Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

IDENTIFICATION OF EIGHT HALOTOLERANT STREPTOMYCETE ISOLATES USING A SUGGESTED NUMERICAL TAXONOMY

[42]

Mohamed Sonya, H.[1]; E.A. Saleh[2] and M.M. Zaki[2]

ABSTRACT

A numerical method was suggested for the identification of eight local halotolerant streptomycete isolates. Cultural, morphological, physiological and antagonistic characteristics of those isolates were determined. Arbitrary scoring of these characters for the eight unknown isolates and those of the more related known *Streptomyces* species in the key of **Pridham and Tresner (1974)** was given and resulted 58 characteristic units. The degree of similarity was determined using the Dice coefficient method and clustering was achieved using the unweighted pair group method average (UPGMA) algorithm. Using this numerical method, six out of the eight *Streptomyces* isolates, i.e., ST08, ST09, ST11, ST13, ST14 and ST15 were identified as *S. longisporus*, *S. janthinus, S. griseochromogenes*, *S. antibioticus S. baarnensis* and *S. albolongus* with 100, 95.8, 95.0, 92.8, 95.0 and 91.1%, respectively**.** Isolates ST10 and ST12 were duplicate of *S. echinatus* with similarities of 93.3 and 94.6%, respectively. Application of the suggested numerical taxonomy on the 14 known *Streptomyces* species revealed that these species fell into three major clusters based on their color of aerial mycelia.

Key words: *Streptomyces*, Numerical identification, Halotolerant, Characteristic units, Taxonomy

INTRODUCTION

Genus *Streptomyces* comprises, by far, the largest number of species of actinomycetes now known to occur in nature **(Williams *et al* 1989**). Many investigators throughout the world are isolating cultures of streptomycetes from soils **(Goodfellow *et al* 1987; Srinivasan *et al* 1991 and Mohamed *et al* 2001)** and other substrates **(Saleh *et al* 1990 and Mohamed *et al* 2000)** and studying their cultural, physiological and biochemical activities **(Abdel-Fattah, 2005).**

Goodfellow (1967) described a numerical taxonomy method and cultures accordingly. Numerical taxonomy methods were thereafter applied to the

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

genus *Streptomyces* by **Paszkiewicz (1972); Kurylowicz *et al* (1975) and Goodfellow *et al* (1992). Goodfellow *et al* (1979)** used a numerical system to classify 156 *Actinomadura* strains, and found marker strains of related taxa, and related isolates from bagasse and fodder *via* the numerical phenetic analyses using 90 unit characters. **Williams *et al* (1983)** suggested a standard numerical classification of 475 strains, of which 394 type cultures of *Streptomyces*, and 14 other actinomycete genera.

The present study suggests a numerical taxonomy method for eight *Streptomyces* isolates by comparing their phylophenetic characters with their corresponding strains in the eighth edition of Bergey's Manual of Determinative Bacteriology **(Pridham and Tresner, 1974).**

MATERIAL AND METHODS

Streptomyces isolates

Eight-halotolerant streptomycete isolates belonging to white (ST08, ST14 and ST15); red (ST09) and gray (ST10, ST11, ST12, and ST13) series were kindly provided from the Department of Agricultural Microbiology, Institute of Soil, Water and Environment Research, ARC, Giza, Egypt.

Characterization of *Streptomyces* isolates

Cultural and morphological characteristics of *Streptomyces* isolates under investigation were determined as proposed by **Pridham and Tresner (1974)** using the media and methods of The International *Streptomyces* Project (ISP) as described by **Shirling and Gottlieb (1966)**. In addition, antagonistic activities of the tested *Streptomyces* isolates against 2 fungi, 2 yeasts and 5 bacteria (kindly provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University) were determined as described by **Mohamed *et al* (2001).** Salt tolerance range was also studied using four NaCl concentrations, i.e., 3.5, 7.0, 10 and 13% as mentioned by **Mohamed *et al* (2000).** Chain type and spore surface of the *Streptomyces* isolates were determined as recommended by **Pridham and Tresner (1974)** using the light and electron microscopy, respectively**.** The abilities of the isolates to produce melanoid pigment, growth on Czapek's medium, to produce diffusible pigments, to tolerate streptomycin (4µg ml^{-1}) and to utilize nine carbon compounds were studied as described by **Saleh *et al* (1990).**

Numerical identification of *Streptomyces*

Characters of *Streptomyces* reported in the key of **Pridham and Tresner (1974)** in the eighth edition of Bergey's Manual of Determinative Bacteriology

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

were divided into two parts. Part I represents the main taxonomical characters that included color of aerial mycelium, spore-chain, melanoid pigment, spore surface and utilization of carbon compounds. Part II includes the other assisted characters for streptomycetes classification, i.e., growth on Czapek's medium, anti-bacterial and -fungal activities, sensitivity to streptomycin, color of substrate mycelium, diffusible pigments and NaCl tolerance.

Suggested scoring of each of the previous characters (main and assisted) was given 1 unit if present or 0 unit if absent **(Table 1).** Relative importances for each of these characters were arbitrary

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

Table 1. Arbitrary numerical scoring for the characters of *Streptomyces* isolates as present (1) or absent (0), for their identification and classification.

Characters for streptomycetes identification	Present	Absent
I-Main taxonomical characters		
a-Color of aerial mycelium (22 units)		
Violet	1 (1)	0
Green	1 (4)	0
Yellow	1 (7)	0
Blue	1 (10)	0
White	1 (13)	0
Red	1 (16)	0
Gray	1 (22)	0
b-Spore-chain (4 units)		
RA	1 (2)	0
S	1 (2)	0
RA-S	1 (3)	0
RF	1 (4)	0
c-Melanoid pigment (1 unit)	1 (1)	0
d-Spore surface (5 units)		
SM	1 (5)	0
WTY	1 (1)	0
SPY	1 (3)	0
H	1 (2)	0
e-Utilization of carbon compounds (17 units)		
No carbon	1 (1)	0
D-Glucose	1 (1)	0
D-Xylose	1 (1)	0
L-Arabinose	1 (1)	0
L-Rhamnose	1 (2)	0
D-Fructose	1 (2)	0
Raffinose	1 (2)	0

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Table 1. Cont.

