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CULTURAL, MORPHOLOGICAL, PHYSIOLOGICAL AND MOLECULAR STUDIES ON SOME STREPTOMYCETE ISOLATES

[15]

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ABSTRACT

In this study, three isolates of *Streptomyces* isolated from Egyptian soil at Giza Governorate and belonging to red series were identified based on their cultural, morphological and physiological characters. According to the key proposed by **Pridham and Tresner (1974)**, the experimental isolates ST55, ST71 and ST86 appeared to be related to *S. lincolnensis*, *S. venezuelae* and *S. umbrinus*, respectively. In addition, random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) was used to amplify the DNA genome of the three applied isolates and an identified strain to determine their fingerprints. This was carried out using 10-decamer oligonucleotides, i.e., OP-A02, OP-D01, OP-D02, OP-D05, OP-D06, OP-D07, OP-D08, OP-D11, OP-D18 and OP-D20. The PCR amplified products were detected by electrophoresing on 1.5% agarose gel and visualized by staining in ethidium bromide and UV trasilluminator. Results showed that the same oligonucleotide flanking fragments differed from one isolate to another with different sizes. Monomorphic and polymorphic fragments were amplified confirming the molecular relationships as well as the genetic diversity between the four applied strains of *Streptomyces*. Based on the statistical analysis of RAPD-PCR polymorphisms a degree of similarities ranged from 33.9 to 59.2 % was found. Furthermore, fragment(s), whatever absent or present, were found to be unique molecular markers and could be used for characterization of some isolates. This result supported the idea for using the RAPD-PCR technique for classification of *Streptomyces* isolates.

Key words: *Streptomyces*, Identification, RAPD-PCR, Molecular markers, Fingerprint

INTRODUCTION

Streptomycetes are gram positive bacteria with high guanine plus cytosine content in their DNA. They exist mainly as spores in their natural habitat, soil, and form vegetative under favorable growth conditions. They constitute the largest actinomycete group in a number of soil **(Goodfellow and Simpson, 1987** and **Srinivasan *et al* 1991)**, and are non-fastidious organisms, which are able to degrade complex biological compounds like cellulose, lignin and chitin, and are

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satisfied with an inorganic nitrogen source. They are known as producers of many secondary metabolities, such as antibiotics, herbicides, insecticides and enzymes **(Srinivasan *et al* 1991** and **Anderson *et al* 1998)**. Also, **Strohl (1997)** mentioned that numerous new bioactive molecules were discovered, in the last decades, through large screening programs of these bacteria, and these substances found their way into various clinical uses ranging from control of infection to cancer treatment.

Thus, strptomycetes are renowned for production of array industrially important metabolites, therefore, the isolation, identification and characterization processes of *Streptomyces* isolates are still very important in search for new strains and/or novel metabolites with economic interest in diverse fields.

In the last ten years, different studies have been reported using random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) technique for identification, characterization and assessment of genetic diversity between isolates of *Streptomyces* (**Porteous *et al* 1994; Mehling *et al* 1995; Malkawi *et al* 1999; Nava *et al* 2001; Rintala *et al* 2002; Tolba *et al* 2002; Wirth and Ulrich, 2002; Cook and Meyers, 2003; Raad *et al* 2003** and **Sony *et al* 2004)**.

In Egypt, numerous researches were performed on these bacteria in diverse branches of agriculture **(Saleh *et al* 1985; Saleh *et al* 1990; Zaki *et al* 1993 Mohamed, 1998 and Mohamed *et al* 2000)**, medicine, pharmaceutics and others, but little studies were carried out using RAPD-PCR technique as a molecular tool for investigating the genetic variation between isolates of *Streptomyces* from soil **(Mohamed *et al* 2000; Mohamed *et al* 2001** and **Mahfouz and Mohamed, 2002)**.

Therefore, in this study, three *Streptomyces* isolates, belonged to the red series were identified and their DNA fingerprinting was determined *via* RAPD-PCR analysis.

MATERIAL AND METHODS

Source of streptomycete isolates

Three streptomycete isolates named ST55, ST71 and ST86, isolated from soil at Giza Governorate in Egypt were kindly obtained from, Department of Agricultural Microbiology, Institute of Soil, Water and Environment Research, ARC, Giza, Egypt. These halotolerant isolates having the ability to grow on the growth medium supplemented with 7% NaCl and belonging to the red series group of *Streptomyces*. In addition, the *S. tuirus* Si-4 that identified by **Mahfouz and Mohamed (2002)** was used as a strain of the red series group in case of RAPD-PCR analysis.

