyogenes* (Ø 14 – 26 mm). Concerning fungi, it is obvious that all these active strepyomycetes were futile to inhibit the growth of *Saccharomyces cerevisiae* as a target organism and only 13 isolates inhibited the growth of *Candida albicans* with variable rates as indicated by the measured inhibition zones (Ø 14 – 30 mm). Pertaining filamentous fungi, all the 25 isolates subdued the growth of *Asperagillus niger* in dissident levels (Ø 14 – 30 mm), 5 against *Asperagillus flavus*, 21 against. *Macrophomina phaseoli*, 22 against *Botrytis allii*, 2 against *Deplodia-oryzae*, 1 against *Fusarium oxysporium* and 9 against *Trichoderma viride*. It is conspicuous that the isolate # 200 was equally superior in potency against *Staphylococcus aureus, Candida albicans* and *Asperagillus niger* as compared to the other active isolates.

3.2. Morphological characteristics

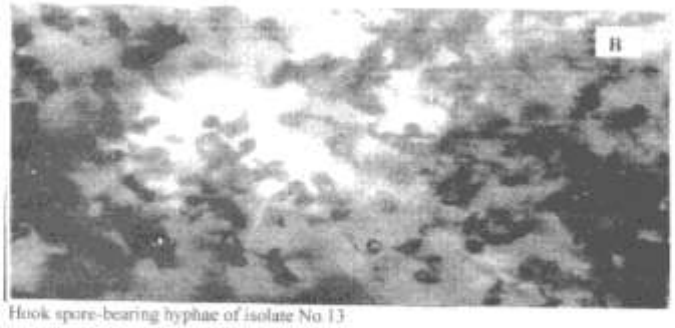
The light microscopic examination of the 25 active streptomycetes isolates indicated that 7 (28%) possessed spore-bearing hyphae of type straight (Fig. 1-A), 1 (4%) possessed hooks (Fig.1-B) and 15 (60%) possessed an extended

Table (1): **Antimicrobial activity of active** *Streptomyces* **isolates against bacteria, yeast and fungi (Zone of inhibition, mm).**

Strain No.	Bacteria					Yeast	Fungi						
	Escherichia coli	*Bacillus cereus*	*Bacillus subtilis*	*Staphylococcus aureus*	*Streptococcus pyogens*	*Candida albicans*	*Aspergillus niger*	*Aspergillus flavus*	*Macrophomina phaseoli*	*Botrytis allii*	*Diplodia oryzae*	*Fusarium oxysporium*	*Trichoderma viride*
1	0	20	20	16	16	20	14	0	14	20	0	0	14
2	0	0	14	14	14	0	14	0	14	14	0	0	0
3	0	0	0	0	0	0	20	0	0	0	0	0	0
5	18	14	20	20	22	14	20	0	16	14	0	0	0
8	0	20	26	24	22	14	14	0	14	20	0	0	14
11	0	15	14	16	22	14	14	0	14	20	0	0	14
13	0	0	17	14	14	0	14	0	14	22	0	0	0
15	0	0	0	0	0	0	20	16	14	0	0	0	0
23	0	0	18	18	24	14	14	0	14	24	0	0	0
24	0	16	16	16	22	14	14	0	14	16	0	0	16
27	0	14	16	16	22	16	14	0	14	18	14	0	12
29	18	0	16	14	26	0	16	0	14	14	0	0	0
31	0	0	0	16	0	16	16	14	0	20	0	0	0
32	0	14	14	16	18	14	14	0	14	14	0	0	14
34	0	0	16	14	14	0	14	0	14	14	0	0	0
44	0	16	16	16	18	14	14	0	14	14	0	0	14
63	16	12	24	24	20	0	24	0	14	18	0	0	0
65	0	0	0	0	0	0	20	16	14	0	0	0	0
81	0	0	16	14	16	14	14	0	14	14	0	0	14
101	0	0	14	14	14	0	16	0	14	16	0	16	0
104	0	0	18	14	14	0	16	0	14	16	0	0	0
106	0	0	16	14	14	0	14	0	14	14	0	0	0
108	0	0	0	0	0	0	16	0	18	20	16	0	14
109	0	0	0	16	0	16	14	14	0	20	0	0	0
200	0	0	0	30	0	30	30	14	0	20	0	0	0