Characters for streptomycetes identification	Present	Absent
II- Other assisted characters		
f- Growth on Czapek's medium (5 units)		
Fair	1 (1)	0
Poor	1 (2)	0
Moderate	1 (3)	0
Good	1 (4)	0
Excellent	1 (5)	0
g- Antibacterial activity (2 units)	1 (2)	0
h- Antifungal activity (2 units)	1 (2)	0
Subtotal	**58 (1)**	**0**
i- Sensitivity to streptomycin (1 uint)	1 (1)	0
j- Color of substrate mycelium (1 unit)	1 (1)	0
k- Diffusible pigments (1 unit)	1 (1)	0
l- NaCl tolerance (4 units)		
0-7%	1 (2)	0
0-10%	1 (3)	0
> 10%	1 (4)	0
Total units	**1 (65)**	

suggested according to their importance in the identification of streptomycetes. Accordingally, color of aerial mycelium was given a maximum of **22** units, type of spore chain **4** units, melanoid pigments **1** unit and spore surface **5** units. Utilization of carbon compounds was given **17** units, growth on Czapek's medium **5** units, antibacterial activity **2** units, antifungal activity **2** units, sensitivity to streptomycin **1** unit, color of substrate mycelium **1** unit, diffusible pigments **1** unit and NaCl tolerance **4** units. Within each character, its units were distributed according to frequency distribution of sub-characters. For example, gray, red, white, blue, yellow, green, and violet aerial mycelia were given 22, 16, 13, 10, 7, 4 and 1 units, respectively.

Cultural, morphological and assisted characters of the eight *Streptomyces* isolates were compared with those of the most similar 14 *Streptomyces* species in the key of **Pridham and Tresner (1974)**. The similarity matrix between the experimental and identified species, in

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

Pridham and Tresner (1974), was determined by Dice Coefficient method. In addition clustering of all characters was determined by the unweighted pair group method with average (UPGMA) algorithm **(Sneath and Sokal, 1973).** Analyses were done using the Diversity Data base™ Version 2.0 from Bio-Rad.

Suggested numerical identification was based on the four characteristics, which are used for streptomycetes identification in **Pridham and Tresner (1974)**. In addition, some of the assisted characters, i.e., growth on Czapek's medium, anti- bacterial and -fungal activities due to their use in the identification of more than 60% of *Streptomyces* species. The unit character for the main characters was 49, and those of selected assisted characters were 9 units making a total of 58 unit characters. The rest of assisted characters, namely, sensitivity to streptomycin, color of substrate mycelium, diffusible pigments, and NaCl tolerance are not usually reported for identification of the majority of *Streptomyces* species in the key of **Pridham and Tresner (1974)**.

RESULTS AND DISCUSSION

Characterization of the *Streptomyces* isolates

Characterization of white, red and gray streptomycete isolates are given in Table (2), Figures (1), (2) and could be represented as follows:

White *Streptomyces* isolates

The two white *Streptomyces* isolates, namely, ST14 and ST15 were characterized with RF chain spores and smooth surface spores, while the third (ST08) had RA chain spores and spiny spore surface. Both of ST08 and ST15 were able to produce melanoid pigment while, ST14 did not. Regarding the utilization of carbon compounds, ST08 utilized all the carbon compounds as sole sources of carbon. On the other hand, ST14 and ST15 varied in their utilization of raffinose and sucrose, and did not utilize mannitol. The three isolates showed good or excellent growth on Czapek's medium. The ST14 and ST15 isolates showed white aerial mycelium and only showed antibacterial activities. On the other hand, ST08 showed colorless aerial mycelium and no antagonistic activities against the bacterial or fungal test organisms. Variation was also observed in the sensitivity of these isolates to streptomycin.

Red *Streptomyces* isolates

The red *Streptomyces* isolate, namely, ST09 was characterized with RA or spiny chains spore with smooth surface, producing melanoid pigments, utilizing of

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

all carbon compounds as sole source of carbon, good growth on Czapek's medium, yellow substrate mycelium, not sensitive to streptomycin, antagonistic activity against the bacterial test organisms.

Gray *Streptomyces* isolates

Three of the four gray *Streptomyces* isolates, namely, ST10, ST11 and ST12 were characterized with RA or spiny chain spores, while the fourth (ST13) showed RF chain spores with smooth spores, and all of them produced melanoid pigment. The color of their aerial mycelia varied between yellow to grayish yellow. They showed variation in

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

Table 2. Taxonomical characters of eight halotolerant streptomycete isolates according to the key of **Pridham and Tresner (1974) .**

Streptomyces isolates	I- Main taxonomical characters							
	a-Color of aerial mycelium		b-Spore-chain*		c-Melanoid pigment		d-Spore surface	
ST08	white		RA		+		spiny	
ST09	red		RA-S		+		spiny	
ST10	gray		RA		+		spiny	
ST11	gray		RA-S		+		spiny	
ST12	gray		RA		+		spiny	
ST13	gray		RF		+		smooth	
ST14	white		RF		-		smooth	
ST15	white		RF		+		smooth	
e-Utilization of carbon compounds								
Carbon sources	*Streptomyces* isolates							
	ST08	ST09	ST10	ST11	ST12	ST13	ST14	ST15
No carbon	-	-	-	-	-	-	-	-
D-Glucose	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+
L-Rhamnose	+	+	+	-	+	+	+	+
D-Fructose	+	+	+	+	+	-	+	+
Raffinose	+	+	-	+	-	+	-	+
D-Mannitol	+	+	+	+	+	+	-	-
i-Inositol	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	-	-	+	-
II- Other assisted characters								
Isolates	Growth on Czapek's medium	Antagonistic activity		Sensitivity to streptomycin	Color of substrate mycelium		Diffusible pigments	NaCl tolerance (Up to 10%)
ST08	Excellent	-		-	Colorless		-	+
ST09	Good	Anti-bacterial		-	Yellow		-	+
ST10	Moderate	-		+	Grayish yellow		-	+
ST11	Excellent	Anti-bacterial		-	Yellow		-	+
ST12	Good	Anti-bacterial		+	Grayish yellow		-	+

