

# Identification and Antiviral Activities of Some Halotolerant *Streptomyces* Isolated from Qaroon Lake

SONYA, H. MOHAMED<sup>1</sup> AND A.M. GALAL<sup>†</sup>

Department of Agriculture Microbiology, Institute of Soil, Water and Environment Research, ARC, Giza, P.O. Box 12619, Egypt

<sup>†</sup>Department of Botany, Faculty of Science, Zagazig University, Zagazig, Egypt

<sup>1</sup>Corresponding author's e-mail: sonya\_hamouda@hotmail.com

## ABSTRACT

Four halotolerant *Streptomyces* isolates (QS01, QS02, QS03 & QS04) were obtained from Qaroon Lake, which had the ability to grow on 7% NaCl concentration in the starch nitrate agar medium. They were identified with a numerical method using two, three and four marker species belonging to red, gray and yellow series color groups. Their antiviral activities against tobacco mosaic tobamovirus (TMV) and potato Y potyvirus (PVY) were also determined. The isolates QS03 and QS04 identified as strains of *S. naganishii* and *S. michigansis* with similarities of 99.0 and 92.3%, respectively. QS01 and QS02 isolates were identified as duplicate strains of *S. erythraeus* with similarities of 93.8 and 90.2%, respectively. The numerical system was found to be a useful method for identification of the streptomycete isolates and could be limit the nomenclature of the new species of streptomycetes as well as the related genera of actinomycetes. As interesting, both of filtrates and pellets (Cells of streptomycetes) were found to contain substances with antiviral activities, as the number of necrotic local lesions (NLL) produced by TMV and PVY on *Nicotiana glutinosa* and *Chenopodium quinoa* respectively were decreased.

**Key Words:** *Streptomyces*; Numerical identification; Halotolerant; Antiviral activities

## INTRODUCTION

Bacteria able to grow in the presence of relatively high salt concentrations are designated halotolerant. The occurrence of nonpigmented halotolerant bacteria was probably first isolated by LeFevre and Round (1919) in their study of the microbiology of cucumber fermentation brines. One of the bacterial groups isolated grew in 0 to 15% NaCl, whereas other bacteria grew over the range of 5 to 25%. To describe microorganisms according to their behavior toward salts, although different classification schemes were proposed, (Trüper & Galinski, 1986; Vreeland, 1987; Ramos-Cormenzana, 1989), the most widely used is that of Kushner, who defined moderate halophiles as organisms growing optimally between 0.5 and 2.5 M salt (Kushner, 1978).

*Streptomyces* spp. are capable of producing microbial antibiotics with a wide variety of chemical structures. In particular, approximately 60% of antibiotics developed for agricultural use were isolated from *Streptomyces* spp. (Tanaka & Omura, 1993). *Streptomyces* strains continue to provide a wide variety of new antibiotics than any other actinomycete genus, suggesting that substantial numbers of *Streptomyces* species or strains with novel antibiotic productivity exist in nature (Okami & Hotta, 1988). In searches for bioactive antibiotics, *Streptomyces* strains have been isolated from various types of soils, including rice paddy, lake mud and water, deciduous forest, tropical forest, wasteland, and cave soils (Tanaka & Omura 1993; Jiang &

Xu, 1996; Xu *et al.*, 1996; Kim *et al.*, 1998). Langó *et al.* (1999) identified *Streptomyces galbus* isolated from the sediment of the high-mountain lake La Caldera (Sierra Nevada) in Spain. In Egypt, a number of studies were achieved corresponding to the halotolerant streptomycetes isolated from soils (Mohamed, 1998; Mohamed *et al.*, 2000; Mahfouz & Mohamed, 2002; Abdel-Fattah, 2005) and marine ecosystems (Saleh *et al.*, 1990; Zaki *et al.*, 1993).

The main goal of this work was to identify four halotolerant *Streptomyces* spp. isolates using the numerical method suggested by Mohamed *et al.*, (2005) and to determine the antiviral activities for the isolates of this study.

## MATERIALS AND METHODS

**Source of streptomycete isolates.** In this study, four halotolerant *Streptomyces* isolates (QS01, QS02, QS03 & QS04) isolated from Qaroon lake, Fayoum Governorate, Egypt were obtained from, Department of Agricultural Microbiology, Institute of Soil, Water and Environment Research, ARC, Giza, Egypt.