Straight spore-bearing hyphae of isolates No. 1,8,11,15, 27, 109,200

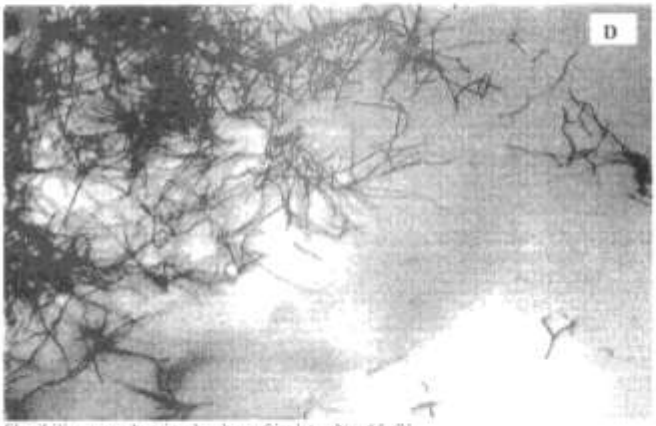


Hook spore-bearing hyphae of isolate No.13

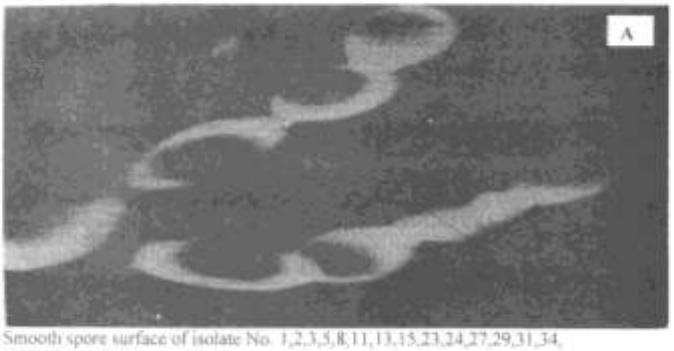
Fig. (1): Morphological characteristics of bearing hyphae of streptomycetes isolates (Photograph 400X). **(Continued)**

Fig. (1): (Continued)

Spiral spore-bearing hyphae of isolates No 2,3, 5,23,24,29,31,32,34,44,63,101,104,106,108

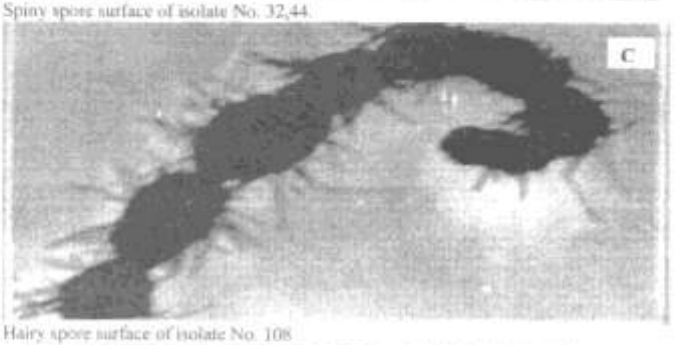


Flexibilis spore- bearing hyphae of isolates No. 65, 81



Smooth spore surface of isolate No. 1,2,3,5,8,11,13,15,23,24,27,29,31,34, 63,65,81,101,104,106,109,200.

Spiny spore surface of isolate No. 32,44.



Hairy spore surface of isolate No. 108.

Fig.(2): Morphological characteristics of spore surface of streptomycetes isolates (Photograph 25 000 X).

Table (2): Morphological and cultural characteristics of active *Streptomyces* isolates