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

*RA: spore chain in the form of open loops, hooks or greatly extended coils of wide. RF: spores in straight (R) or flexuous (F) chains. S: spira; spore chain in form of hooks, open loops and coils

Figure 1. Microphotographs of spore chain of some *Streptomyces* isolates (x-400).

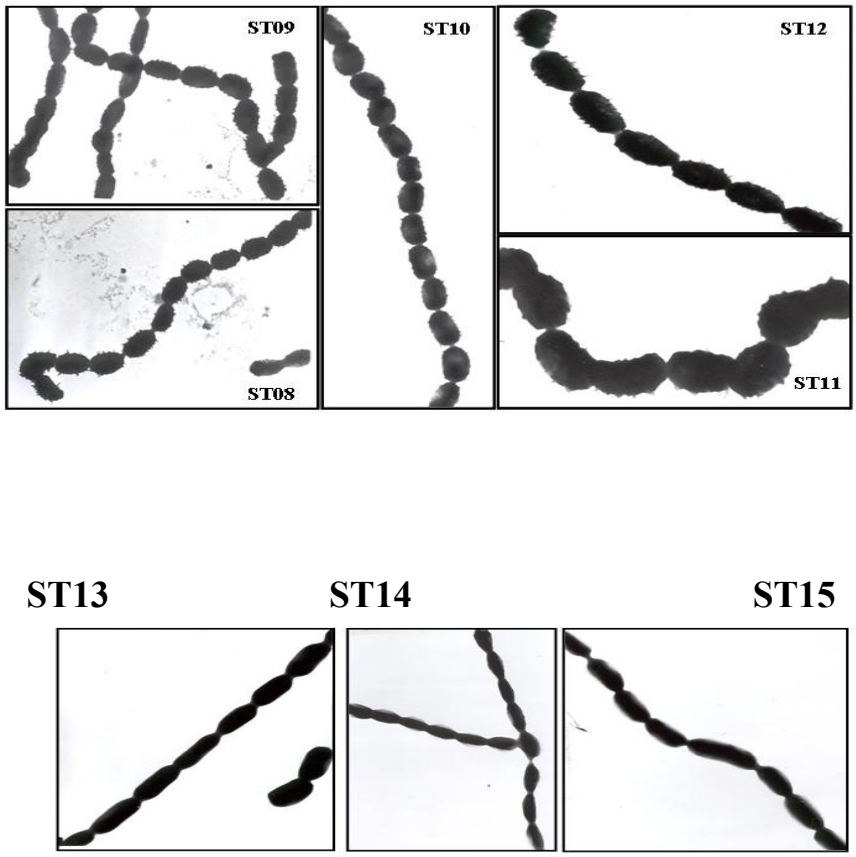

Figure 2. Electron micrographs (X-10000) of spore surface of some *Streptomyces* isolates.

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

the utilization of sucrose, raffinose, rhamnose and fructose as sole carbon sources. Growth on Czapek's medium was moderate (ST10 and ST13), good (ST12) or excellent (ST11). They showed antagonistic activities against the bacterial test organisms except for ST10. They were also sensitive to streptomycin except for ST11.

All the isolates under investigation were able to grow in the presence of 10% NaCl in the growth medium and could not produce diffusible pigments.

Numerical identification of the *Streptomyces* isolates

According to the proposed numerical taxonomy in this study, cultural, morphological, physiological and antagonistic characteristics of the eight *Streptomyces* isolates and related *Streptomyces* species recorded in the proposed key of **Pridham and Tresner (1974)** were given 1 unit if present or 0 unit if absent. Scoring results for all characters of the eight *Streptomyces* isolates are given in **Table (3)**. Accordingly, isolates numbers ST13, ST11, ST09, ST12, ST10, ST14, ST15 and ST08 had the following scoring in descending order, being 50, 50, 45, 45, 42, 41, 40 and 40 units, respectively.

Clustering of all scoring units was determined **(Sneath and Sokal, 1973)** and the results as phylophenetic tree are given in **Figure (3)**. The data reveal the presence of two major related clusters, one includes**,** ST08, ST09, ST14 and ST15 and the second includes ST10, ST11, ST12 and ST13. It was also found that each major cluster contained two subclusters. Subcluster A contained ST14 and ST15; subcluster B contained ST08 and ST09, subcluster C contained ST11 and ST13 and finally subcluster D contained ST10 and ST12.

Numerical identification of white series isolates

Characters of the three white *Streptomyces* isolates, namely, ST08, ST14 and ST15 and most similar *Streptomyces* species, i.e., *S. albolongus, S. alboniger*, *S. baarnensis*, *S. longisporus* and *S. viridaris* in the key proposed by **Pridham and Tresner (1974)** were scored **(Tables 4 and 5).**

The analysis of the scored data showed that ST08, ST14 and ST15 isolates were most related to *S. longisporus*, *S. baarnensis* and *S. albolongus*. Results in **Table (6)** and **Figure (4)** showed that ST08 & *S. longisporus***;** ST14 & *S. baarnensis* and ST15 & *S. albolongus* fell in three subclusters with similarities of 100, 95 and 91.1%, respectively**.** However, slight differences were found between ST14 and *S. baarnensis* in the utilization of raffinose and antibacterial activities.

