ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 47(2): Bo. 51-58, (2009).

CLASSIFICATION PROFILE OF THREE DIFFERENT STREPTOMYCETE ISOLATES FROM SHARKIA GOVERNORATE SOILS

BY

Howaida M.L. Abdel-Basit
Dept. of Agric. Microbiology, Faculty of Agric., Zagazig Univ., Zagazig, Egypt

ABSTRACT

A total number of 45 actinomycete isolates was isolated from different soil samples collected from Fakous, Abo-Kibeer and Salhia, Sharkia Governorate, Egypt. Only three isolates of streptomycetes belonged to red, violet and yellow series and designated ASH1, ASH2 and ASH3, respectively were identified based on their cultural, morpholgical and physiological characters as reported by the keys of Bridhamd and Tresner (1974) in Bergey's Manual. The streptomycete isolates were found to belong to different *Streptomyces* strains. The isolate ASH1 was completely identified as *S. melanogenes*, which exhibited similar characters with *S. melanogenes* with slight differences in growth on Czapek's agar medium. The isolate ASH2 was identified as *S. violans*, exhibiting similar characters with slight differences in the sensitivity to streptomycin and the ability to grow in the presence of NaCl up to 7.0%. While, the isolate ASH3 was identified as *S. alboflavus*, which exhibited similar characters except for the ability to grow at zero to 7.0% NaCl . The three strains were capable of producing antibiotics which highly active *in vitro* against the tested Gram-positive and Gram-negative bacteria, yeast and fungi. This study focused on actinomycetes and their importance in producing antibiotics.

INTRODUCTION

Among the genera of actinomycetes, the genus *Streptomyces* is represented in nature by the largest number of species and varieties, which differ greatly in their morphological, physiological and biochemical activities. Interestingly, the majority of the antibiotic-producing actinomycetes are found among these species, which led to a growing economic importance for this group of organisms (Taddei *et al.*, 2006).

Streptomycetes exist mainly as spores in their natural habitat, soil, and form vegetative mycelia under favorable growth conditions. They constitute the largest actinomycete group in a number of soils (Srinivasan *et al.*, 1991). Also, they are nonfastidious organisms, which are able to degrade complex biological compounds like cellulose, lignin and chitin, and are satisfied with an inorganic nitrogen source (Anderson *et al.*, 1998).

However, *Streptomyces* species are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites, such as antibiotics, herbicides, insecticides and enzymes (Srinivasan *et al.*, 1991; Anderson *et al.*, 1998 and Hapwood, 2006). Thus, approximately 10,000 antibiotics have been identified, and almost half of them are generated by streptomycetes (Demain, 1999 and Lazzarini *et al.*, 2000). A variety of bioactivities is associated with secondary metabolites generated by streptomycetes, including antibacterial, antifungal, antiviral, antitumor and enzyme inhibitory activities (Wu *et al.*, 2007).

Thus, streptomycetes are renowned for production of array industrially important metabolites, therefore, the isolation, identification and characterization processes of *Streptomyces* isolates are still very important in

search for new strains and/or novel metabolites with economic interest in diverse fields. In Egypt, numerous researches were performed on these bacteria in diverse branches of agriculture (Zaki *et al.*, 1993; Mohamed, 1998; Mohamed *et al.*, 2000 and 2001 and Mahfous and Mohamed, 2002).

Therefore, the aim of the present study is to describe the cultural, morphological and physiological characters as well as the antagonistic activity, in addition to, the classification of three different streptomycete isolates from local soils.

MATERIALS AND METHODS

Sampling:

Soil samples were collected from different locations at Sharkia Governorate. A total number of 45 soil samples was collected from Fakous, Abo-Kibeer and Salhia soils. At each location, 15 samples were randomly taken from different sites and kept individually in a sterile bag. Samples were kept at 4°C in the laboratory until isolation. A soil sample from each location was used for soil analysis (Table 1).