No. of active isolates	Spore chain morphology	Spore chain ornamentation	Colour of spore mass	Pigmentation of substrate mycelium	Diffusible pigment
1	Straight	Smooth	Medium grey	Light brown	Colorless
2	Spiral	Smooth	Greyish brown	Light brown	Colorless
3	Spiral	Smooth	Light grey	Orange yellow	Colorless
5	Spiral	Smooth	Medium grey	Light brown	Colorless
8	Straight	Smooth	Medium grey	Light brown	Colorless
11	Straight	Smooth	Medium grey	Pale yellow	Colorless
13	Hook	Smooth	Medium grey	Pale yellow	Colorless
15	Straight	Smooth	Yellowish white	Orange yellow	Colorless
23	Spiral	Smooth	Medium grey	Light brown	Colorless
24	Spiral	Smooth	Dark grey	Brownish yellow	Colorless
27	Straight	Smooth	Medium grey	Pale yellow	Colorless
29	Spiral	Smooth	Dark grey	Dark brownish yellow	Colorless
31	Spiral	Smooth	Yellowish white	Orange yellow	Colorless
32	Spiral	Spiny	Dark grey	Pale yellow	Colorless
34	Spiral	Smooth	Light grey	Pale yellow	Colorless
44	Spiral	Spiny	Dark grey	Yellowish white	Colorless
63	Spiral	Smooth	Medium grey	Pale yellow	Colorless
65	Flexibilis	Smooth	Yellowish white	Orange yellow	Colorless
81	Flexibilis	Smooth	Light grey	Pale yellow	Colorless
101	Spiral	Smooth	Dark grey	Dark brown	Colorless
104	Spiral	Smooth	Light grey	Dark orange brown	Colorless
106	Spiral	Smooth	Medium grey	Pale yellow	Colorless
108	Spiral	Hairy	Medium grey	Pale yellow	Colorless
109	Straight	Smooth	Yellowish white	Light yellow brown	Colorless
200	Straight	Smooth	Light grey	Light yellow brown	Colorless

Table (3): **Biochemical and physiological characteristics of active *Streptomyces* isolates.**

Characteristics	Number of strains	
	(Positive)	(Negative)
Utilization of different carbon source Glucose	1,2,3,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109	200
D-fructose	1,2,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109	3, 200
Sucrose	1,2,3,5,8,11,13,15,23,24,27,29,31, 32,34,44,63,65,81,101,104, 106,108,109	200
Xylose	1,2,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109	3,200
L-Arabinose	1,2,3,5,8,11,15,13,23,24,27,29 31,32,34,44,63,65,81,101.104, 106,108,109	200
Raffinose	1,2,3,5,8,11,13,23,24,27,29 31,32,34,63,81,101, 104,106	15,44,65,108,109, 200
Galactose	1,2,3,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109,200	None
Rhamnose	1,2,3,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109,200	None
D-mannitol	1,2,3,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109,200	None
Enzyme Activities: Proteolytic	2,5,11,13,15,23,27,29 31,32,34,44,63,65,81,101,104, 109,200	1,3,8,24,106,108
Lipolytic	1,5,11,23,27,29,34,81,106, 108	2,3,8,13,15,24,31,32,4 4,63,65,101,104, 109,200
Lecithinase	2,5,8,15,23,24,27,29 31,34,63, 65, 81,101,104,108,109, 200	1,3,11,13,32,44,106

(Continued)

Table (3), Continued.

Characteristics	Number of strains	
	(Positive)	(Negative)
Degradition of Pectin	1,2,3,5,8,11,13, 23,27,29 31,32,34,44,63,81,101,104, 106,108	15,24,65,109,200
Chitin	None	1,2,3,5,8,11,13,15,23,2 4,27,29,31,32,34,44,63 ,65,81,101,104,106,10 8,109,200
Xanthine	2,5,11,13,24,27,29,32,200	1,3,8,15,23,31,34,44,6 3,65,81,101,104,106,1 08, 109
Arbutin	1,2,3,5,8,11,13,15,23,24,27,29 31,32,34,44,63,65,81,101,104, 106,108,109,200	None
Melanine production: Iron peptone	108	1,2,3,5,8,11,13,15,23,2 4,27,29,31,32,34,44,63 ,65,81,101,104,106,10 9,200
Tyrosine	None	1,2,3,5,8,11,13,15,23,2 4,27,29,31,32,34,44,63 ,65,81,101,104,106,10 8,109,200
Nitrate reduction	2,3,5,8,11,13,15,23,24,27, 31,63,65,101,104, 108,109,200	1,29,32,34,44,81,106
Hydrogen sulphide production	1,2,5,8,11,13,15,23,27,29 31,32,34,44,63,65,81,101,104, 106,108,109,200	3,24

spirals (Fig.1-C) and 2 (8%) possessed flexibilis or flexous (Fig.1-D). The majority of the isolates (88%) showed smooth spore surface (Fig.2-A) as indicated by the transmission electron microscope; however, only 8 and 4% showed spiny (Fig.2-B) and hairy spore chain ornamentation, respectively (Fig.2-C).

3.3. Cultural characteristics of active isolates

As shown in Table (2), the majority of the isolates 21 (84%) belonged to the grey color series and only 4 (16%) were yellowish white. Diffusible pigments were not detected for all these isolates.

