Identification and molecular studies on some halotolerant streptomycetes isolated from Sinai sandy soil

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ABSTRACT

Five streptomycete isolates obtained from Sinai sandy soil in Egypt, having the ability to tolerate 9-12 % NaCl in the growth medium, were identified. Results showed that two out of them are belonging to the violet series (Si-1 and Si-9) and the other three isolates are belonging to the red series (Si-4, Si-6 and Si-11). Results of the cultural, morphological and physiological characteristics showed that Streptomyces isolate Si-1 a novel violet species. Four isolates were identified as S. tuirus Si-4, S. lateritius Si-6, S. mauvecolor Si-9 and S. melanogenes Si-11. Data of RAPD-PCR identification revealed that Streptomyces strain Si-1 had dissimilarities ranging from 60.9 to 65.1% with the red series isolates and 58.5% with the Si-9 violet series isolate. The dissimilarities between the three red series isolates were 33.3, 46.9 and 50.3%. Unique and specific PCR fragments were obtained for each strain; therefore, the results of this work paid an attention to encourage the use of RAPD-PCR technique as a new molecular tool for identification of actinomycetes.

Key words: *Halotolerant, Actinomycetes, Streptomyces, Identification, RAPD, PCR.*

INTRODUCTION

Streptomycetes are widely distributed in terrestrial and aquatic habitats. Soil, fodder and composts appear to be the primary reservoirs for streptomycetes. Indeed, it appears that streptomycetes exist in soil for long periods as resting arthrospores that germinate giving the occasional presence of exogenous nutrients (Mayfield *et al.*, 1972). Streptomycete spores have a net negative surface except at low pH levels (Douglas *et al.*, 1970) and are generally more resistant to heat than the corresponding hyphae (Goodfellow and Simpson, 1987).

Streptomycetes are usually considered to be strict aerobes and they can grow in sterile soil at low oxygen concentration and in dry soil, their counts decrease. But, their proportion to other bacteria may be higher as their spores are more resistant to desiccation than the vegetative cells of bacteria (Wong and Griffin, 1974).

Several recent investigations reported the presence and importance of actinomycetes belonging to the genus *Streptomyces* in different soil types (Paul and Banerjee, 1983; De and Gupta, 1991; Watanabe *et al.*, 1994; El-Abyad *et al.*, 1996 a&b; Mohamed, 1998). In Egypt, Hussein and Abbas (1986), Saleh *et al.* (1990) and Zaki *et al.* (1993) studied halotolerant streptomycetes isolated from soil and/or marine ecosystem. Recently, Mohamed *et al.* (2000) isolated a number of 58 halotolerant streptomycete isolates collected from sandy soils of Damietta, Ismailia, Port Said and Sinai governorates. The isolates varied greatly in their salt tolerance, in which salt concentration increased in the growth medium from 3.0 to 21 %.

Few studies have documented the use of RAPDs for the typing of bacterial species and strains. Application of the RAPD assay is to find genetic variation where it had not previously been recognized, and supports differences that have been detected by other methods of characterization. Mehling *et al.* (1995) applied RAPD assays in identifying conserved regions of actinomycete genomes using various arbitrary primers as well as pUC18/19 reverse sequencing primers. Use of a modified reverse primer led to amplification of one major band (1100 bp), which was not found when DNAs from other bacteria were used in comparable experiments.

This study was designed to identify some halotolerant actinomycete isolates obtained from sandy soil in Sinai, Egypt, having the ability to tolerate a concentration of NaCl ranging from 9 to 12%. A trail to use the RAPD-PCR analysis as a molecular tool was also included for differentiation between the selected actinomycete isolates.

MATERIALS AND METHODS

Streptomycete isolates source

Five halotolerant streptomycete isolates from Sinai sandy soil (Egypt), having the ability to grow on starch nitrate agar medium with different concentrations of NaCl ranging from 9 to 12%, were provided from Department of Agricultural Microbiology, Institute of Soil, Water and Environment Research, ARC, Giza, Egypt.

Test organisms used

Different test organisms, including fungi, i.e., *Fusarium oxysporum F. sp.* Lycopersci-123 and *Helminthosporium gramenium*-133; yeast, 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 test organisms were kindly provided by Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Streptomycetes identification

Cultural, morphological and physiological characteristics of the streptomycete strains under investigation were determined using the media and methods of the International *Streptomyces* Project (ISP) as described by Shirling and Gottlieb (1966). The type of spore chain for each isolate was determined according to Pridham *et al.* (1958). The keys given by Shirling and Gottlieb (1968 a & b 1972), Kster (1972) and Pridham and Tresner (1974), were used for identification.

Extraction of total nucleic acids from streptomycete isolates

Starch nitrate broth medium (Waksman and Lechevalier, 1961) (50 ml in 250-ml conical flask) inoculated separately with 2 ml of the spore inoculum of each of the five *Streptomyces* isolates, was incubated at 28°C2 for 6 days on a rotary shaker (160-rpm). Two flasks were used and a flask was left without inoculation to serve as control. The streptomycete mycelium was collected by centrifugation of 5 ml of cell suspension for 20 min at 14000 rpm at 4°C. The supernatant was discarded and the pellets were then pulverized in liquid nitrogen and then subjected to nucleic acids extraction as described by Marmur (1961).

Purification of DNA extracts

To the nucleic acids suspension, the ribonuclease (DNase free) was added with a final concentration of 1 µg/ml followed by digestion for 30 min at 37°C in a water bath. The protein molecules were removed by adding the proteinase K with a final concentration of 50 µg/ml followed by incubation for 1 hr in a water bath. The DNA was then re-extracted with phenol/chloroform and precipitated as given by Marmur (1961).

Measurement of DNA concentration

The concentration and purity of DNA extracts of the five identified streptomycete isolates were determined as recommended by Brown (1990) using the ultraviolet absorbence spectrophotometer (Model Du-40 spectrophotometer Beckman). With a pure sample of DNA, the $A_{260/280}$ is 1.8-2. The DNA concentration was then adjusted to 100 ng/µl.

Primers used for RAPD-PCR

Ten decamer oligonucleotide primers; OP-B15, OP-B17, OP-E08, OP-Z04, OP-Z06, OP-Z07, OP-Z08, OP-Z10, OP-Z13 and OP-Z14 as nomenclatured OPERON Technologies, Alameda, CA., were used for RAPD-PCR.