Isolation and purification of actinomycete isolates:

Soil samples were air-dried for three days and then ground in a mortar using pestle. Isolation of actinomycetes was performed by serial dilutions and spread plate technique. One gram soil was suspended in 9 ml of sterile double distilled water. Aliquots were spread on the plates containing starch casein agar (Berman *et al.*, 1994) and incubated at 28°C for 7 to 14 days. A total number of 45 actinomycetes isolates was taken as one isolate from each soil sample.

The cultural characteristics of these isolates were tested on the basis of observation, made after 7, 14 and 21 days of incubation on Czapek-dox agar, nutrient agar and International *Streptomyces* Project (ISP) media as described by Shirling and Gottlieb (1966). Only three *Streptomyces* isolates were selected and purified according to their color of aerial mycelium . These selected isolates were designated as ASH1, ASH2 and ASH3.

Table (1): Mechanical, physical and chemical analysis of soil samples in different locations of Sharkia Governorate.

Type of soil analysis	Properties	Fakous	Abo-Kibeer	Salhia
Mechanical analysis	Sand%	38.8	70.8	89.0
	Silt%	5.60	7.60	5.70
	Clay%	55.6	21.6	5.10
	Type of soil	Clay	Sandy-clay loam	Sandy
Physical analysis	pH	8.00	8.01	7.75
	E.C. dsm^{4}	6.40	8.80	4.20
	O.M%	1.82	1.52	1.33
Chemical analysis	Cations			
	Ca^{++}	10.7	11.4	27.1
	Mg^{++}	11.5	13.8	15.3
	Na^{+}	43.5	71.7	20.1
	K^{+}	0.54	0.36	0.31
	Anions			
	HCO_3^-	3.00	3.1	2.80
	Cl^-	32.6	8.0	43.3
	SO_4^-	30.6	3.2	18.1

Morphological characteristics

Visual observation of both morphological and microscopic characteristics using light and electron microscopy (Tresner *et al.*, 1961) and Gram-stain reaction were performed for each of these three chosen isolates. All the morphological characters observed on media described by Shirling and Gottlieb (1966) were used for classification and differentiation as follows: the color of aerial mycelium, color of substrate mycelium, production of melanoid pigments and spore chain morphology. Spore chain type for each isolate was determined according to Pridham and Tresner (1974). Spore ornamentation was observed by electron microscopy (Tresner *et al.*, 1961).

Physiological characteristics

The key given by Pridham and Tresner (1974) for identification of *Streptomyces* isolates was followed. Physiological characteristics were determined after growth at 28±2°C for the recommended incubation periods. All the carbon-sources for carbon-utilization tests were filter-sterilized and tested at the concentrations recommended by Shirling and Gottlieb (1966). Also, sensitivity to streptomycin antibiotic (4 μg ml^{-1}) in the medium was tested.

Salt tolerance

The ability of the *Streptomyces* isolates to grow at different gradient salt concentrations ranged from normal salt concentration of the medium (0.05%) increased to 3, 5, 7, 9, 10, 14, 16 and 18 % salt (NaCl) was tested using starch nitrate agar medium (Waksman and Lechevalier, 1961). The method described by Saleh *et al.* (1990) was adopted for determination of salt-tolerance . The inoculated plates were incubated at 28±2°C up to 15 days and monitored for growth of the isolates.

Antagonistic activity

The antimicrobial activities of the three applied isolates (ASH1, ASH2 and ASH3) were examined using the agar diffusion method (Wu, 1984). The tested microorganisms represented fungi, *i.e.*, *Fusarium oxysporum* f. sp. Lycopersci-123, *Rhizoctonia solani, Alternaria solani* and *Helminthosporium gramenium*-133; yeasts, *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; G-ve rods, *i.e.*, *Escherichia coli*-1319 and G +ve cocci, *i.e.*, *Staphylococcus aureus* coagulase +ve. These organisms were kindly provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University. The bacterial strains were grown on nutrient agar medium, yeast on peptone glucose agar medium (Jacobs and Gerstein, 1960) and fungi on potato glucose agar medium (Waksman and Lechevalier, 1961). The antimicrobial activity was determined by measuring the diameter of inhibition zones (in mm). Uninoculated starch nitrate broth was used as a control.