**Characterization of *Streptomyces* isolates.** According to the key proposed by Pridham & Tresner (1974) the characters of *Streptomyces* isolates in this study were determined using the media and methods of the International *Streptomyces* Project (ISP) as described by Shirling and Gottlieb (1966). Using the light and electron microscopy, the properties of their spores and mycelia were

determined. To evaluate the abilities of the *Streptomyces* isolates for pigmentation, growth on Czapek's medium, tolerance to streptomycin ( $4 \mu\text{g mL}^{-1}$ ) and utilization of sugars as a sole source of carbon, the method of Saleh *et al.* (1990) was applied. The antibiosis activities of the isolates under investigation, against a number of 9 microorganisms of fungi, yeasts and bacteria; which were provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University; were tested based on the method of Pridham and Tresner (1974) and Mohamed *et al.* (2000).

**Isolates identification.** The numerical method suggested by Mohamed *et al.* (2005) was used for identification of the four *Streptomyces* isolates of this study. Characters were scored as present (1 unit) or absent (0 unit) and then 52 units were used to determine the similarity matrix by Dice Coefficient method between the *Streptomyces* isolates, while 42 units were used in case of using the marker species in Pridham and Tresner (1974). On the other hand, clustering was achieved by the unweighted pair group method with average (UPGMA) algorithm (Sneath & Sokal, 1973). The computer analysis was done using the Diversity Database™ Version 2.0 from Bio-Rad.

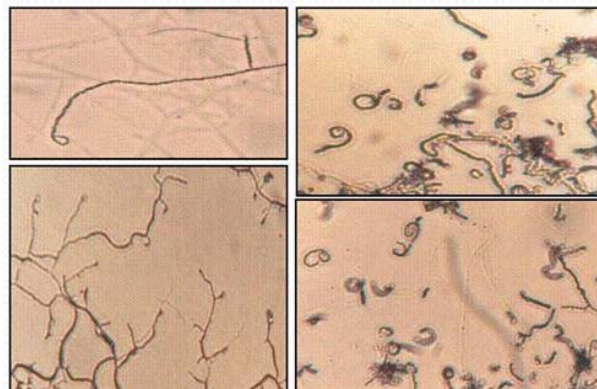
The antiviral activities of the isolates against the tobacco mosaic tobamovirus (TMV) and potato Y potyvirus (PVY) were determined with the method given by Mahfouz and Mohamed (2002). The standard inocula for each *Streptomyces* species were prepared as described by Mohamed (1998) and an aliquot of 2 ml were transferred aseptically to 50 ml starch nitrate broth medium (Waksman & Lechevalier, 1961). After incubation at  $28^{\circ}\text{C} \pm 2$  for 6 days on a rotary shaker ( $160 \text{ rpm min}^{-1}$ ), the supernatants were obtained by centrifugation at 14000 rpm at  $4^{\circ}\text{C}$  for 5 min and four dilutions, i.e., 1/50, 1/100, 1/200 and 1/400 were prepared for each *Streptomyces* supernatant. The previous pellets were separately pulverized in liquid nitrogen into a fine powder followed by resuspension in a volume of 1 ml d.H<sub>2</sub>O. Similarly, four dilutions were prepared.

Equal volumes of each of dilution and virus infectious sap were prepared. Then, for each mixture, five leaves of *Nicotiana glutinosa* and *Chenopodium quinoa* plants were inoculated in case of TMV and PVY, respectively. The inoculated plants were maintained in an insect proof glasshouse for necrotic local lesions (NLL) development.

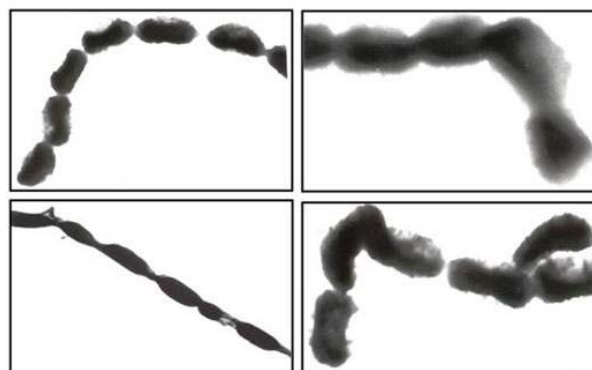
## RESULTS AND DISCUSSION

**Description of *Streptomyces* isolates.** Data obtained based on cultural and morphological tests using the key proposed by Pridham and Tresner (1974) confirmed that all the isolates belonged to the genus *Streptomyces*. Of these QS01 belonged to the red colour series and the substrate mycelium produced yellow pigment on the standard media (Table I; Fig. 1 & 2). The spores were characterized by spiral and spiny surface. Melanoid pigment was not detected on the standard media. This isolate characterized by good growth