RAPD-PCR

RAPD-PCR was carried out according to the procedure given by Williams *et al.* (1990) with minor modification. The amplification reaction was conducted on a volume of 50 µl. Each reaction mixture contained 100 ng genomic DNA (as a template), 50 pmole primer, 2 units of *Taq* DNA polymerase (Promega Corp., Madison, WI, USA), 5 µl of 10X buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs and deionized distilled water (dd.H_2O). The reaction was overlaid with a drop of mineral oil. The PCR amplification was performed in a Perkin-Elmer/DNA Thermal Cycler 480 (Norwalk, CT) for 35 cycles after initial denaturation for 4 min at 94°C. Each cycle consisted of denaturation at 94°C for 1 min, annealing temperature at 37°C for 2 min and extension at 72°C for 2 min. The final primer extension cycle was extended to 5 min. The amplified products were resolved by electrophoresis in a 1 % agarose at 60 volts for 2.5 hr with TBE buffer (Sambrook *et al.*, 1989). PCR products were visualized by staining gel ethidium bromide (0.5 µg/ml) and photographed under UV light using a Polaroid camera.

RAPD analysis

Amplified products were visually examined and scored as present (1) or absence (0). Bands of the same mobility were scored as identical. The similarity coefficient (F) between isolates was defined by the formula of Nei and Li (1979). A dendrogram was derived from the distance by unweighted paired-group method,

arithmetic mean (UPGMA) contained in the computer program package NTSYS 1.5 (Rohlf, 1990).

RESULTS AND DISCUSSION

In this study, the applied isolates were re-examined by the light microscope as recommended by Bergey s Manual (1994). Results showed that they are belonging to the genus *Streptomyces*, as they formed well-developed branching non-septate aerial mycelia carrying long spore chains and the non-motile spores were not borne on verticillate sporophores. Based on the color of aerial mycelium (Tresner and Backus, 1963), the *Streptomyces* isolates were divided into two groups. *Streptomyces* isolates Si-1 and Si-9 were categorized in violet group, while isolates Si-4, Si-6 and Si-11 were categorized in red group.

Identification of streptomycetes belonging to violet series

Streptomyces isolate Si-1

Data in Table (1) show that the halotolerant *Streptomyces* isolate Si-1 was able to tolerate NaCl up to 9% in starch nitrate agar medium and has violet aerial mycelium (the violet color series). Its substrate mycelium produced violet pigment, aerial spore chains belonged to section RA (Figure 1A) with warty

Table (1): Characteristics of Streptomyces isolate Si-1 compared with those of similar species reported in different keys.

Character	Isolate Si-1	*violans** Pridham and Tresner (1974)	*S. rectivrolaceus** Pridham and Tresner (1974)
Color of aerial mycelium	Violet	Violet	Violet
Spore-chain	RA	S	RF
Melanoid pigment	+	+	-
Spore surface	WTY	SPY	SM
Growth on Czapek s medium	Good	Good	ND
Color of substrate mycelium	Violet	Violet	Violet
Diffusable pigments	-	+ on some media	ND
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	Antibacterial and antifungal
Sensitivity to streptomycin	NS	ND	ND
NaCl tolerance	0-9%	ND	ND

+ = Growth. - = No growth. NS: Not sensitive. RA= Retnaculiaperti. S= Spiral or coiled. RF= Spores in straight (R) or flexuous (F) chains. ND= No data. SM= Smooth. *: Not found in Shirling and Gottlieb (1968a & b; 1972) and (1972).

surface (Figure 1D). This isolate has the ability to produce melanoid pigment, while failed to produce diffusable pigments on the standard media, utilized all used sugar as a sole carbon source for growth and characterized by good growth on Czapek's agar medium. Data also indicated that this isolate showed antagonistic activities against test organisms used, i.e., bacteria, fungi and yeast and no sensitivity to streptomycin (4 g ml^{-1}). The characteristics of this isolate were compared with the cultural, morphological and physiological characteristics of the *Streptomyces* spp. (Shirling and Gottlieb, 1968a & b; 1972; Kuster, 1972 and Pridham and Tresner, 1974). As shown in Table (1), *Streptomyces* isolate Si-1 is unlikely to be S. *violans* or S. *rectivrolaceus* mainly because of the differences in spore chain morphology, spore surface ornamentation and melanoid pigment production. Accordingly, the tested isolate Si-1 differs from the described violet pigmented *Streptomyces* species. Therefore, it could be identified as a new species.

Streptomyces isolate Si-9

Results in Table (2) of the halotolerant *Streptomyces* isolate Si-9, having the ability to tolerate salt concentration up to 12%, show that this isolate has violet aerial mycelium (violet color series) and substrate mycelium producing a deep yellow pigment. It has straight and long spore chains (section RF) (Figure 1B) and spores are characterized by spiny surface (Figure 1C). This isolate is also characterized by poor growth on Czapek's agar medium and sensitivity to streptomycin (4 ug

Table (2): Characteristics of Streptomyces isolate Si-9 compared with those of similar species reported in different keys.

Character	Isolate Si-9	*S. mauvecolor** Pridham and Tresner (1974)
Color of aerial mycelium	Violet	Violet
Spore-chain	RF	S
Melanoid pigment	+	+
Spore surface	SPY	SPY
	Poor	Poor
Color of substrate mycelium	Deep yellow	ND
Diffusable pigments	-	ND
Utilization of carbon:		
No carbon	-	ND
D-glucose	+	+
D-xylose		-
L-arabinose	-	+
L-rhamnose		-
D-fructose	-	+
Raffinose	-	-
D-mannitol		-
i-inositol	-	-
Sucrose		-
Antagonistic activity	Antibacterial and antifungal	ND
Sensitivity to streptomycin	Se	ND
NaCl tolerance	0-12%	ND

+ = Growth. - = No growth. = Indoubt. Se: Sensitive. ND: No data. RF = Spores in straight (R) or flexuous (F) chains. S= Spirals or coiled. SPY= Spiny. *= Not found in Shirling and Gottlieb (1968 a & b; 1972) and Kuster (1972).