Identification of red series isolates

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

The scored characters of the red ST09 *Streptomyces* isolate under investigation was compared with the scored characters of three red *Streptomyces* species, namely, *S. purpurascens, S. yokosukanensis* and *S. janthinus* in **Pridham and Tresner (1974)** key **(Table 7).** Result in **Table (8)** and **Figure (5)** show that ST09 could be identified as a strain of *S. janthinus* with similarity of 95.8 %**.** The 4.2% differences between them could be due to the type of spore-chain, growth on Czapek's medium, antagonistic activities and production of diffusible pigments as shown in **Table (7).**

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

Table 3. Scoring of the characters of the eight *Streptomyces* isolates under investigation.

Characters	*Streptomyces* isolates							
	ST08	ST09	ST10	ST11	ST12	ST13	ST14	ST15
a- Color of aerial mycelium (22)	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	0	1	1	1	1	1	0	0
	0	1	1	1	1	1	0	0
	0	1	1	1	1	1	0	0
	0	0	1	1	1	1	0	0
	0	0	1	1	1	1	0	0
	0	0	1	1	1	1	0	0
	0	0	1	1	1	1	0	0
	0	0	1	1	1	1	0	0
	0	0	1	1	1	1	0	0
b-Spore-chain (4)	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	0	1	0	1	0	1	1	1
	0	0	0	0	0	1	1	1
c-Melanoid pigment (1)	1	1	1	1	1	1	0	1

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Table 3. Cont.

Characters	*Streptomyces* isolates							
	ST08	ST09	ST10	ST11	ST12	ST13	ST14	ST15
e-Utilization of carbon compounds (17)								
No carbon	0	0	0	0	0	0	0	0
D-Glucose	1	1	1	1	1	1	1	1
D-Xylose	1	1	1	1	1	1	1	1
L-Arabinose	1	1	1	1	1	1	1	1
L-Rhamnose	1	1	1	1	0	1	1	1
	1	1	1	1	0	1	1	1
D-Fructose	1	1	1	0	1	0	1	1
	1	1	1	0	1	0	1	1
Raffinose	1	1	0	1	1	1	0	1
	1	1	0	1	1	1	0	1
D-Mannitol	1	1	1	1	1	1	0	0
	1	1	1	1	1	1	0	0
i-Inositol	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
Sucrose	1	1	0	1	0	0	1	0
	1	1	0	1	0	0	1	0
	1	1	0	1	0	0	1	0
f- Growth on Czapek's medium (5)	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	1	0	1	1	0	1	1
	1	0	0	1	0	0	1	0
g- Anti-bacterial activity (2)	0	1	0	1	1	1	1	1
	0	1	0	1	1	1	1	1
h- Anti-fungal activity (2)	0	0	0	0	0	1	0	0
	0	0	0	0	0	1	0	0
Total (58 Units)	**40**	**45**	**42**	**50**	**45**	**50**	**41**	**40**

1: Present. 0: Absent.

Figure 3. Phylophenetic tree of eight *Streptomyces* isolates based on analysis of their selected characters.

Table 4. Scoring of the characters of ST08 isolate compared with those of related species in **Pridham and Tresner (1974)** key.

Characters	ST08 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key		
		S. alboniger	*S. longisporus*	*S. viridaris*
a- Color of aerial mycelium **(22)**	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
b-Spore-chain **(4)**	1	1	1	1
	1	1	1	1
	0	1	0	1
	0	1	0	1
c-Melanoid pigment **(1)**	1	0	1	1
d-Spore surface **(5)**	1	1	1	1
	1	1	1	1
	1	1	1	1
	0	1	0	1
	0	1	0	1

Table 4. Cont.

Characters	ST08 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key		
		S. alboniger	*S. longisporus*	*S. viridaris*
e- Utilization of carbon compounds **(17)**				
No carbon	0	0	0	0
D-Glucose	1	1	1	0
D-Xylose	1	1	1	1
L-Arabinose	1	1	1	0
L-Rhamnose	1	0	1	1
	1	0	1	1
D-Fructose	1	1	1	0
	1	1	1	0
Raffinose	1	0	1	1
	1	0	1	1
D-Mannitol	1	1	1	0
	1	1	1	0
i-Inositol	1	1	1	0
	1	1	1	0
Sucrose	1	0	1	1
	1	0	1	1
	1	0	1	1
f- Growth on Czapek's meium **(5)**	1	0	1	1
	1	0	1	1
	1	0	1	1
	1	0	1	1
	1	0	1	1
g- Anti-bacterial activity **(2)**	0	1	0	1
	0	1	0	1
h- Anti-fungal activity **(2)**	0	0	0	0
	0	0	0	0
Total (58 Units)	**40**	**33**	**40**	**38**

1: Present.0: Absent.

Table 5. Scoring of the characters of white ST14 and ST15 isolates compared with those of related species in **Pridham and Tresner (1974)** key.

Characters	ST14	*Streptomyces* species in **Pridham and Tresner (1974)** key		ST15
		S. albolongus	*S. baarnensis*	
a- Color of aerial mycelium **(22)**	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
b-Spore-chain **(4)**	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
c-Melanoid pigment **(1)**	0	1	0	1
d-Spore surface **(5)**	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1

Table 5. Cont.

Characters	ST14	*Streptomyces* species in **Pridham and Tresner (1974)** key		ST15
		S. albolongus	*S. baarnensis*	
e- Utilization of carbon compounds **(17)**				
No carbon	0	0	0	0
D-Glucose	1	1	1	1
D-Xylose	1	1	1	1
L-Arabinose	1	1	1	1
L-Rhamnose	1	0	1	1
	1	0	1	1
D-Fructose	1	1	1	1
	1	1	1	1
Raffinose	1	0	0	1
	1	0	0	1
D-Mannitol	1	1	1	0
	1	1	1	0
i-Inositol	1	1	1	1
	1	1	1	1
Sucrose	0	0	0	0
	0	0	0	0
	0	0	0	0
f- Growth on Czapek's medium **(5)**	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	0
g- Anti-bacterial activity **(2)**	1	1	0	1
	1	1	0	1
h- Anti-fungal activity **(2)**	0	0	0	0
	0	0	0	0
Total (58 Units)	**42**	**39**	**38**	**40**

1: Present. 0: Absent.