3.4. Physiological and biochemical characteristics of active isolates

The physiological properties of the active isolates are transcribed in Table (3). The 25 isolates varied in their ability to assimilate various carbon sources, all of them utilized D manitol, rhamnose and galactose ; 24 utilized glucose, sucrose and L arabinose; 23 utilized D fructose and Xylose; however, only 18 isolates utilized raffinose. Concerning the enzyme production, it was found that 76% of the active isolates were proteolytic, 40% lypolytic, 68% produced lecithinase, 84% degraded pectin, 36% degraded xanthine and 100% degraded arbutin however, none of them degraded the chitin. Most isolates (23) produced H_2S and 19 were nitrate reducers. None of the isolates synthesized melanoid pigments on tyrosine agar medium; however, it was produced by only one isolate on peptone-yeast extract-iron agar.

3. 5. Chemotaxonomy

Scrutiny of the whole-cell hydrolysate of the active isolates proved that all have a chemotype I cell wall characterized by LL-DAP acid. No diagnostic sugars were found.

3. 6. Identification of *Streptomyces* strains

On the basis of morphological, physiological, biochemical and chemotaxonomical characteristics and using the computerized data base to compare the biological properties of the active isolates with those of other *Streptomyces* spp., the strains were subjected to hierarchical cluster analysis using the similarity matching coefficient (S_{sm}) and clustered by UPGMA. At 94% similarity many clusters were formed. The first cluster contained ten strains all united at 97% similarity level and identified as *Streptomyces lydicus.* The second cluster is connected with a single member phenon of *Str. griseoflavus*. The third cluster containing 8 strains united at 94% similarity matrix, was identified as *Str.* a*troolivaceus*. The fourth cluster contained one strain and was nearly identical to *Str. violacensniger*. The fifth cluster contained two strains which were identical to *Streptomyces microflavus*. The last cluster contained three strains,all of which were identical to *Steptomyces anulatus*. The obtained results are illustrated in a dendogram (Fig. 3).

4. DISCUSSION

Streptomyces have been recognized as the most plentiful source of microorganisms for all types of secondary bioactive metabolites that have crucial applications in human medicine as anti-microbial and anti-cancer compounds and in agriculture fields as herbicides, insecticides and antiparasitic compounds (Watve *et al.*, 2001). *Streptomyces* are widely represented in nature by the largest number of species and varieties. They differ greatly in their morphology, physiology and biochemical acitvities producing the majority of known antibiotics (Taddei *et al*., 2006). As correspond to their habitat, these bacteria are nutritionally quite versatile and the most to produce extracellular hydrolytic enzymes that permit the utilization of high molecular weight biopolymers such as proteins, polysaccharides, fats and other substrates (Antanova- Nikolova *et al.*, 2004).

Depending upon colony characteristics of streptomycetes, 200 putative isolates were obtained, only 25 showed variable antimicrobial activities against the tested organisms of Gram negative, Gram positive bacteria, yeasts and filamenous fungi. The degree of antimicrobial activity of the antagonestic isolates was evaluated depending on the mean diameter of the inhibition zone in mm (Ø mm) and divided to the following groups: very weak (Ø <16 mm), weak (Ø 16 – 19 mm), moderate (Ø 20 – 30 mm) and high activity (Ø 30 mm or more) according to Landerkin *et al.*, (1950).

Various classifications were contrived to conciliate the increasing number of *Streptomyces* species, most of them are based on a few intuitively chosen morphological and pigmentation properties which were rarely studied under standardized growth conditions (Atalan *et al.,* 2000). Biochemical, nutritional and physiological characters used in streptomycetes taxonomy, usually had been applied to only selected species (Williams *et al.*, 1983; Kutzner *et al.*, 1989 and Schlegel, 1992)

A comparative analysis of the obtained results concerning morphological, physiological, biochemical and chemotaxonomical characteristics using SPSS statistical software devided the 25 bioactive streptomycetes into 6 clusters. The

first major cluster contained ten isolates nearly identical to *Streptomyces lydicus*, showing activity (Crawford *et al.*, 1993; Yuan and Crawford, 1995 and Tokala *et al.*, 2002).