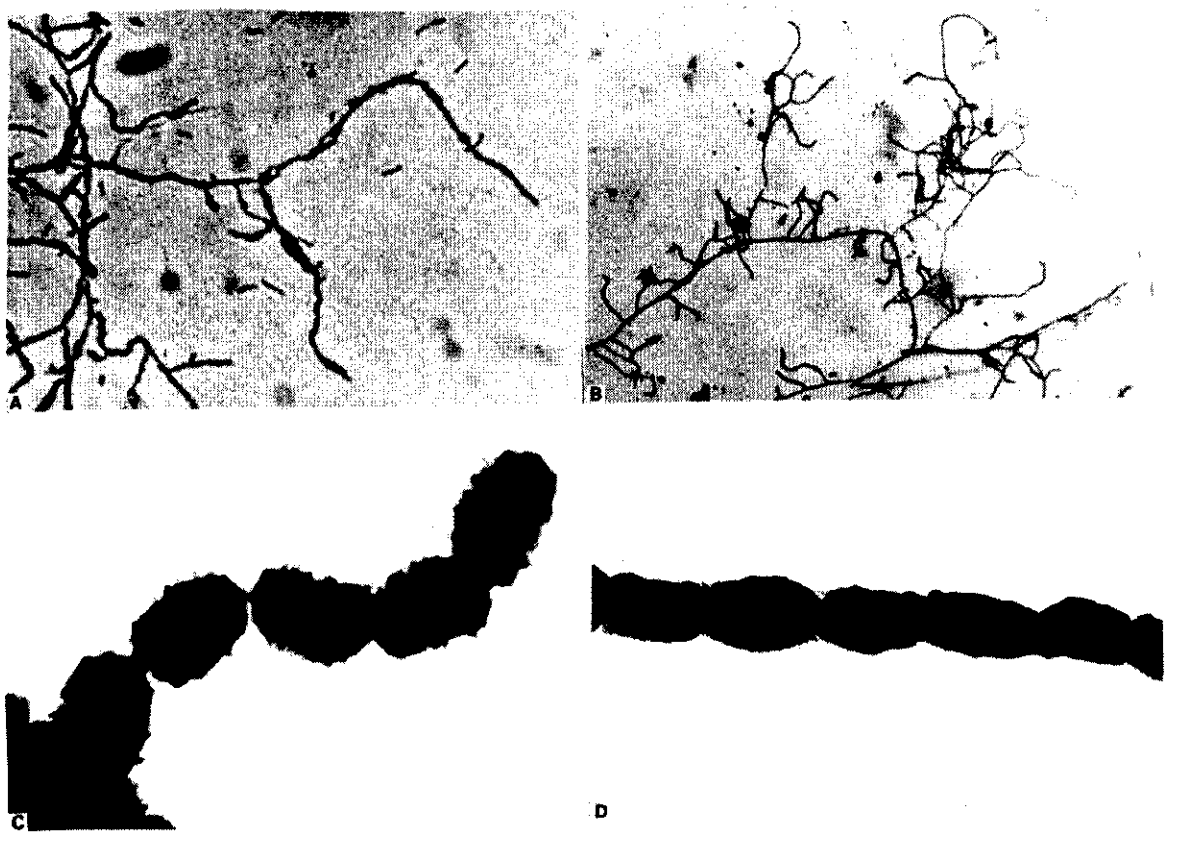

Fig. (1): Microphotographs (Top, X-1000) and electron micrographs (Bottom, X-10000) of spore chain of Streptomyces isolates Si-1 and Si-9 belonging to violet series. Note, spores in retnaculiaperti chains (RA) with a warty (WTY) surface (A & D, respectively) or in flexuous chains (RF) with a spiny (SPY) surface (B & C, respectively).

ml^{-1}). The isolate is also characterized by utilizing the D-glucose, doubtful to utilize sucrose, D-mannitol, L-rhamnose and D-xylose, failing to utilize L-arabinose, D-fructose, Raffinose and i-inositol sugars as sole carbon sources, no diffusable pigments and antagonized all test organisms used.

Considering the description keys proposed by Pridham and Tresner (1974), the isolate could be closely related to *S. mauvecolor*, although there were some differences in the utilization of L-arabinose and D-fructose for growth and the spore chain morphology. No similar species were found in Shirling and Gottlieb (1968a &b and 1972) and Kuster (1972). Therefore, isolate Si-9 could be considered as a strain of *S. mauvecolor*.

Identification of *Streptomyces* belonging to red series

Streptomyces isolate Si-4

Results in Table (3) show the characteristics of *Streptomyces* isolate Si-4. The cultural, morphological and physiological characteristics of the tested isolate clearly showed that the color of aerial mycelium was red (red color series), while the reverse side of substrate mycelium was deep orange. Spore chains are belonging to spiral section (Figure 2A) with smooth surface (Figure 2D). Light brown soluble pigments were produced on

Table (3): Characteristics of Streptomyces isolate Si-4 compared with those of similar species reported in different keys.

Character	Isolate Si-4	*S. tuirus** Pridham & Tresner (1974)	*S. massasporeus*** Shirling and Gottlieb (1968,a)	*S. massasporeus*
Color of aerial mycelium	Red	Red	Red	Red
Spore-chain	S	S	S or RA	S
Melanoid pigment	+	+	+	+
Spore surface	SM Good	SM Moderate	SM ND	SM
Color of substrate mycelium	Deep orange	Red to violet	Grayed yellow modified by red or violet	ND
Diffusable pigments	Light brown	Red to red-brown on some media	Red to violet	+
Utilization of carbon:				
No carbon	-	ND		ND
D-glucose	+	+	+	ND
D-xylose	+	+	+	ND
L-arabinose	+	+	+	ND
L-rhamnose	+	+	+	ND
D-fructose	+	+	+	ND
Raffinose	+	+	+	ND
D-mannitol	+	+	+	ND
i-inositol	+	+	+	ND
Sucrose	+	+	+	ND
Antagonistic activity	Antibacterial and antifungal	Antibacterial	ND	ND
Sensitivity to streptomycin	Se	ND	ND	ND
NaCl tolerance	0-12%	ND	ND	ND

+= Growth. -= No growth. ND= No data. RA= Retnaculiaperti. S= Spiral or coiled. RF= Spores in straight (R) or flexuous (F) chains. SM= Smooth. Se = Sensitive. *= Not found in Shirling and Gottlieb (1968 a & b) and Kuster (1972). ** = Not found in Pridham and Tresner (1974).

some media used. This isolate utilized all the sugars used as sole carbon sources for growth. It had antibacterial and fungal activities, ability to grow on 12% NaCl on starch nitrate agar medium and characterized by a good growth on Czapek's agar medium. This isolate showed no sensitivity to streptomycin (4 μg ml^{-1}).

Using the keys of Shirling and Gottlieb (1968a) and Kuster (1972), the tested isolate is unlikely to be *S. massasporeus*. Slight differences were reported, such as the morphology of spore chains, the reverse side of colonies and production of soluble pigments on standard media.