Table 6. Similarities between the white *Streptomyces* isolates and related species in **Pridham and Tresner (1974)** key.

Streptomyces isolates	Related white *Streptomycetes* species in **Pridham and Tresner (1974)** key				
	S. longisporus	*S. baarnensis*	*S. albolongus*	*S. viridaris*	*S. alboniger*
ST08	**100**	87.2	83.5	82.1	74.0
ST14	87.8	**95.0**	93.8	85.0	88.0
ST15	85.0	89.7	**91.1**	87.2	84.9

Bold number represents the most similar species.

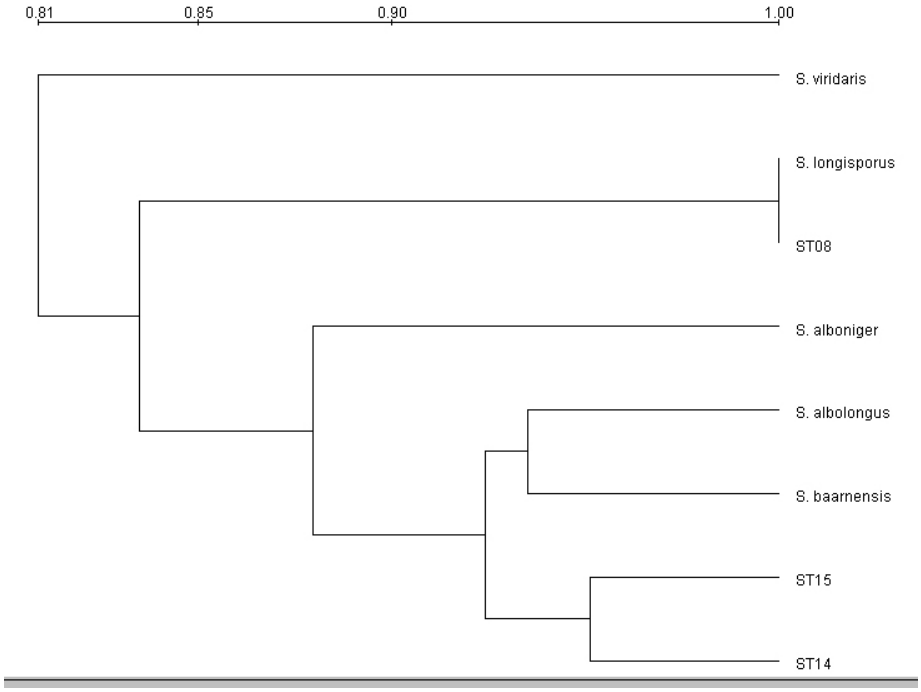

Figure 4. Phylophenetic tree of three *Streptomyces* isolates belonging to white series and related species in **Pridham and Tresner (1974).**

Table 7. Scoring of the characters of ST09 isolate compared with those of related species in **Pridham and Tresner (1974)** key.

Characters	ST09 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key		
		S. janthinus	*S. purpurascens*	*S. yokosukanensis*
a- Color of aerial mycelium (22)	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
b-Spore-chain (4)	1	1	1	1
	1	1	1	1
	1	0	0	0
	0	0	0	0
c-Melanoid pigment (1)	1	1	1	1
d-Spore surface (5)	1	1	1	1
	1	1	1	1
	1	1	1	1
	0	0	0	0

	0	0	0	0

Table 7. Cont.

Characters	ST09 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key		
		S. janthinus	*S. purpurascens*	*S. yokosukanensis*
e- Utilization of carbon compounds (17)				
No carbon	0	0	0	0
D-Glucose	1	1	1	1
D-Xylose	1	1	1	1
L-Arabinose	1	1	1	1
L-Rhamnose	1	1	1	1
	1	1	1	1
D-Fructose	1	1	1	1
	1	1	1	1
Raffinose	1	1	1	1
	1	1	1	1
D-Mannitol	1	1	1	1
	1	1	1	1
i-Inositol	1	1	1	1
	1	1	1	1
Sucrose	1	1	1	1
	1	1	1	1
	1	1	1	1
f- Growth on Czapek's medium (5)	1	1	0	1
	1	1	0	1
	1	1	0	0
	1	1	0	0
	0	1	0	0
g- Anti-bacterial activity	1	1	1	1
	1	1	1	1
h- Anti-fungal activity	0	1	0	1
	0	1	0	1
Total (58 Units)	**45**	**47**	**40**	**44**

1: Present.0: Absent.

Table 8. Similarities between the red *Streptomyces* isolate and those related species in **Pridham and Tresner (1974)** key.

Streptomyces Isolate	Related red *Streptomyces species* in **Pridham and Tresner (1974)** key		
	S. janthinus	*S. yokosukanensis*	*S. purpurascens*
ST09	**95.8**	94.6	94.4

Bold number represents the most similar species.