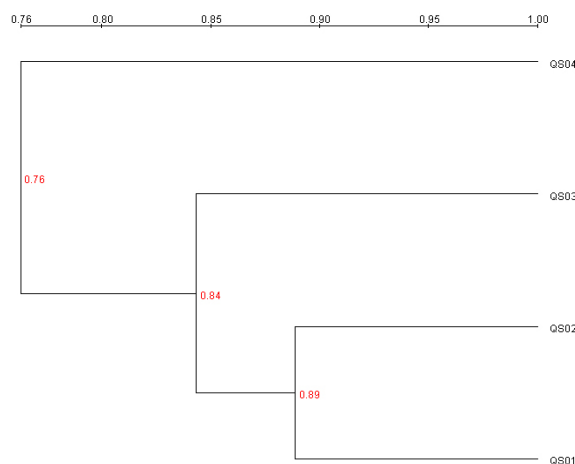
**Fig. 1. Microphotographs of spore chain of *Streptomyces* isolates QS01, QS02, QS03 and QS04**



**Fig. 2. Electron micrographs (X-10000) of spore surface of *Streptomyces* isolates QS01, QS02, QS03 and QS04**



**Fig. 3. Phylogenetic tree of four *Streptomyces* isolates belonging to different series based on the analysis of the selected characters.**



on Czapek's agar medium. The QS01 isolate was able to utilize seven out of the nine sugars as sole carbon source for

**Table I. Taxonomical characters of four halotolerant streptomycetes isolated from Qaroon lake as recommended by the key of Pridham and Tresner (1974)**

<i>Streptomyces isolate</i>		I- Main taxonomical characters				
	a-Color of aerial mycelium	b-Spore-chain	c-Melanoid pigments (C)	d-Spore surface		
QS01	Red	Spiral	C -	Spiny		
QS02	Red	Spiral	C -	Warty		
QS03	Gray	RA	C +	Smooth		
QS04	Yellow	RF	C +	Smooth		
e-Utilization of carbon compounds						
No carbon	-	+	+	+		
D-Glucose	+	+	+	+		
D-Xylose	+	+	+	+		
L-Arabinose	+	+	+	+		
L-Rhamnose	+	-	+	+		
D-Fructose	-	+	+	+		
Raffinose	+	+	+	+		
D-Mannitol	+	+	+	+		
i-Inositol	+	+	+	+		
Sucrose	-	-	-	-		
II- Other assisted characters						
Isolate	Growth on Czapek's medium	Antagonistic activity	Sensitivity to streptomycin	Color of substrate mycelium	Diffusible pigments	NaCl tolerance
QS01	Good	Antibacterial	Not sensitive	Yellow	-	7%
QS02	Excellent	Antibacterial & Antifungal	Sensitive	Yellow	-	≥7%, but < 10%
QS03	Moderate	Antibacterial & Antifungal	Sensitive	Yellow	-	7%
QS04	Poor	Antifungal	Not sensitive	Bright yellow	-	≥7%, but < 10%

C+: produced. C-: not produced. 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. -: not utilized or not produced. +: utilized or produced.

**Table II. Scoring the characters of the four *Streptomyces* isolates under investigation for their identification via the suggested statistical system and those related in Pridham and Tresner (1974)**

Characters	Units	Scoring of <i>Streptomyces</i> isolates belonging to red series			
		Isolates of this study		<i>Streptomyces</i> spp. in Pridham and Tresner (1974)	
		QS01	QS02	<i>S. erythraeus</i>	<i>S. luteofluorescens</i>
a- CarMy	22	16	16	16	16
b-Spore-chain	4	4	4	4	4
c-Melanoid pigments	1	0	0	0	0
d-Spore surface	5	3	1	3	1
e- UCC	17	11	11	13	12
f- GCzM	5	4	5	5	0
g- Anti-B activity	2	2	2	0	0
h- Anti-F activity	2	0	2	0	0
Total	58	40	41	41	33
%	100	68.96	70.69	70.69	56.90
Characters	Units	Scoring of <i>Streptomyces</i> isolates belonging to gray series			
		QS03	<i>S. naganishii</i>	<i>S. griseoruber</i>	<i>S. galbus</i>
		<i>S. hygroscopicus</i>			
a- CarMy	22	22	22	22	22
b-Spore-chain	4	4	4	4	4
c-Melanoid pigments	1	1	1	1	1
d-Spore surface	5	5	5	5	5
e- UCC	17	13	13	9	16
f- GCzM	5	3	2	2	3
g- Anti-B activity	2	2	2	0	0
h- Anti-F activity	2	2	2	0	2
Total	58	52	51	43	53
%	100	89.65	87.93	74.14	91.37
Characters	Units	Scoring of <i>Streptomyces</i> isolates belonging to yellow series			
		QS04	<i>Streptomyces</i> spp. in Pridham and Tresner (1974)		
		<i>S. michigansis</i>	<i>S. griseobrunneus</i>	<i>S. alboflavus</i>	
a- CarMy	22	7	7	7	
b-Spore-chain	4	3	3	3	
c-Melanoid pigments	1	1	1	1	
d-Spore surface	5	5	5	5	
e-UCC	17	13	10	13	
f- GCzM	5	2	2	5	
g- Anti-B activity	2	0	2	2	
h- Anti-F activity	2	2	0	2	
Total	58	33	30	38	
%	100	56.89	51.72	65.52	

UCC: Utilization of carbon compounds. I: Antiviral activity, not tested.