Based on the description keys proposed by Pridham and Tresner (1974), the isolate Si-4 could be closely related to *S. tuirus* with slight differences in producing red to violet color of substrate mycelium and red to red-brown soluble pigments on some media. Therefore, isolate Si-4 could be considered as a strain of *S. tuirus*.

Streptomyces isolate Si-6

Data in Table (4) show that isolate Si-6 is belonging to the red color series and the vegetative mycelium was also pigmented with pale rose color. This isolate had RA spore chains (Figure 2B) with warty surface (Figure

Table (4): Characteristics of Streptomyces isolate Si-6 compared with those of similar species reported in different keys.

Character	Isolate Si-6	*S. lateritius** Pridham and Tresner (1974)	*S. lateritius* Shirling and Gottlieb (1968a)
Color of aerial Mycelium	Red	Red	Red
Spore-chain	RA	RA	RF
Melanoid pigment	+	+	+
Spore surface	Warty	Warty	Warty
	Poor	ND	ND
Color of substrate mycelium	Pale rose	ND	Grayed yellow to violet
Diffusable pigments	-	ND	Blue or violet
Utilization of carbon:			
No carbon	-		ND
D-glucose	+	+	+
D-xylose	-	+	+
L-arabinose	-	+	+
L-rhamnose	+	+	+
D-fructose		+	+
Raffinose	+	-	No growth or trace
D-mannitol	-	-	No growth or trace
i-inositol		+	
Sucrose		-	No growth or trace
Antagonistic activity	Anti bacterlia and antifungal	ND	ND
Sensitivity to streptomycin	NS	ND	ND
NaCl tolerance	0-12%	4%-but <7%	ND

+= Growth. - = No growth = Doubtful. ND = No data. RA=Retnaculiaperti. RF = Spores in straight (R) or flexuous (F) chains. NS=Nρt sensitive. * = Not found in Shirling and Gottlieb (1968 b and 1972) and Kuster (1972). ND = No data.

2E). It produces a melanoid pigment, given also a poor growth on Czapek's agar medium and produces pale rose soluble pigments. The isolate actively utilized D-glucose, L-Rhamnose and Raffinose, but failed to utilize D-xylose, L-arabinose and D-mannitol and doubtful in utilizing D-fructose, i-inositol and sucrose as sole carbon sources for growth. This isolate showed antagonistic activity against the test microorganisms used and a good growth in the presence of 4 $\mu g\ ml^{-1}$ streptomycin antibiotic as well as 12% NaCl in the growth medium.

On the basis of the keys proposed by Pridham and Tresner (1974), the selected isolate Si-6 appeared to be close to *S. lateritius* as illustrated in Table (4). Although there were some differences in the utilization of D-xylose, L-arabinose, D-fructose, L-raffinose and sucrose as sole carbon sources for growth, it showed antagonistic activities and salt tolerance. In Shirling and Gottlieb (1968a), *S. lateritius* showed RF spore chain section, grayed yellow to violet or blue substrate mycelium producing blue or violet soluble pigments on the standard media. They also reported that this isolate was able to utilize D-glucose, L-arabinose, D-xylose, D-fructose and rhamnose as sole carbon sources for growth, but no growth or very weak growth on sucrose, D-mannitol and raffinose was observed and growth on i-inositol was doubtful. Therefore, isolate Si-6 could be considered as a strain of *S. lateritius*.

Streptomyces isolate Si-11

Results in Table (5) showed that the *Streptomyces* isolate Si-11 has the ability to olerate 12% salt concentration. The isolate appeared with aerial mycelium belonging to red color series and pale red substrate vegetative mycelium. The spore chains belonged to RA section (Figure 2C) with smooth surface (Figure 2F). Light brown soluble pigments were produced on some media used. This isolate utilized all the sugars used as sole carbon sources for growth except raffinose. It has antibacterial and anti fungal antagonism, giving a good growth on Czapek's agar medium and showed no sensitivity to streptomycin (4-$\mu g\ ml^{-1}$) in the growth medium. Slight differences in utilizing of raffinose as a sole carbon source for growth, antagonistic activity and tolerance to NaCl in the growth medium were observed.

Streptomyces isolate Si-11 is unlikely to be *S. linolnensis* (Pridham and Tresner, 1974), mainly because of the differnces in utilization of raffinose as a sole carbon source for growth and antifungal activity.

Consequently, the tested isolate Si-11 appeared, according to Pridham and Tresner (1974), Shirling and Gottlieb (1968b) and Kuster (1972) to be close to *S. melanogenes* with slight difference in utilizing rhamnose. Therefore, it could be considered that the experimental isolate Si-11 is very likely to be a strain of *S. melanogenes.*

RAPD analysis

Highly purified DNA extracts of *Streptomyces* strains were prepared and successfully used as templates for RAPD-PCR. Data reveal that no amplified bands were observed in any of the negative controls, which indicates that the reaction mixtures were free from any strange DNA contamination.

For each isolate, the number of amplified fragments differed with different primers, which is expected as shown in Figures (3, 4 and 5). On the other direction, the number and size of amplified fragments differed from one species to another for the same primer used. This is clear since they differed in their DNA sequences.

Results in Table (6) revealed that the streptomycete isolate Si-1 had dissimilarities ranging from 60.9 to 65.1 % with the red series isolates and 58.5% with Si-9 violet series isolate. The dissimilarities between the three red series isolates were 33.3, 46.9 and 50.3%. This result supported the cultural, morphological and physiological identification based on the keys proposed by Pridham and Tresner (1974), Shirling and Gottlieb (1968a & b; 1972) and Kuster (1972). As the isolate Si-1 belongs to the violet series, it showed no similarity with those isolates of the proposed keys. This confirmed that this isolate could be a novel species. Moreover, each species was characterized by unique band(s) with the primer used (Table 7). However, some bands were common for all tested species.

Table (5): Characteristics of Streptomyces isolate Si-11 compared with those of similar species reported in different keys.