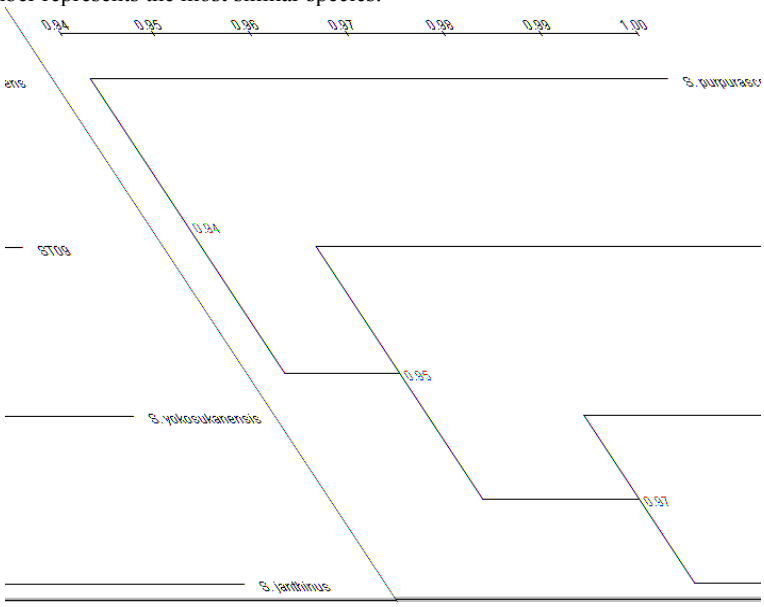

Figure 5. Phylophenetic tree of ST09 isolate belonging to red series and related species in **Pridham and Tresner (1974)**.

Identification of gray series isolates

Characters of ten gray streptomycetes, i.e., the four isolates under investigation (ST10, ST11, ST12 and ST13) and most related six species in the key of **Pridham and Tresner (1974)** (*S. durhamensis*, *S. filipinensis*, *S. griseochromogenes*, *S. chromofuscus*, *S. echinatus* and *S. antibioticus*) were scored (**Tables 9 and 10**). Clustering (**Figure 6)** and similarities (**Table 11**) analyses showed that ST11 and ST13 isolates were strains of *S. griseochromogenes*, and *S. antibioticus* with similarities of 95.0 and 92.8%, respectively. Isolates ST10 and ST12 were duplicate strains of *S. echinatus* with similarities of 93.3 and 94.6%, respectively. This result confirmed the previous results in **Figure (3)**. As these two *Streptomyces* isolates were fell in one subcluster with a similarity of 92%.

Streptomyces isolate ST11 differed from *S. griseochromogenes* in the utilization of L-rhamnose and fructose as sole carbon sources and color of substrate mycelium. ST11 tolerated the presence of NaCl up to 10% in the growth medium, while, *S. griseochromogenes* did not. There were some differences between ST13 and *S. antibioticus*, in utilization of raffinose, D-fructose, growth on Czapek's medium, antagonistic activities and salt tolerance (**Table 9**).

Application of the suggested numerical taxonomy on some known *Streptomyces* species

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

The suggested numerical taxomomy under investigation was applied for

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

Table 9. Scoring of the characters of ST11 isolate compared with those of related species in **Pridham and Tresner (1974)** key.

Characters	ST11 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key		
		S. durhamensis	*S. filipinensis*	*S. griseochromogenes*
a- Color of aerial mycelium (22)	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
b-Spore-chain (4)	1	1	1	1
	1	1	1	1
	1	1	1	1
	0	0	1	0

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

	0	0	0	0

Table 9. Cont.

Characters	ST11 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key		
		S. durhamensis	*S. filipinensis*	*S. griseochromogenes*
e- Utilization of carbon compounds (17)				
No carbon	0	0	0	0
D-Glucose	1	1	1	1
D-Xylose	1	1	1	1
L-Arabinose	1	1	1	1
L-Rhamnose	1	0	0	0
	1	0	0	0
D-Fructose	0	1	1	1
	0	1	1	1
Raffinose	1	1	1	1
	1	1	1	1
D-Mannitol	1	1	1	1
	1	1	1	1
i-Inositol	1	1	1	1
	1	1	1	1
Sucrose	1	0	1	1
	1	0	1	1
	1	0	1	1
F- Growth on Czapek's medium (5)	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
	1	1	1	1
g- Anti-bacterial activity (2)	1	0	0	1
	1	0	0	1
h- Anti-fungal activity (2)	0	1	1	0
	0	1	1	0
Total (58 Units)	**49**	**47**	**51**	**50**

Table 10. Scoring of the characters of ST10, ST12 and ST13 isolates compared with those of related species in **Pridham and Tresner (1974)** key.

Characters	ST10 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key			ST13 isolate	ST12 isolate
		S. chromofuscus	*S. echinatus*	*S. antibioticus*		
a- Color of aerial mycelium (22)	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
b-Spore-chain (4)	1	1	1	1	1	1
	1	1	1	1	1	1
	0	0	1	1	1	1
	0	0	1	1	1	1
c-Melanoid pigments (1)	1	1	1	1	1	1
d-Spore surface (5)	1	1	1	1	1	1
	1	1	1	1	1	1
	1	1	1	1	1	1
	0	0	0	1	1	0
	0	0	0	1	1	0

Table 10. Cont.

Characters	ST10 isolate	*Streptomyces* species in **Pridham and Tresner (1974)** key			ST13 isolate	ST12 isolate
		S. chromofuscus	*S. echinatus*	*S. antibioticus*		
e- Utilization of carbon compounds (17)						
No carbon	0	0	0	0	0	0
D-Glucose	1	1	1	1	1	1
D-Xylose	1	1	1	1	1	1
L-Arabinose	1	1	1	1	1	1
L-Rhamnose	1	1	1	1	1	0
	1	1	1	1	1	0
D-Fructose	1	1	1	1	0	1
	1	1	1	1	0	1
Raffinose	1	0	1	0	1	1
	1	0	1	0	1	1
D-Mannitol	1	1	1	1	1	1
	1	1	1	1	1	1
i-Inositol	1	1	1	1	1	1
	1	1	1	1	1	1
Sucrose	0	1	0	0	0	0
	0	1	0	0	0	0
	0	1	0	0	0	0
f- Growth on Czapek's medium (5)	1	0	1	1	1	1
	1	0	0	1	1	1
	1	0	0	0	1	1
	0	0	0	0	0	1
	0	0	0	0	0	0
g- Anti-bacterial activity (2)	0	1	1	1	1	1
	0	1	1	1	1	1
h- Anti-fungal activity (2)	0	0	0	0	1	0
	0	0	0	0	1	0
Total (58 Units)	**44**	**44**	**46**	**48**	**50**	**46**

Table 11. Similarities between the gray *Streptomyces* isolates and related species in **Pridham and Tresner (1974)** key.