**Table III. Similarities between the *Streptomyces* isolates of this study and those in Pridham and Tresner (1974)**

<i>Streptomyces</i> Species	<i>Streptomyces</i> belonging to gray series			
	<i>S. naganishii</i>	<i>S. hygroscopicus</i>	<i>S. galbus</i>	<i>S. griseoruber</i>
QS03	99.0	95.2	90.5	90.5
	<i>Streptomyces</i> belonging to red series			
	<i>S. erythraeus</i>		<i>S. luteofluorescens</i>	
QS01	93.8		76.7	
QS02	90.2		75.7	
	<i>Streptomyces</i> belonging to yellow series			
	<i>S. michigansis</i>	<i>S. alboflavus</i>	<i>S. griseobrunneus</i>	
QS04	92.3	91.7		89.2

**Table IV. Antiviral activities of some halotolerant *Streptomyces* isolates against TMV and PVY viruses.**

Source of substances	Dilutions	Antiviral activities of some <i>Streptomyces</i> isolates			
		QS01	QS02	QS03	QS04
		<b>In case of TMV</b>			
	<b>Crude</b>	<b>000*</b>	<b>000</b>	<b>013</b>	<b>028</b>
Supernatant	1/50	018	028	050	057
	1/100	047	065	073	089
	1/200	103	116	108	122
	1/400	145	153	172	142
	Crude	026	029	028	024
Pellet	1/50	043	055	056	062
	1/100	086	088	091	093
	1/200	121	125	118	132
	1/400	151	149	167	155
Control	Positive			175	
	Negative			000	
	Healthy			000	
		<b>In case of PVY</b>			
	<b>Crude</b>	<b>000**</b>	<b>000</b>	<b>029</b>	<b>030</b>
Supernatant	1/50	026	034	058	061
	1/100	057	082	083	090
	1/200	123	121	117	127
	1/400	164	157	166	151
	Crude	000	000	031	032
Pellet	1/50	029	036	060	067
	1/100	062	087	093	099
	1/200	132	129	128	132
	1/400	169	163	172	162
Control	Positive			190	
	Negative			000	
	Healthy			000	

\*: Average number of NLL on treated leaf of *N. glutinosa*. \*\*: Average number of NLL on treated leaf of *Chenopodium quinoa*. Positive control: leaf inoculated with only infectious sap. Negative control: leaf inoculated with only buffer. Healthy: uninoculated leaf.

Note: The bold number of NLL reflects high antagonistic activities.

growth. Moreover, the isolate showed antibacterial activities and no sensitivity to streptomycin ( $4 \mu\text{g mL}^{-1}$ ) was observed.

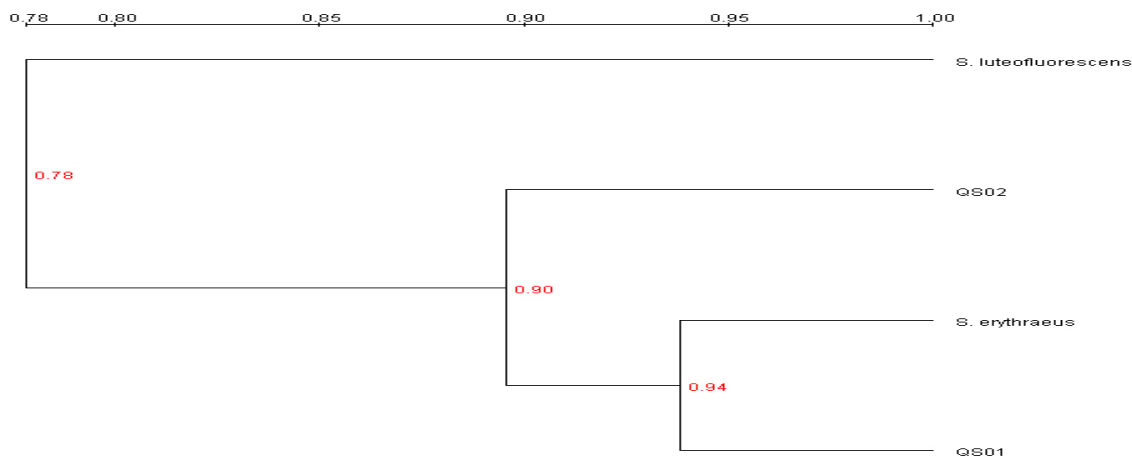
Data on isolate QS02 show that it had red aerial mycelium (red colour series), and its spores were with a type-chain belonging to spiral section with warty surface (Table I; Fig. 1 & 2). This isolate was also characterized by excellent growth on Czapek's agar medium, producing no pigment, utilizing 7/9 sugars used as sole carbon source, and sensitivity to streptomycin ( $4 \mu\text{g mL}^{-1}$ ). Antibacterial and antifungal activities also scored for this isolate.