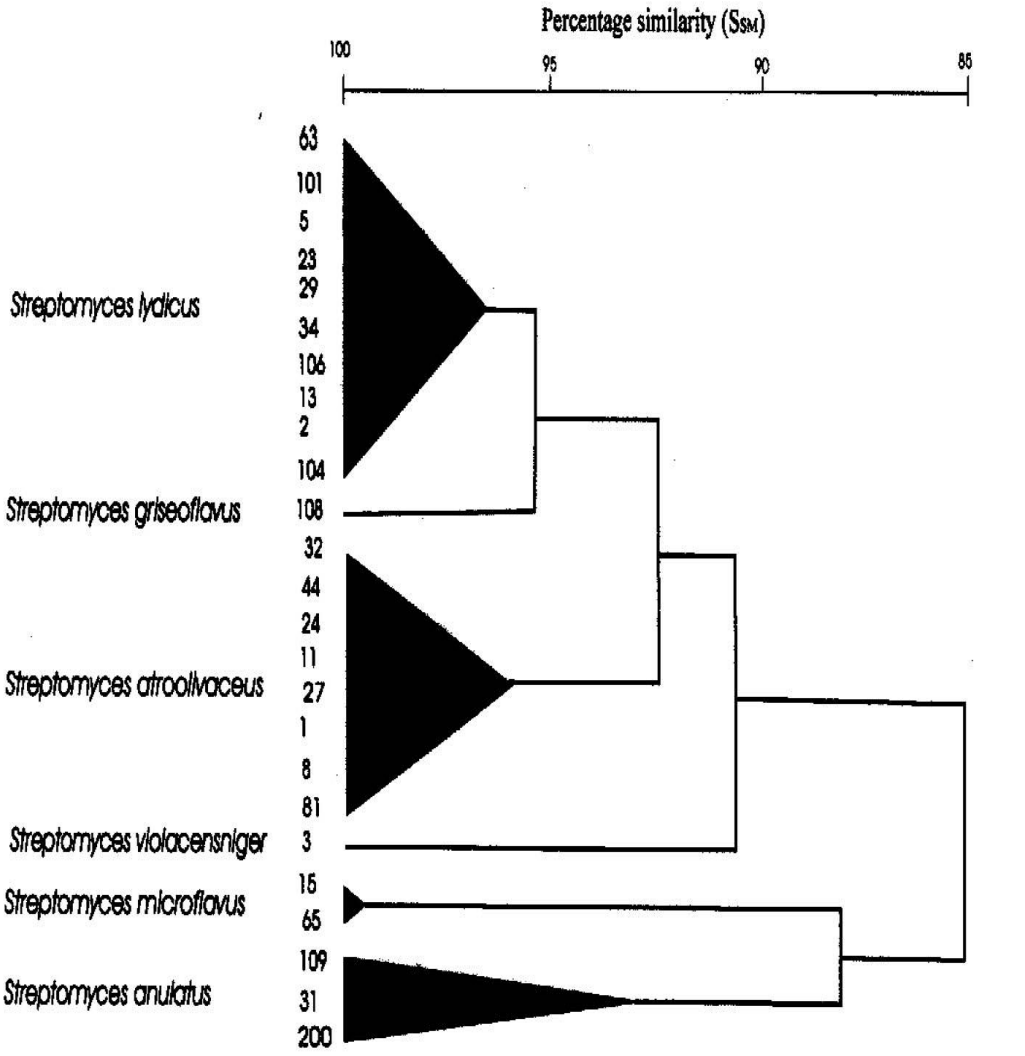


Fig (3): Dendrogram of active *Streptomyces* species isolated from the different Egyptian soils.

against Gram-positive bacteria and fungi. *Streptomyces lydicus* isolated by Singh and Gurusiddaiah (1984) from the deep-pitted lesion of potato tubers, was found to produce a new polypeptide antibiotic named chandramycin which showed activity against several of Gram-positive and a few Gram negative species of bacteria. It also showed a strong activity against anaerobic microorganisms. The strain of *Streptomyces lydicus* WYEC 108 was found to act as antifungal biocontrol agent and as a plant growth promoting bacterium in the absence of fungal pathogen challenge as well as a root colonizing active actinomycete which influence the pea root nodulation by increasing nodulation frequency at the infection level by *Rhizobium* spp.