RESULTS AND DISCUSSION

Results in Table (2) and Figure (1) indicated that the *Streptomyces* isolate ASH1 belonged to the red color series group (Fig. 1-A). The substrate mycelium produced bluish pigment on the standard media used. Aerial spore chains belonged to section RA and the spores were characterized by smooth surface without any ornamentation. Melanoid pigment was detected on the standard media. This isolate (ASH1) was characterized by good growth on Czapek's agar medium.

The physiological characteristics showed that this isolate (ASH1) was able to utilize all tested sugars including D-glucose, D-xylose, L-arabinose, L-rhamnose, D-fructose, raffinose, D-mannitol and i-inositol except sucrose as a sole carbon source for growth . Also, this isolate showed antibacterial activities (Table 5). The sensitivity to streptomycin (4 μg ml^{-1}) and the NaCl tolerance were not detected.

Comparing the cultural, morphological and physiological characteristics of the *Streptomyces* spp. in Pridham and Tresner (1974) with those of this isolate, it is very likely to be a strain of *S. melanogenes* with slight differences in the growth on Czapek's agar medium and the color of substrate mycelium.

Table (2): Classification of streptomycete isolate ASH1

Character	Isolate-ASH1	*S. melanogenes*
Color of aerial mycelium	Red	Red
Spore-chain	RA	RA
Melanoid pigment	+	+
Spore surface	SM	SM
Growth on Czapek's medium	Excellent	ND
Color of substrate mycelium	Bluish	Red, bluish or bluish green
Diffusible pigments	-	ND
Utilization of carbon:		
No carbon	-	+
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	±	-
D-Fructose	+	+
Raffinose	+	+
D-Mannitol	+	+
i-Inositol	+	+
Sucrose	-	±
Antagonistic activity	Antibacterial	Antibacterial and antitumor
Sensitivity to streptomycin	ND	ND
NaCl tolerance	ND	ND

+: Growth. -: No growth. **ND:** Not determined. **SM**: Smooth.
±: Indoubt. **RA:** Spore chain in form of open loops, hooks or greatly extended coils of wide

Results presented in Table (3) and Figure(1) clearly indicate that the characteristics of *Streptomyces* isolate ASH2 belonged to the violet color series (Fig. 1-B) and the substrate mycelium produced violet pigment on the standard medium used. Aerial spore chains belonged to section RF and the spores were characterized by smooth surface without any ornamentation. Also, melanoid pigment was not detected on the standard media. This isolate was characterized by moderate growth on Czapek's agar medium.

The physiological characteristics of this isolate (ASH2) proved its ability to utilize all used sugars including D-glucose, L-arabinose, L-rhamnose, D-fructose, raffinose and i-inositol as carbon sucrose for growth. But, the growth on D-xylose and D-mannitol was not detected (Table 3). Moreover, this isolate showed antibacterial and antifungal activities. The sensitivity to streptomycin (4 $\mu g\ ml^{-1}$) was observed. Also this strain had the ability to grow at NaCl concentrations up to 7.0%.

Comparing the cultural, morphological and physiological characteristics of the *Streptomyces* spp in Pridham and Tresner (1974) with those of isolate ASH2, it is likely to be a strain of *S. violans* with slight differences in the sensitivity to streptomycin and the ability to grow in the presence of NaCl up to 7% in the growth medium .

Results of *Streptomyces* isolate ASH3 presented in Table (4) show that this isolate has yellow color series (Fig. 1-C) and the substrate mycelium produced a brownish pigment. Also, this isolate had straight and long spore chains, section RF, and the spore characterized by smooth surface without any ornamentation. This isolate was also characterized by moderate growth on Czapek's agar medium, produced melanoid pigment and utilized all sugars used as sole carbon sources, except L-rhamnose. Also, this strain was sensitive to streptomycin (4 $\mu g\ ml^{-1}$) and able to tolerate NaCl up to 7% in the growth medium. In addition, the isolate showed antibacterial and antifungal activities (Table 5).