The isolate QS03 indicated gray color of aerial mycelium while the reverse side of substrate mycelium was yellow. Spore chains belonged to RA section with smooth surface (Table I; Fig. 1 & 2). No soluble pigment produced

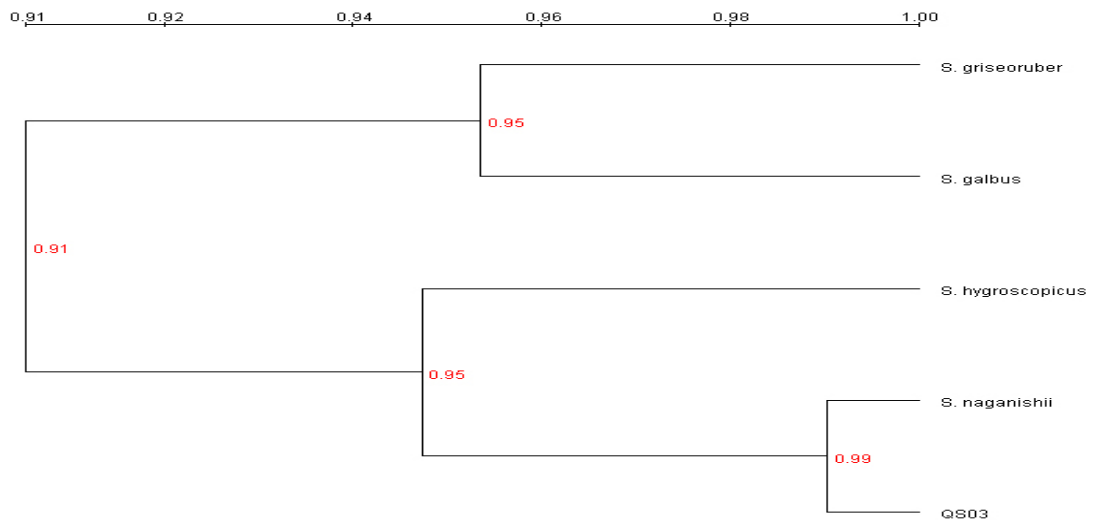
in all standard media used. Moderate growth on Czapek's agar medium was noted. This isolate actively utilized all of the carbon sources for growth except sucrose. The isolates showed antibacterial and antifungal activities and was not affected by streptomycin ( $4 \mu\text{g mL}^{-1}$ ).

The last isolate, i.e., QS04 was belonged to the yellow colour series and the vegetative mycelium was also pigmented and bright yellow. This isolate had RF spore chain with smooth surface with any ornamentation (Table I; Fig. 1 & 2). It grew poorly on Czapek's agar medium and actively utilized all the applied sugars except sucrose. This isolate showed antifungal activity and exhibited a good growth in the presence of  $4 \mu\text{g mL}^{-1}$  streptomycin in the growth medium.

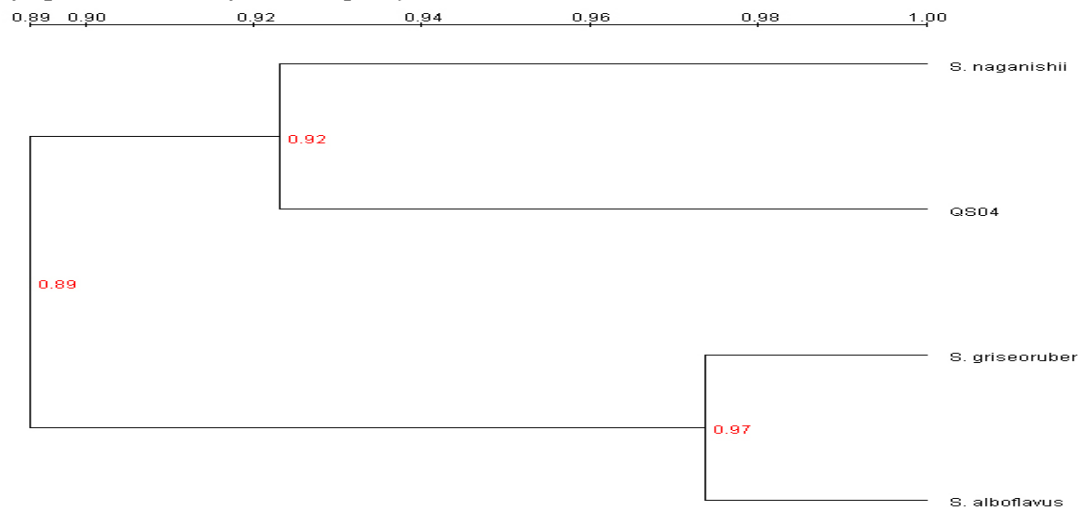
**Fig. 4. Phylophentic tree of two red *Streptomyces* isolates (QS01 and QS02) and those related in Pridham and Tresner (1974).**