Only one strain belongs to the second cluster and is nearly identical to *Streptomyces griseoflavus* and was found to be active against fungi only. In 1995, Ubukata and his coworkers isolated three novel 36-membered macrolide active antibiotics against fungi from *Streptomyces griseoflavus*. As well, Grote and Zeeck (1988) found that colabomycin A (1) as an antimicrobial agent, produced by *Str. griseoflavus*, belongs to the manumycin group of antibiotics. S-adenosylmethionine had been found to cause overproduction of bicozamycin by *Streptomyces griseoflavus* when added to the medium at an appropriate concentration (Saito *et al.*, 2003).

The third cluster contained eight isolates are nearly identical to *Str. atroolivaceus*, showed

activity against Gram-positive bacteria, yeast and filamentous fungi (Stajner, *et al*., 1973).

The strain no. 3 which belongs to the fourth cluster is nearly identical to *Str. violacensniger*, showed activity against *Aspergillus niger* only. Höltzel *et al.* (1998) isolated spirofungin as a new antifungal antibiotic from *Streptomyces violacensniger* Tü 4113 which shows various activities, particularly against yeasts.

In the same year, Trejo-Estrada *et al.*(1998) discovered that the strain *Streptomyces violacensniger* YCED-9 produces three antimicrobial compounds with antifungal activity, AFA (Anti-*Fusarium* activity) a fungicidal active against most fungi except oomycetes; nigricin, a fungistatic polyether; and geldanamycin, a benzoquinoid polyketide highly inhibitory on mycelial growth indpendently. Hayakawa *et al.*, (2004) reported that 77% of the total streptomycete isolates were assigned to the *Streptomyces violacensniger* cluster, these isolates had broad antimicrobial spectra as they inhibited the growth of all tested Gram positive bacteria, yeasts and filamentous fungi.

The fifth cluster contained two strains that are identical to *Streptomyces microflavus*, showed activity against fungi only. Fattiviracins (FV) as antiherpetic antibiotics are produced by *Streptomyces microflavus* as reported by Uyeda (2003) the strain found to produce at least 13 derivatives (FV-1 to FV-13). He added that fattiviracins have a potent activity against enveloped DNA viruses such as herpes family and enveloped RNA viruses such as influenza A and B viruses.

The three strains identical to *Steptomyces anulatus* are found in the last cluster and showed activity against Gram-positive bacteria, yeast and fungi. Praveen *et al.*,(2008**)** found that the biologically active strains: *Streptomyces halstedii* MTCC 6817 and *Streptomyces anulatus* MTCC 6818 produced the same antibiotic that was chemically characterized as actinomycin-D. As reported by Philipp *et al.*,(2002), a detailed screening of the secondary metabolite pattern produced by different athropod associated strains of the species *Streptomyces anulatus* resulted in the isolation and structure elucidation of the endophenazines A-D (2, 4-6).

Conclusion

The 200 putative streptomycetes were isolated from different fertile soil samples. Screening was carried out according to their biological activity revealed that only 25 isolates showed uneven antagonistic effect against the forecited test organisms. A comparative analysis of the obtained results concerning morphological, physiological, biochemical and chemotaxonomical characteristics using SPSS statistical software divided the 25 bioactive streptomycetes into 6 clusters: *Streptomyces lydicus, Streptomyces griseoflavus, Str. atroolivaceus, Str.violace-nsniger, Streptomyces microflavus* and *Steptomyces anulatus.*

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دراسات تقسيمية على العزلات الفعالة من الإستربتوميسيس المعزولة من الأراضى المصرية

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ملخص

أجرى هذا البحث بغرض عزل الإستربتوميسيتات النشطة بيولوجيا من عينات تربة زراعية من مناطق مختلفة (القوصية/أسيوط و بلقاس/دقهلية، والجيزة) حيث تم الحصول على 200 عزلة. و بدراسة النشاط البيولوجى لهذه العزلات وجد أن 25 عزلة فقط هي التي لها نشاط بيولوجى مضاد للبكتريا السالبة لجرام، والموجبة لجرام، والخميرة والفطريات الخيطية0 لذا تم دراسة الخواص المورفولوجية، والفسيولوجية، والنشاط الإنزيمى ، بالإضافة إلى تركيب الجدار الخلوى لهذه العزلات النشطة0 وبناءاً على هذه الخواص تم تقسيم ال 25 عزلة النشطة الى 6 تجمعات عنقودية و بتحليل النتائج إحصائيا باستخدام برنامج SPSS أوضحت النتائج أن التجمع العنقودى السائد هو *Streptomyces lydicus* يليه على التوالى *S. 0 atroolivaceus*