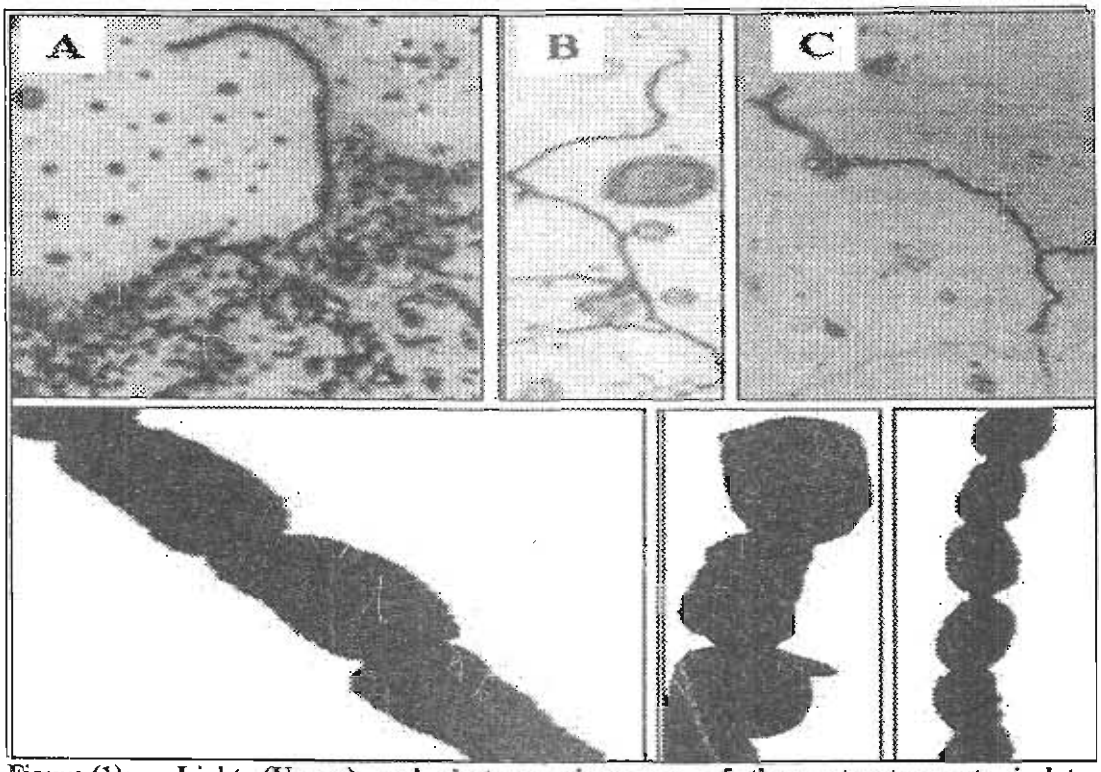

Figure (1): Light (Upper) and electron microscopy of three streptomycete isolates belonging to red (A), violet (B) and yellow (C) series, respectively, showing type of spore chains and spores surface, respectively.

Table (3): Classification of streptomycete isolate ASH2.

Character	Isolate-ASH2	*S. violans*
Color of aerial mycelium	Violet	Violet
Spore-chain	RF	RF
Melanoid pigment	-	-
Spore surface	SM	SM
Growth on Czapek's medium	Moderate	Good
Color of substrate mycelium	Violet	Violet
Diffusible pigments	-	+on some media
Utilization of carbon:		
No carbon	-	-
D-Glucose	+	+
D-Xylose	-	ND
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
Raffinose	+	+
D-Mannitol	-	ND
i-Inositol	+	+
Sucrose	±	+
Antagonistic activity	Antibacterial and antifungal	Antibacterial and antifungal
Sensitivity to streptomycin	S	ND
NaCl tolerance	0-7%	ND

+: Growth. -: No growth. ±: Indoubt RF: Spores in straight (R) or flexous (F) chains.
SM: Smooth. S: Sensitive. ND: Not determined.