**Fig. 5. Phylogenetic tree of a gray *Streptomyces* isolate (QS03) and its related in Pridham and Tresner (1974).**



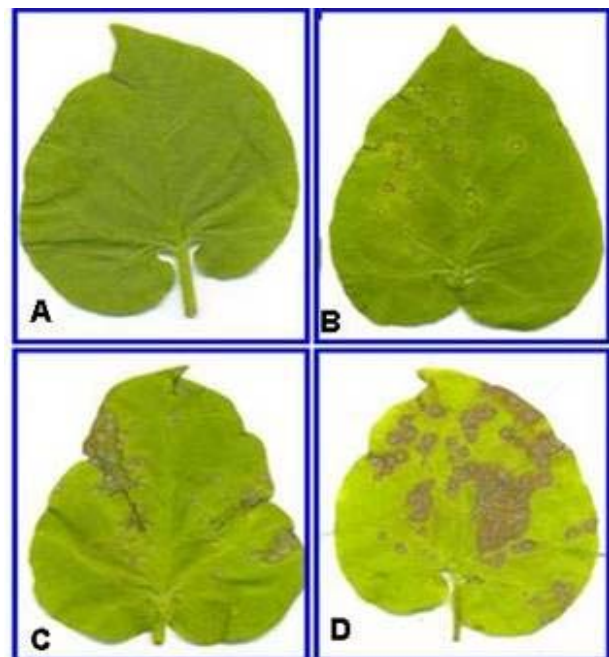
**Fig. 6. Phylophentic tree of a yellow *Streptomyces* isolate (QS04) and its related in Pridham and Tresner (1974).**



**Numerical identification of *Streptomyces* isolates.**

Mohamed *et al.* (2005) suggested a numerical method for the first time in Egypt for identification of eight unknown *Streptomyces* isolates as species using 14 related *Streptomyces* species in Pridham and Tresner (1974). They successfully identified six (ST08, ST09, ST11, ST13, ST14 & ST15) out of the eight applied *Streptomyces* isolates 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% similarities, respectively. While, two *Streptomyces* isolates (ST10 & ST12) were classified as duplicate strains of *S. echinatus* with similarities of 93.3 and 94.6%, respectively. Using this identification method, the cultural, morphological physiological and antibiosis characteristics of the *Streptomyces* isolates of this study were compared with two (*S. erythraeus* and *S. luteofluorescens*), three (*S. naganishii*, *S. griseoruber* and *S. galbus*) and four (*S. hygrosopicus*, *S. michigansis*, *S. griseobrunneus* and *S. alboflavus*) *Streptomyces* species (Pridham & Tresner, 1974). Results showed that the three *Streptomyces* isolates which belonged to three different series color groups, i.e., yellow, gray and red were fell in three major clusters (Fig. 3). The third cluster represented the red series color group showed the presences of QS01 and QS02 in one cluster. The result also indicated the importance of color of aerial mycelium as main taxonomical character for identification of

**Fig. 7. Necrotic local lesions (NLL) induced by TMV on *N. glutinosa* (as a NLL host) leaves treated with mixtures of different concentrations (1/50, 1/100, 1/200 and 1/400; A, B, C and D, respectively) of the supernatant of the halotolerant *Streptomyces* QS01 isolate and TMV-infectious sap (1:1, v:v).**