Character	Isolate Si-11	*S. linolnensis** Pridham and Tresner (1974)	*S. melanogenes* Pridham and Tresner (1974)	*S. melanogenes* Shirling and Gottlieb (1968b)	*S. melanogenes*
Color of aerial Mycelium	Red	Red	Red	Red	Red
Spore-chain	RF	RF	RA	RF	Straight
Melanoid pigment	+	+	+	ND	+
Spore surface	SM	SM	SM	SM	SM
Growth on Medium	Good	Excellent	ND	ND	ND
Color of substrate Mycelium	Pale red	ND	Red, bluish or bluish green	Grayed yellow to yellow-brown	ND
Diffusable pigments	Light brown	ND	ND	Yellow or red	+
Utilization of Carbon:					
No carbon	-	-	-	ND	ND
D-glucose	+	+	+	+	ND
D-xylose	+	+	+	+	ND
L-arabinose	+	+	+	+	ND
L-rhamnose	+	+	-	No growth or only trace of growth	ND
D-fructose	+	+	+	+	ND
Raffinose	-	+	+	+	ND
D-mannitol	+	+	+	+	+
i-inositol	+	+	+	+	
Sucrose	+	+		Reports vary on utilization of sucrose	+
Antagonistic activity	Antibacterial and antifungal	Antibacterial	Antibacterial and antitumor	ND	ND
Sensitivity to Streptomycin	NS	ND	ND	ND	ND
NaCl tolerance	0-12%	4%-but <10%	ND	ND	ND

+= Growth. -= No growth. ND= No data. . RA= Retnaculiaperti. RF= Spores in straight (R) or flexuous (F) chains. SM=Smooth. NS : Not sensitive. * = Not found in Shirling and Gottlieb (1968 a, b & 1972) and Kuster (1972).

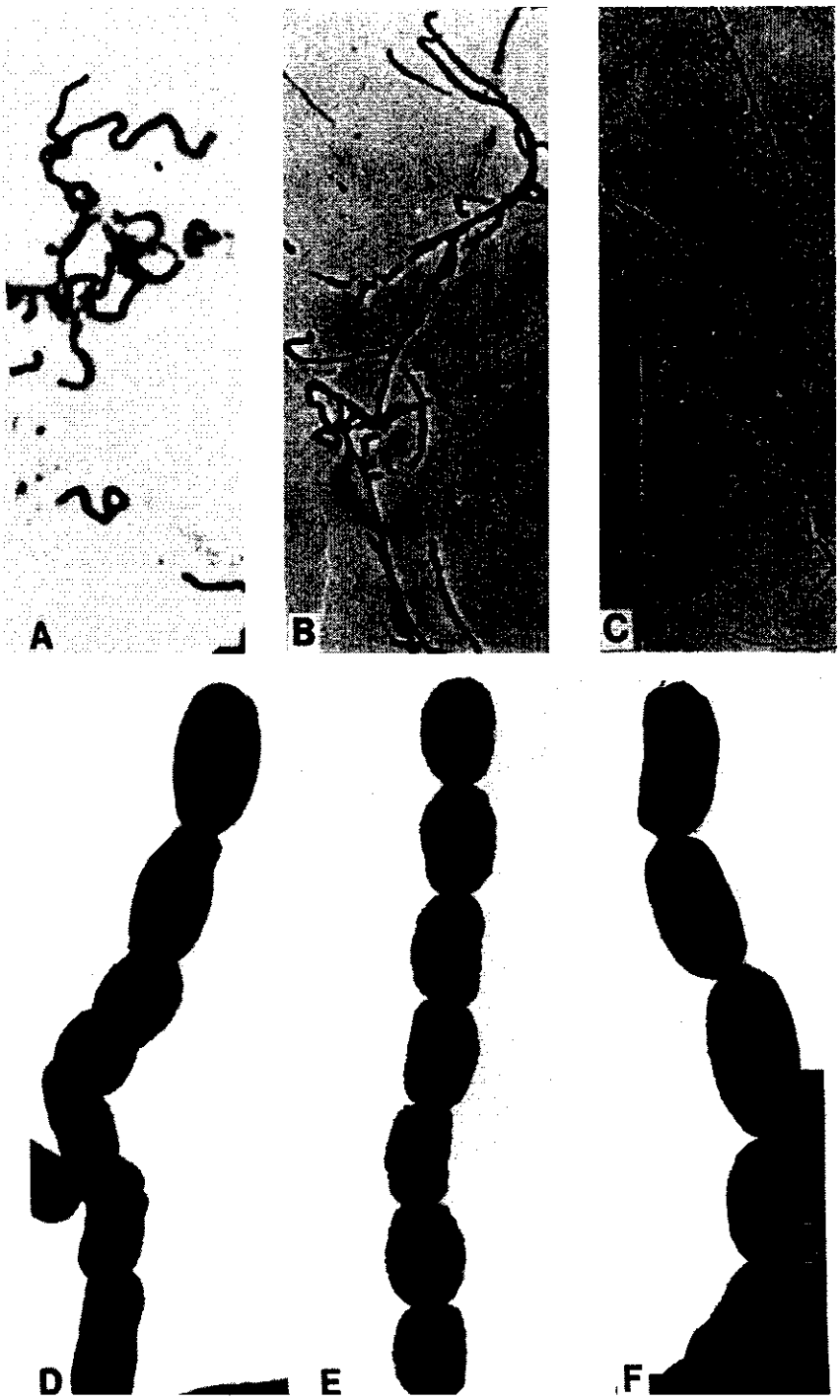

Fig. (2): Microphotographs (Top, X-1000) and electron micrographs (Bottom, X-10000) of spore chain of Streptomyces isolates Si-4 (A & D); Si-6 (B & E) and Si-11 (C& F) belonging to red series. Note, spores in spiral chains with a smooth surface (A & D, respectively) or in retnaculiaperti chains with a warty surface (B & E, respectively) and atypical chains with a smooth surface (C & F), respectively.