Table (4): Classification of streptomycete isolate ASH3.

Character	Isolate-ASH3	*S. alboflavus*
Color of aerial mycelium	Yellow	Yellow
Spore-chain	RF	RF
Melanoid pigment	+	+
Spore surface	SM	SM
Growth on Czapek's medium	Moderate	Moderate
Color of substrate mycelium	Brownish	ND
Diffusible pigments	-	ND
Utilization of carbon:		
No carbon	-	-
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	-	-
D-Fructose	+	+
Raffinose	+	+
D-Mannitol	+	+
i-Inositol	+	+
Sucrose	+	+
Antagonistic activity	Antibacterial and antifungal	Antibacterial and antifungal
Sensitivity to streptomycin	S	S
NaCl tolerance	0-7%	ND

+: Growth. -: No growth. **RF:** Spores in straight (R) or flexous (F) chains
SM: Smooth. **S:** Sensitive. **ND**: Not determined.

Comparing the description proposed by Pridham and Tresner (1974), the isolate ASH3 was closely related to *S. alboflavus* with slight difference represents in the ability to grow at zero to 7.0% NaCl concentrations.

The *Streptomyces* isolates were subjected for a trial to identification using International keys (Shirling and Gottlieb 1966 and Pridham and Tresner, 1974). From the above mentioned keys, the *Streptomyces* isolates ASH1, ASH2 and ASH3 were found closely related to *S. melanogenes, S. violans* and *S. alboulavus* respectively. The three *Streptomyces* isolates produce antibiotics which highly active against bacteria, fungi and yeasts .In general, it is anticipated that the isolation, characterization and the study of actinomycetes can be useful in searching for new antibiotic resources and the discovery of novel species of actinomycetes (Asha Devi *et al.*, 2006).

Data presented in Table (5) revealed the antagonoistic activities of ASH1, ASH2 and ASH3 isolates against the test organisms. In general, actinomycetes are universally distributed and considered as the most important sources of antibiotics (Saleh *et al.*, 1985 and Zaki *et al.*, 1993). The strain *S. violans* ASH2 showed the most inhibitory activities against the test organisms following *S. alboflavus* ASH3, as they were able to antagonize 11 and 8 out of 12 test organisms, respectively. On the other hand, *S. melanogenes* ASH1 showed no antiagonistic activity against all fungi and yeasts used in this study.

Activity against *Staph. aureus* was not observed for *S. violans* ASH2, while *S. alboflavus* ASH3 was not able to antagonize *B. subtilis*-1007. *H. gramenium*-133, *R. solani* and *C. tropicalis* CAIM-2 were not sensitive to the antimicrobial substances produced by *S. alboflavus*. These results are in agreement with those obtained by Lim *et al.* (2000), Rifaat and Kansoh (2004), Asha Devi *et al.* (2006) and Wu *et al.* (2007). They emphasized the capability of *Streptomyces* strains for producing numerous antibiotics that are highly active against Gram-positive and Gram-negative bacteria, yeasts and fungi.

It is concluded that searching for new *Streptomyces* isolates from different soil types led to selecting best indigenous isolates with antifungal and antibacterial abilities.

Table (5): Antimicrobial activities of *Streptomyces* strains against tested microorganisms.

Test organisms	*Streptomyces* strains		
	ASH1	ASH2	ASH3
Bacteria:			
B. cereus-1283	12*	13	14
B. megatherium-1066	12	16	14
B. mycoides-1084	14	14	14
B. subtilis-1007	11	14	0
B. coli	7	13	13
Staph. Aureus coagulase +ve	14	0	13
Fungi:			
A. solani	0	14	16
H. gramnenium-133	0	16	0
F. oxysporum f.sp. Lycopersci	0	14	14
R. solani	0	14	0
Yeast:			
C. albicans CAIM-35	0	13	14
C. tropicalis CAIM-2	0	13	0

* Zone of inhibition (mm).