***Streptomyces*.**

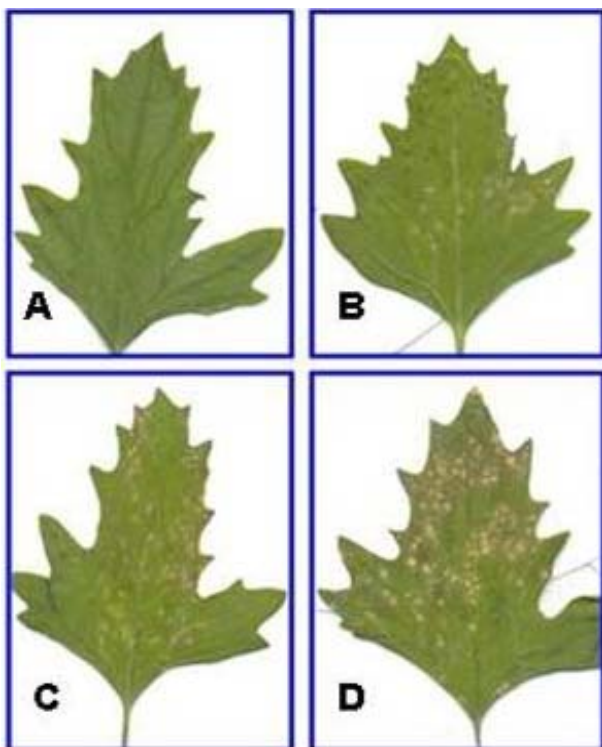
Data revealed that QS03 and QS04 were the strains of *S. naganishii* and *S. michigansis* with similarities of 99.0 and 92.3%, respectively (Tables II & III; Figs. 4, 5, 6). Isolate QS03 differed from *S. naganishii* in the ability to grow on Czapek's medium, CSubMy and type of spore chain (RA & S, respectively). Isolate QS01 and QS02 were considered as strains of *S. erythraeus* with similarities of 93.8 and 90.2%, respectively. This suggestion confirmed by result shown in the dendrogram (Fig. 3) as QS01 and QS02 isolates fell in one major cluster. Mohamed *et al.* (2005) found similar results. Slight differences in growth on Czapek's medium, utilization of D-fructose as a sole source of carbon and sensitivity to streptomycin were recorded between QS01 and *S. erythraeus*.

Some investigators proposed different numerical systems for identification the actinomycetes as well as streptomycetes. Goodfellow and Pirouz (1982) compared the numerical phenetic survey of 130 actinomycetes representing 19 genera and 50 species using 108 unit characters. They mentioned that over 80% of the strains were assigned to two clusters containing between two and 25 organisms. Most of the clusters were distinct and homogeneous though two were divided into subclusters. Williams *et al.* (1983) used a numerical classification of *Streptomyces* and related genera. The found that most of the *Streptomyces* type cultures (475 strains which included 394 type cultures of *Streptomyces* and representatives of 14 other actinomycete genera) fell into one large cluster-group. Their data provided a basis for the reduction of the large number of *Streptomyces* species which have been described. Moreover, they showed that the phenetic data obtained in their study, together with those from previous diverse studies, indicated that the genera *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa* and *Microellobosporia* should be reduced to synonyms of *Streptomyces*, while *Intrasporangium*, *Nocardioides* and *Streptoverticillium* remained as distinct genera in the family *Streptomycetaceae*.

Data obtained for the phonetic relationship of this study agreed with the findings of Goodfellow and Pirouz (1982), Williams *et al.* (1983) and Mohamed *et al.* (2005) as the applied classification systems could be limited to the nomenclature of the new species of streptomycete isolates as well as the related genera of actinomycetes.

**Antagonistic activities of the *Streptomyces* isolates.** The antiviral activities of some *Streptomyces* species in the key of Pridham and Tresner (1974) were reported. Mahfouz and Mohamed (2002) found similar results for some local *Streptomyces* species isolated from Sinai sandy soil against TMV using *D. metel* as a local lesion host. In the present study, the antagonism activities of the four *Streptomyces* isolates under investigation and obtained from Qaroon lake were determined against two different viruses belonging to two different plant viuses groups, i.e., tobamoviruses (TMV) and potyviruses (PVY). QS01 and QS02 isolates

**Fig. 8. Necrotic local lesions (NLL) induced by PVY on *Ch. quinoa* (as a NLL host) leaves treated with mixtures of different concentrations (1/50, 1/100, 1/200 & 1/400; A, B, C & D, respectively) of the supernatant of the halotolerant *Streptomyces* QS01 isolate and PVY-infectious sap (1:1, v:v).**



showed greater activities, as their crude supernatants completely inhibited the inducing of NLL by the two tested viruses (Table IV; Fig. 7 & 8). By dilution of the supernatants up to 1/50, 1/100, 1/200 and 1/400, the NLL appeared on the inoculated leaves. This could be due to the decreased amount of antiviral substance(s), which excreted out of the streptomycete cells and released into the supernatants. Isolates QS03 and QS04 showed moderate antiviral activities as a few number of NLL were appeared using the crude supernatants. The results indicated the possibilities of extraction, purification and identification of such substances. Mahfouz and Mohamed (2002) also expressed the similar views. The results also encourage the idea for the use of such active streptomycetes as a biopesticide for controlling such plant pathogens, i.e., viruses.