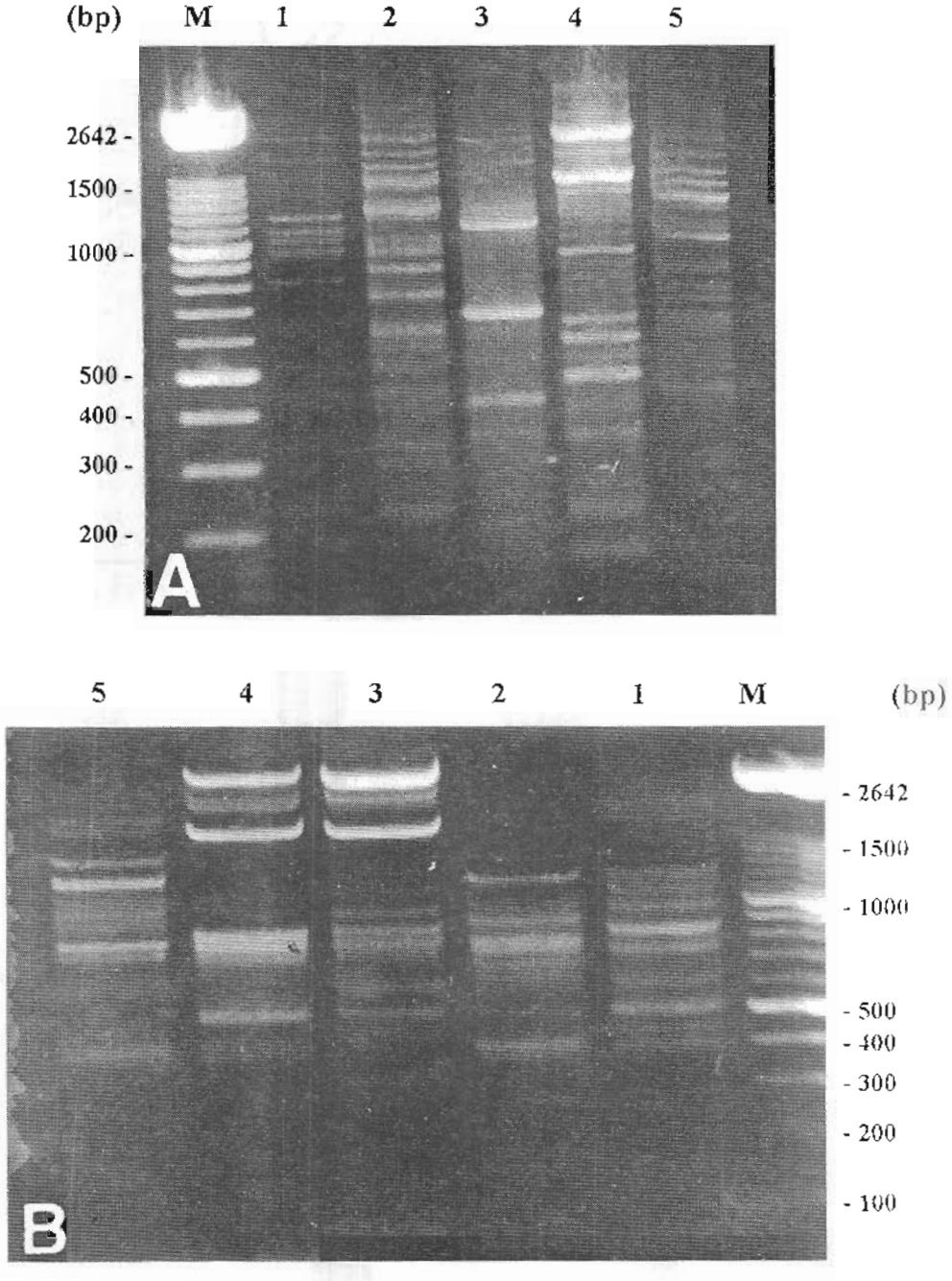

Fig. (3): Agarose gel (1%) in TBE buffer stained with ethidium bromide showing RAPD-PCR polymorphisms of DNA of Streptomyces strains (Lanes 1-5, Si-6, Si-4, Si-1, Si-9 and Si-11, respectively) using OP-Z10 (A) and OP-B15 (B). M: DNA marker.

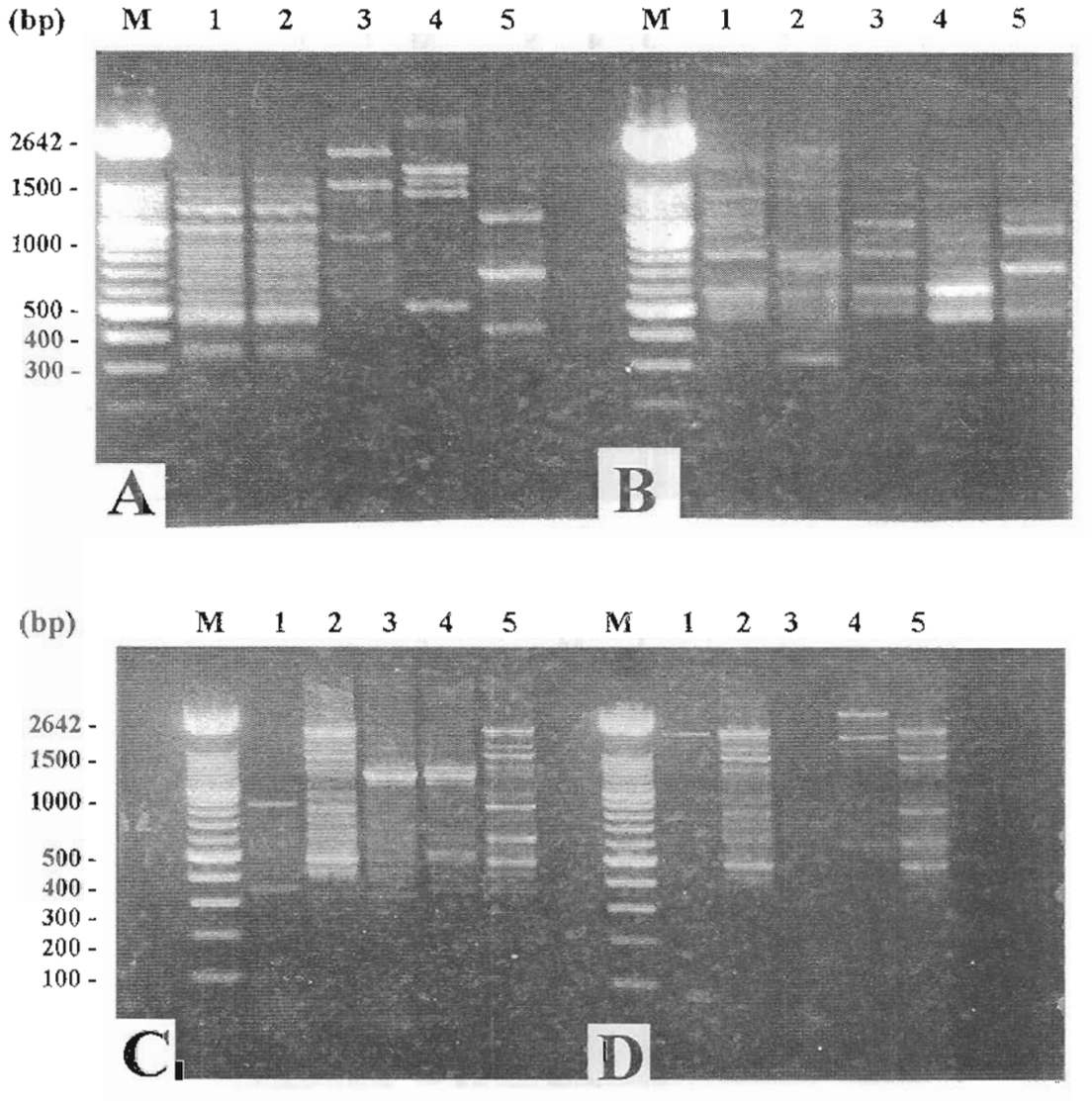

Fig. (4): Agarose gel (1%) in TBE buffer stained with ethidium bromide showing RAPD-PCR polymorphisms of DNA of Streptomyces strains (Lanes 1-5, Si-6, Si-4, Si-1, Si-9 and Si-11, respectively) using OP-Z07 (A), OP-Z13 (B) and OP-Z06 (C) and OP-Z14 (D). M: DNA marker.