REFERENCES

Anderson, M.A.; Mikkola, R. and Kroppenstedt, R.M. (1998): The mitochodnrial toxin produced by *Streptomyces griseus* isolated from an indoor environment is valinomycin. Appl. Environ. Microbiol., 64: 4767-4773 .

Asha Devi, N.K.; Jeyarani, M. and Balakrishnan, K. (2006): Isolation and identification of marine actinomycetes and their potential in antimicrobial activity. Bakistan J. Biol. Sci., 9 (3): 470-472 .

Berman, V.S.; Montenergro, D.A.; Korshalla, J.D.; Maiese, W.M.; Steinberg, D.A. and Greenstein, M. (1994): Bioxalomyclins new antibiotics produced by the marine *Streptomyces* spp. LL. 31F508: taxonomy and fermentation. J. Antibiot., 47: 1417-1424.

Demain, A.L.(1999): Pharmaceutically active secondary metabolites of microorganisms. Appl. Microbiol. Biotechnol., 52: 455-463.

Hapwood, D.A.(2006): Soil to genomics: the *Streptomyces chromosome*. Annu. Rev. Genet., 40.

Jacobs, M.B. and Gerstein, M.J. (1960): Handbook of Microbiology. D. Van Nostrand Co., Inc., New York, pp. 139-207.

Lazzarini, A.; Cavaletti, L.; Toppo, G.and Marinelli, F. (2000): Rare genera of actinomycetes as potential producers of new antibiotics. Antonie Van Leeuwenhoek, 78: 399-405.

Lim, S.W.; Kim, J.D.; Kim, B.S. and Hwang, B.K.(2000): Isolation and numerical identification of *Streptomyces humidus* strain S5-55 antagonistic to plant pathogenic fungi. Plant Pathol J., 16 (4): 181-199.

Mahfouz, H.T. and Mohamed, Sonya H. (2002) Physiological, antagonistic and finger printing studies on some halotolerant *Streptomyces* strains. Arab Journal of Biotechnol., 5: 103-120.

Mohamed, H. Sonya (1998): Role of actinomycetes in the biodegradation of some pesticides. Ph..D. Thesis, Agric. Microbiol., Dept., Agric.Microbiol.,Faculty of Agric., Ain Shams University,Cairo,Egypt,pp151

Mohamed H. Sonya; Salim, Sh.M. and Saleh, E.A. (2000): Taxonomical and biochemical studies on some halotolerant actinomycetes isolated from sandy soil in Egypt. Arab Univ. J. Agric. Sci. Ain Shams Univ., Cairo 8 (1): 41-61.

Mohamed, H. Sonya; Abdel-Fattah, H.I.; Selim, Sh.M. and Sharaf, M.S. (2001): Identification and molecular studies on some halotolerant actinomycetes isolated from Sinai sandy soil. Arab. J. Biotechnol., 4: 179-196.

Pridham, T.G. and Tresner, H.D. (1974): Family Streptomycetaceae. In Bergey's Manual of Determinative Bacteriology (1974), 8th ed. Buchanan R.E. and N.E. Gibbons (eds.), Williams and Wilkins Co., Baltmore, USA, pp. 792-793,804-805 & 826-827.

Riffaat, Hala M. and Amany L. Kansoh (2004): *Streptomyces virginiae*: Taxonomy, identification and biological activities. Arab J. Biotech., 8 (1): 29-34.

Saleh, E.A.; El-Haddad, M.E.; Mahmoud, S.A.Z.; Abdel-Fattah, M.K. and Sawsan F. Shehata (1985): Studies on the enzymatic activities of some local *Streptomyces* isolates. Proc. Egypt. Bot. Soc. Ismaillia Con. 16-19 April, 1985. Egypt, Part 1, No. 4: 10-14.