**Acknowledgment.** We thank A.S. Sadik, Ain Shams Univ. and Gh.A. Gad El-Karim and Mr. A. Faway, 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.*, (in press).

- Goodfellow, M. and T. Pirouz, 1982. Numerical classification of sporoactinomycetes containing meso-diaminopimelic acid in the cell wall. *J. Gen. Microbiol.*, 128: 503–27
- Jiang, C.L. and L.H. Xu, 1996. Diversity of aquatic actinomycetes in lakes of the middle plateau, Yunnan, China. *Appl. Environ. Microbiol.*, 62: 249–53
- Kushner, D.J., 1978. Life in high salt and solute concentrations: *Halophilic bacteria*. In: Kushner, D.J. (ed.) *Microbial Life in Extreme Environments*. pp. 317–68. Academic Press, Ltd., London, United Kingdom.
- Langó, Zs., L.G. Tóth, G. Kovács and L. Cruz–Pizarro, 1999. Identification and biochemical characterization of *Streptomyces galbus* from the sediment of the high–mountain lake La Caldera (Sierra Nevada, Spain). *Fol. Microbiol.*, 44: 651–6
- LeFevre, E. and L.A. Round, 1919. A preliminary report upon some halophilic bacteria. *J. Bacteriol.*, 4: 177–82
- Mahfouz, H.T. and S.H. Mohamed, 2002. Physiological, antagonistic and fingerprinting studies on some halotolerant *Streptomyces* strains. *Arab J. Biotechnol.*, 5: 103–20
- Mohamed, S.H., 1998. Role of actinomycetes in the biodegradation of some pesticides, p. 151. *Ph.D. Thesis*, Agriculture Microbiology, Department of Agriculture Microbiology, Faculty of Agriculture, Ain Shams University.
- Mohamed, S.H., 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.*, 8: 41–61
- Mohamed, S.H., E.A. Saleh and M.M. Zaki, 2005. Identification of eight halotolerant *Streptomyces* isolates using a suggested numerical system. *Arab Univ. J. Agric. Sci.*, (In press).
- Okami, Y. and K. Hotta, 1988. Search and discovery of new antibiotics, In: Goodfellow, M., S.T. Williams and M. Mordaski, (eds.) *Actinomycetes in Biotechnol.*, pp. 33–67. Academic Press, London, United Kingdom
- Pridham, T.G. and H.D. Tresner, 1974. Family *Streptomycetaceae*. In: *Bergey's Manual of Determinative Bacteriology*, 8<sup>th</sup> Williams and Wilkins Co., Baltimore, USA.
- Ramos–Cormenzana, A., 1989. Ecological distribution and biotechnological potential of halophilic microorganisms, In: Da Costa, M.S., J.C. Duarte and R.A.D. Williams, (eds.) *Microbiology of Extreme Environments and its Potential for Biotechnology*, pp. 289–309. Elsevier Applied Science, London, United Kingdom.
- Saleh, E.A., M.M. Zaki, M.E. El–Demerdash and Mohamed H. Sonya, 1990. Identification of some halotolerant streptomycetes isolated from marine ecosystems in Egypt. *Ann. Agric. Sci.*, Special Issue, 409–25
- Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.*, 16: 313–40
- Sneath, P.H.A. and R.R. Sokal, 1973. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*, pp. 1–573. San Francisco: W.H. Freeman
- Tanaka, Y.T. and S. Oomura, 1993. Agroactive compounds of microbial origin. *Annu. Rev. Microbiol.*, 47: 57–87
- Trüper, H.G. and E.A. Galinski, 1986. Concentrated brines as habitats for microorganisms. *Experientia*, 42: 1182–7
- Vreeland, R.H., 1987. Mechanisms of halotolerance in microorganisms. *Crit. Rev. Microbiol.*, 14: 311–56
- Waksman, S.A. and H.A. Lechevalier, 1961. Classification, identification and description of genera and species. In: *The Actinomycetes. Vol. II*. The Williams and Wilkins, Co., Baltimore, USA.
- 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: 1743–813
- Xu, L.H., Q.R. Li and C.L. Jiang, 1996. Diversity of soil actinomycetes in Yunnan, China. *Appl. Environ. Microbiol.*, 62: 244–8
- Zaki, M.M., E.A. Saleh, M.E. El–Demerdash and S.H. Mohamed, 1993. Antimicrobial activities of some halotolerant streptomycete strains as affected by incubation period and medium composition. *Ann. Agric. Sci., Sp. Issue*, 2: 519–29

(Received 18 March 2005; Accepted 22 June 2005)