Streptomyces isolates	Related gray *Streptomycetes* species in **Pridham and Tresner (1974)** key					
	S. griseochromogenes	*S. echinatus*	*S. filipinensis*	*S. chromofuscus*	*S. durhamensis*	*S. antibioticus*
ST10	88.4	**93.3**	87.5	88.6	91.3	90.1
ST11	**95.0**	90.2	89.6	89.4	87.8	86.6
ST12	93.9	**94.6**	90.9	85.7	92.6	91.5
ST13	87.1	91.7	88.2	83.0	89.8	**92.8**

Bold number represents the most similar species.

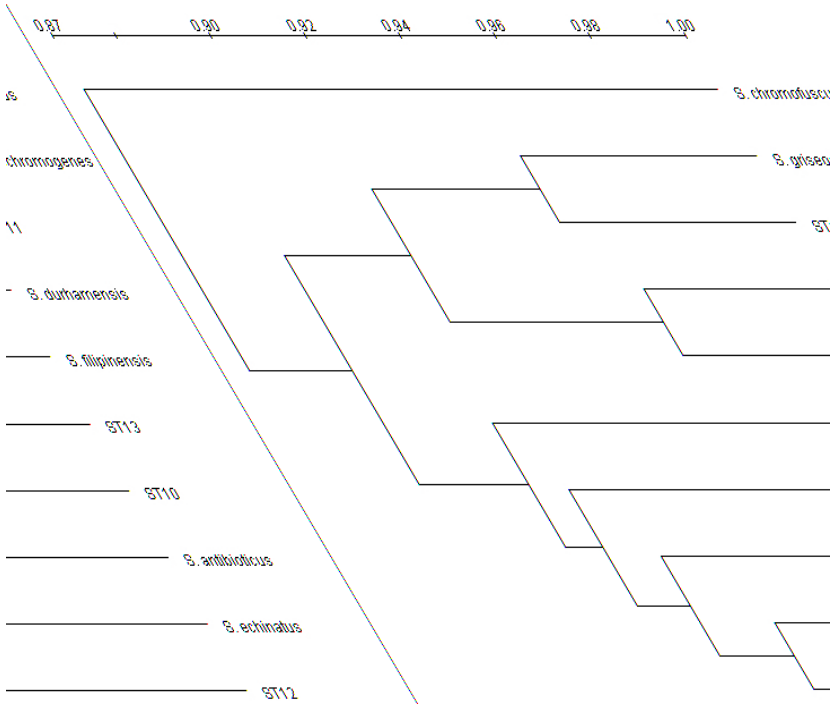

Figure 6. Phylophenetic tree of four isolates belonging to gray series and related species in **Pridham and Tresner (1974).**

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

determination of the relationship between the 14 known *Streptomyces* species used in the present study. Data presented in the phylophenetic tree (**Figure 9**) reveal that the *Streptomyces* species fell into three major clusters based on their color of aerial mycelia as follows:

First: includes white series species, i.e., *S. viridaris*, *S. alboniger*, *S. baarnensis*, *S. albolongus* and *S. longisporus*.

Second: includes red series species, i.e., *S. purpurascens, S. yokosukanensis* and *S. janthinus*

Third: includes gray series species, i.e., *S. chromofuscus*, *S. antibioticus*, *S. echinatus*, *S. griseochromogenes*, *S. durhamensis* and *S. filipinensis*.

Our results reveal that the suggested numerical taxonomy proved valiable as a base for the identification of *Streptomyces*. However, further studies are needed for more evaluation of this method by its application on the all known *Streptomyces* species presented in **Pridham and Tresner (1974)** key.

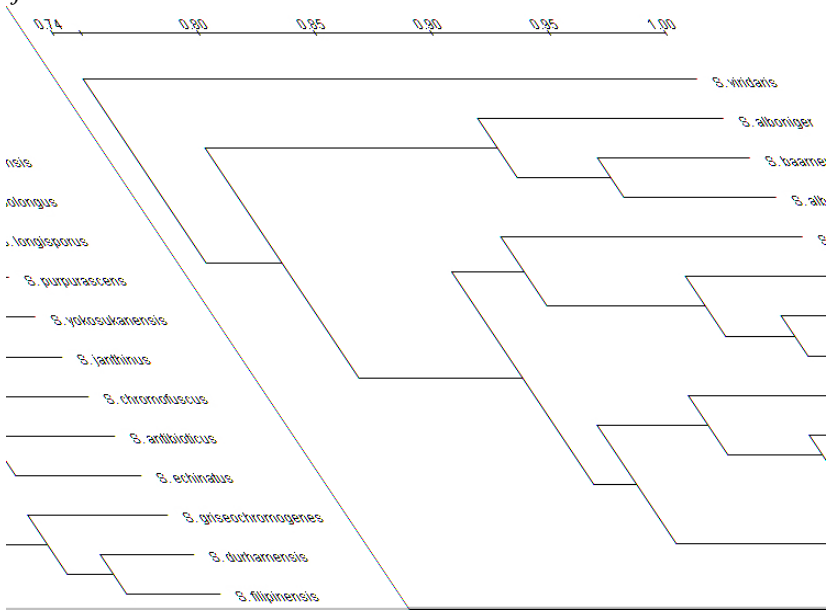

Figure 7. Phylophenetic tree of 14 *Streptomyces* species in the key of **Pridham and Tresner (1974)** achieved using suggested numerical taxonomy.