Saleh, E.A.; Zaki, M.M.; El-Demerdash, M.E. and Sonya H. Mohamed (1990): Identification of some halotolerant streptomycetes isolated from marine ecosystems in Egypt. Annals Agric. Sci., Ain Shams Univ., Cairo, Special Issue: 409-425.

Shilring, E.B. and Gottlieb, D. (1966): Methods for characterization of *Streptomyces* species. Inst. J. Syst. Bacteriol., 16: 313-340.

Srinivasan, M.C.; Laxman, R.S. and Deshpande, M.V. (1991): Physiology and nutritional aspects of actinomycetes: an overview. World J. Microbiology and Biotechnolgy, 7: 171-189.

Taddei, A.; Maria Jose Rodriguez; Vilchez, E.M. and Cristina Castrelli(2006): Isolation and identification of *Streptomyces* spp. from Venezuelan soils. Morphological and biochemical studies. Microbiol. Res., 161: 222-231.

Tresener, H.D.; Davies, M.C. and Backus, E.J. (1961): Electron microscopy of *Streptomyces* spore morphology and its role in species differentiation. J. Bacteriol., 81: 70-80.

Waksman, S.A. and Lechevalier, H.A. (1961): The actinomycetes. Vol. II. Classification, identification and description of genera and species. The Williams and Wilkins, Co., Baltimore, USA, p. 340.

Wu, R.Y. (1984): Studies on the *Streptomyces* SC4. II Taxonomic and biological characteristics of *Streptomyces* strain SC4. Bot. Bull. Acad. Sin., 25: 111-123.

Wu, X.C.; Chen, W.F.; Qian, C.D.; Li,O. and Wen, Y.P. (2007): Isolation and identification of newly isolated antagonistic *Streptomyces* sp. Strain AP 19-2 producing chromomycins. Journal of Microbiology, 45 (6): 499-504

Zaki, M.M.; Saleh, E.A.; El-Demerdash, M.E. and Sonya, H. Mohamed (1993): Antimicrobial activities of some halotolerant streptomycetes strains as affected by inoculation period and medium composition. 4th Conf. Agric. Dev. Res., Ain Shams Univ., Cairo, feb. 13-18, 1993. Annals Agric. Sci, Sp. Issue, 2, 519-529.

الوضع التقسيمى لثلاثة أنواع من الإستربتوميسيتات المعزولة من أراضي محافظة الشرقية

هويدا محمد لبيب عبد الباسط
قسم الميكروبيولوجيا الزراعية- كلية الزراعة- جامعة الزقازيق

تم عزل ٤٥ عزلة من الاكتينوميسيتات من عينات تربة جمعت من فاقوس وأبو كبير والصالحية من محافظة الشرقية ج.م.ع. وقد تم إختيار وتعريف ثلاثة عزلات بإستخدام الصفات الظاهرية والفسيولوجية والبيوكيميائية بإستخدام مفاتيح الموصى بها عالميا لهذا الغرض حيث تبين أنها تنتمي إلى جنس الاستربتوميسيس. وقد وجد أن العزلة ASH1 تنتمي إلى المجموعة الحمراء ومطابقة للنوع *S. melanogenes* مع إختلاف بسيط في قدرتها على النمو على بيئة زابكس وكذلك العزلة ASH2 والتي تنتمي إلى المجموعة البنفسيجية مطابقة للنوع *S. violans* مع إختلافها في عدم قدرتها على تحمل المضاد الحيوي الاستربتوميسين. أما العزلة ASH3 والتي تنتمي إلى المجموعة الصفراء وهى مطابقة للنوع *S. alboflavus* مع إختلاف بسيط في عدم تحملها لتركيز ملح كلوريد الصوديوم من صفر إلى ٧%. وقد أظهرت الثلاثة عزلات المختارة نشاطاً إحيائيا ضد مجموعة من البكتريا (الموجبة والسالبة لصبغة جرام) والفطريات والخمائر مما يوضح أهمية عزل ودراسة أنواع جديدة من الأكتينوميسيتات المنتجة للمضادات الحيوية.