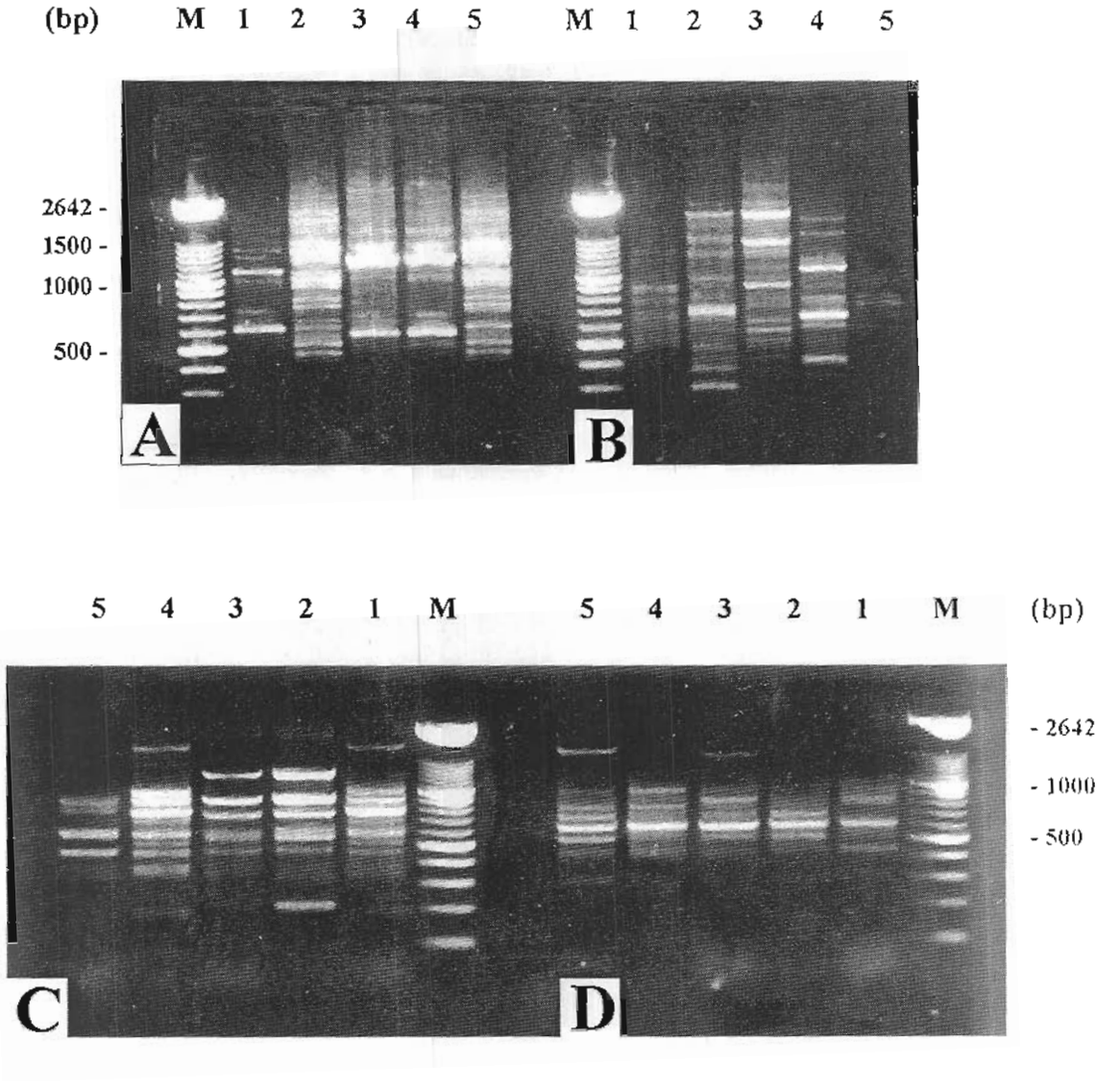

Fig. (5): Agarose gel (1%) in TBE buffer stained with ethidium bromide showing RAPD-PCR polymorphisms of DNA of Streptomyces strains (Lanes 1-5, Si-6, Si-4, Si-1, Si-9 and Si-11, respectively) using OP-Z04 (A), OP-B17 (B) and OP-E08 (C) and OP-Z08 (D). M: DNA marker.