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

ACKNOWLEDGMENT

The authors would like to thank Prof. Dr. Atef S. Sadik, Agric. Microbiol. Dept., Faculty of Agric., Ain Shams Univ. and Mr. Ahmed Fawzy, AGERI, ARC, Giza, Egypt for their sincere help to accomplish this study.

REFERENCES

Abdel-Fattah, H.I. (2005). Cultural, morphological, physiological and molecular studies on some streptomycete isolates. ***Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(2): 249-268.***

Goodfellow M. (1967). Numerical taxonomy of some named bacterial cultures. ***Can. J. Microbiol. 13(10):1365-74.***

Goodfellow, M.; G. Alderson and J. Lacey (1979). Numerical taxonomy of *Actinomadura* and related actinomycetes. ***J. Gen. Microbiol. 112(1): 95-111.***

Goodfellow, M.; J. Lacey and C. Todd (1987). Numerical classification of thermophilic streptomycetes. ***J Gen. Microbiol. 133(11): 3135-3149.***

Goodfellow, M.; E.V. Ferguson and J.J. Sanglier (1992). Numerical classification and identification of *Streptomyces* species-a review. ***Gene 115(1-2): 225-233.***

Kurylowicz W.; A. Paszkiewicz; T. Szulga; W. Woznicka and W. Kurzatkowski (1975). Classification of streptomycetes by different numerical methods. ***Postepy. Hig. Med. Dosw. 197529(3): 281-355.***

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

Paszkiewicz A. (1972). Application ofnumerical methods to the taxonomy of the genus *Streptomyces*. ***Arch. Immunol. Ther. Exp. (Warsz).20(3):307-32.***

Pridham, T.G. and H.D. Tresner (1974). Family *Streptomycetacae*. In; ***Bergey's Manual of Determinative Bacteriology (1974), p751, 793, 802 & 826***. (Buchanan R.E. and N.E. Gibbons, 8th Eds.), Williams and Wilkins, Baltmore, USA.

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

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.

2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

Shirling, E.B. and D. Gottlieb (1966). Methods for characterization of *Streptomyces* species. ***Int. J. Syst. Bacteriol. 16(3): 313-340.***

Sneath, P.H.A. and R.R. Sokal (1973). ***Numreical Taxonomy: The Principles and Practice of Numerical Classification, pp. 1-573.*** San Francisco: W.H. Freeman.

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

Williams, S.T.; M. Goodfellow; G. AlDerson; E.M.H. Wellington; P.H.A. Sneath and M.J. Sackin (1983). Numerical classification of *Streptomyces* and related genera ***J. Gen. Microbiol. 129 (6): 1743-1813.***

Williams, S.T.; M. Goodfellow and G. Alderson (1989). Genus *Streptomyces*. In: ***Bergey's Manual of Determinative Bacteriology. pp. 2452-2492.*** (Williams, S.T.; M.E. Sharpe and J.P. Holt eds), Williams and Wilkins, Baltimore, USA.

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.

2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 641-668, 2005

مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية، جامعة عين شمس، القاهرة، 13(3)، 641-668، 2005

تعريف بعض عزلات الاستربتوميسيتات المحللة للشيتين باستخدام طرق التقسيم الرقمي

]42[

سونيا محمود محمد[1]، السيد محمد صالح[2]، محمد محمود زكي[2]

1. قسم الميكروبيولوجيا الزراعية، معهد بحوث الأراضي والمياه والبيئة، مركز البحوث الزراعية، الجيزة، مصر
2. قسم الميكروبيولوجيا الزراعية، كلية الزراعة، جامعة عين شمس، شبرا الخيمة، القاهرة، مصر

تم تطبيق طريقة التقسيم الرقمي لتعريف ثمانية من العزلات المحللة للشيتين والتابعة لجنس الاستربتوميسيتات، وقد تم تقدير الخواص المزرعية والمورفولوجية والفسيولوجية والقدرة على التضاد لهذه العزلات ضد الدراسة. تبع ذلك عطاء كل من هذه الصفات الثمانية والتي تمثل كل الأنواع من الاستربتوميسيتات المعروفة والتي تم الحصول عليها من تقسيم برايدام وترسون عام 1974 والأكثر توافقاً لبرنامج الكمبيوتر والذي يحتوي على 58 صفة لتمثل أعلى صفة لها وخواصها مع العزلات موضع الدراسة لها وأرقام اعتبارية. كما تم تقدير درجة التشابه باستخدام طريقة Dice coefficient وشجرة القرابة باستخدام طريقة UPGMA algorithm .

وباستخدام الطرق الرقمية في تعريف من العزلات الثمانية من الاستربتوميسيتات ST08, ST09, ST11, ST13, ST14 and ST15 إلى *S. longisporus*, *S. janthinus, S. griseochromogenes*, *S. antibioticus S. baarnensis* and *S. albolongus* بنسب تشابه 100، 95.8، 95، 92.8، 95، 91.1% على التوالي والعزلات ST12، ST10 بنسب تشابه مع النوع *S. echinatus* بنسبة تشابه 94.6% و 93.3% لكل منها على التوالي. وكذلك تم تطبيق التحليل العنقودي على الأنواع من الاستربتوميسيتات وتوافقها في التقسيم وفقاً لبرايدام وترسون عام 1974 والمستخدمة في هذه الدراسة وقد وضحت النتائج أن هذه الأنواع قد وقعت ضمن ثلاث Clusters رئيسية باستخدام التحليل العنقودي وهو ما يؤكد نجاح مثل هذه الطريقة في التقسيم الرقمي.

تحكيم: أ.د يوسف الحمدي ... أ.د عبد الرحمن الدين علي فارس

1- Agricultural Microbiology Department, Institute of Soil, Water and Environment Research, Agricultural Research Center, Giza, Egypt.
2- Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

(Received April 16, 2005)
(Accepted May 14, 2005)