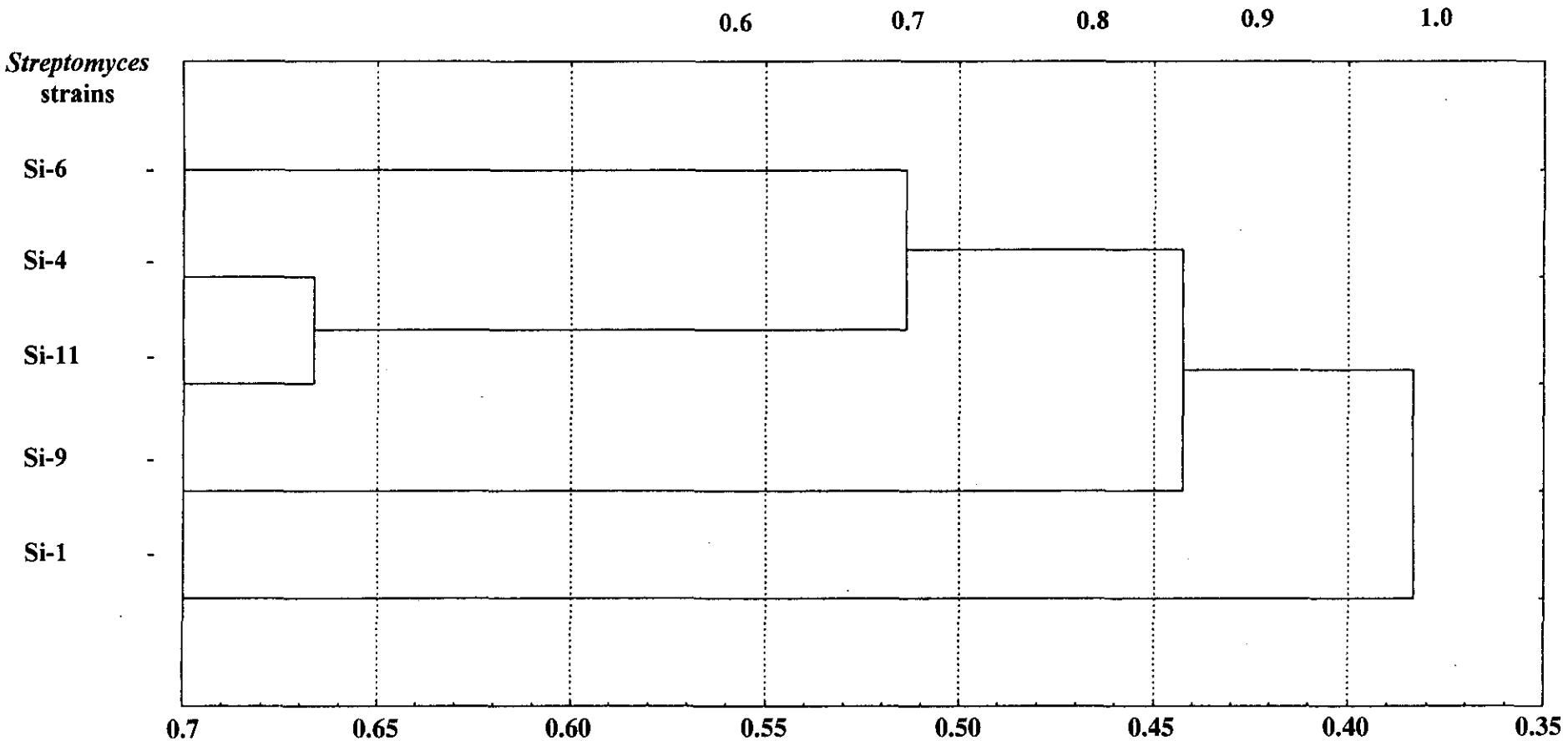

Fig. (6): Dendrogram showing relationship between the five identified Streptomyces strains (Si-1. Si-4. Si-6. Si-9 and Si-11) based on RAPD analysis.

Table (6): Similarity and dissimilarity between the five identified Streptomyces strains based on RAPD-PCR analysis.

Streptomyces strains	Similarity (Sim) and Dissimilarity (Dis) between *Streptomyces* strains (%)				
	Violet series		Red series		
	Si-1	Si-9	Si-4	Si-6	Si-11
	Sim	Sim	Sim	Sim	Sim
Violet series					
Si-1	100				
Si-9	41.5	100			
Red series					
Si-4	38.0	39.1	100		
Si-6	39.1	47.5	53.1	100	
Si-11	34.9	46.9	66.7	49.7	100

Table (7): Unique amplified fragments for each Streptomyces strain using ten primers.

Primers used	No. of unique bands				
	Streptomyces strains				
	Si-1	Si-9	Si-4	Si-6	Si-11
OP-B 15 (5 GGAGGGTGTT3)	1	0	1	1	0
OP-B 17 (5 AGGGAACGAG3)	4	2	4	0	0
OP-E 08 (5 TCACCACGGT3)	0	1	1	0	0
OP-Z 04 (5 AGGCTGTGCT3)	0	0	0	0	0
OP-Z 06 (5 GAACGGACTC3)	1	2	0	0	1
OP-Z 07 (5 CCAGGAGGAC3)	3	3	0	0	4
OP-Z 08 (5 TGGACCGGTG3)	0	1	0	0	1
OP-Z 10 (5 CCGACAAACC3)	1	4	3	2	0
OP-Z 13 (5 GACTAAGCCC3)	1	1	2	0	1
OP-Z 14 (5 TGCGTGCTTG3)	1	2	1	0	0
Total	**12**	**16**	**12**	**3**	**7**

The data of the present experiment could be considered as an additional proof that the tested isolates are different species of the genus *Streptomyces*. This work paid an attention to encourage use of RAPD-PCR technique as a new molecular tool for identification of actinomycetes.

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الملخص العربي

دراسات تعريفية وجزيئية علي بعض الأستربتوميسيتات المتحملة للملوحة المعزولة من التربة الرملية في سيناء

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تم اختيار وتعريف خمسة عزلات من الأستربتوميسيتات المتحصل عليها من التربة الرملية من سيناء لها القدرة علي تحمل الملح بتركيز ٩-١٢% في بيئة النمو. وقد ثبت أن هذه العزلات تابعة لجنس الاستربتوميسيس وأن اثنين منهما تابعين لمجموعة الاستربتوميسيتات البنفسجية اللون وهما العزلتين Si-9 & Si-1 وثلاثة تابعين لمجموعة الاستربتوميسيتات الحمراء اللون وهم العزلات Si-11 & Si-6, Si-4. وقد أوضحت نتائج الخواص المز رعية والمورفولوجية و الفسيولوجية أن عزلة الاستربتوميسيس Si-1 تمثل نوع جديد ، بينما تم تعريف أربعة عزلات علي أنهم *Streptomyces tuirus* Si-4, *S. lateritius* Si-6, *S. mauvecolor* Si-9, *S. melanogenes* Si-11. ومن نتائج التعريف الجزيئي باستخدام تكنيك الـ RAPD-PCR فقد اتضح أن عزلة الاستربتوميسيس Si-1 تختلف بنسبة تتراوح ما بين ٦٠,٩ إلى ٦٥,١ % مع العزلات التابعة لمجموعة الاستربتوميسيتات الحمراء اللون وبنسبة ٥٨,٥% مع عزلة Si-9 التابعة لمجموعة الاستربتوميسيتات البنفسجية اللون. وقد بلغت الاختلافات بين الثلاثة أنواع التابعة لمجموعة الاستربتوميسيتات الحمراء اللون ٣٣,٣ ، ٤٦,٩ ، ٥٠,٣%. و نظرا للحصول علي Fragments بأعداد وأحجام مختلفة مميزة لكل نوع من الاستربتوميسيتات فقد دفعت نتائج هذا العمل الانتباه إلى تشجيع استخدام تكنيك الـ RAPD-PCR كأداة جزيئية جديدة يمكن استخدامها في تعريف الأكتينوميسيتات.