Identification of streptomycete isolates

In this experiment, the key given by **Pridham and Tresner (1974)** for identification was followed. For determination of the cultural, morphological and physiological characteristics of the applied streptomycete isolates, the media and methods of the International *Streptomyces* Project (ISP) as described by **Shirling and Gottlieb (1966)** were applied. Spore chain type for each isolate was determined according to **Pridham and Tresner (1974).** The antibiosis activities of the applied isolates were tested using two fungi **(***Fusarium oxysporum F. sp*. Lyco-

persci-123 and *Helminthosporium gramenium*-133, 2 yeasts (*Candida albicans* CAIM-352 and *C. tropicalis* CAIM-2); and 5 bacteria (*Bacillus cereus*-1283, *B. megaterium*-1066, *B. mycoides*-1084, *B. subtilis*-1007; and *Escherichia coli*-1319) as employed by **Mohamed *et al* (2001).** These microorganisms were obtained from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University.

Primers used

In this study, a number of 10-decamer oligonucleotide primers; OP-A02, OP-D01, OP-D02, OP-D05, OP-D06, OP-D07, OP-D08, OP-D11, OP-D18, and OP-D20 from OPERON Technologies, Alameda, CA., kits A and D were used. The nucleotide sequences of the applied primers was as follows:

Primer	Sequences (5'----------3')
OP-A02	TGC CGA GCT G
OP-D01	ACC GCG AAG G
OP-D02	GGA CCC AAC C
OP-D05	TGA GCG GAC A
OP-D06	ACC TGA ACG G
OP-D07	TTG GCA CGG G
OP-D08	GTG TGC CCC A
OP-D11	AGC GCC ATT G
OP-D18	GAG AGC CAA C
OP-D20	ACC CGG TCA C

RAPD-PCR analysis of the applied *Streptomyces* isolates

In order to extract the DNA genome of the three *Streptomyces* isolates, 50 ml in 250-ml conical flask of starch nitrate broth medium (**Waksman and Lechevalier, 1961**) were inoculated separately by the *Streptomyces* isolates, then incubated at 28^{o}C±2 for 6 days on a rotary shaker (160-rpm), and the mycelium was then collected and pulverized in liquid nitrogen. The nucleic acid was extracted, purified and its concentration was determined and adjusted as described by **Brown (1990)** and **Mahfouz and Mohamed (2002)** to 100 ng/µl.

According to the method of **Williams *et al* (1990)** and **Mohamed *et al* (2001)**, 10 decamer oligonucleotide primers from OPERON Technologies, Alameda, CA., kits A and D, were used for amplification of the DNA genome of the three applied *Streptomyces* isolates. The amplification reaction conducted on a volume of 50 µl. PCR amplification performed in a Perkin-Elmer (Gene Amp PCR System 2400) for 35 cycles after initial denaturation for 4 min at 94^{o}C. Each cycle consisted of denaturation at 94^{o}C for 1 min, annealing at 37^{o}C for 1 min, extension at 72^{o}C for 2 min. The primer extension extended to 7 min at 72^{o}C in the final cycle. The PCR amplified products were detected by electrophoresing on 1.5% agarose gel in 1X TAE buffer at 60 volts for two hours **(Sambrook *et al* 1989)**. PCR fragments visualized by staining gel in ethidium bromide (0.5 µg/ml) and photographed under UV light using a Polaroid camera.

Analysis was carried out by visually examination of the amplified fragments and scoring as present (1) or absent (0). The similarity coefficient (F) between isolates was defined by the formula of **Nei and Li (1979).** From the distance by unweighted paired-group method, arithmetic mean (UPGMA) contained in the computer program package NTSYS 1.5 **(Rohlf, 1990)**, the phylogenetic tree was derived.

RESULTS AND DISCUSSION

Isolate ST55 identification

Results in **Table (1)** indicate that the *Streptomyces* isolate ST55 belonged to the red series group and the substrate mycelium produced yellow pigment on the standard media used. Aerial spore chains belonged to section RF and the spores were characterized by smooth surface without any ornamentation. Melanoid pigment was detected on the standard media. This isolate was characterized by good growth on Czapek's agar medium. The physiological characteristics showed that this isolate was able to utilize all used sugar except for D-mannitol and sucrose as carbon sources for growth. In addition, this isolate showed antibacterial and antifungal activities against the tested organisms used under study and no sensitivity to streptomycin (4 μg ml^{-1}) was observed.

Comparing the cultural, morphological and physiological characteristics of the ***Streptomyces*** spp in **Pridham and Tresner (1974)** with those of this isolate, it was very likely to be strain of ***S. lincolnensis*** with slight differences in the colour of substrate mycelium, and in the utilization of D-mannitol and sucrose as carbon source for growth.

Isolate ST71 identification

Results presented in **Table (2)** reveal that the characteristics of *Streptomyces* isolate ST71 appeared to be closely resemble to ***S. venezuelae*** based on the description keys proposed by **Pridham and Tresner (1974)** with slight differences in the utilization of i-Inositol and D-mannitol as a carbon source for growth.

The cultural, morphological and physiological characteristics of the tested isolate clearly showed that the color of aerial mycelium was red (red colour series) while the reverse side of substrate mycelium was yellow. Spore chains are belong to RF section with smooth surface. No soluble pigment was produced in all standard media used. Moderate growth on Czapek's agar medium was noted. It was noted that this isolate actively utilized all of the carbon sources used for growth. It is of importance to mention that this isolate antagonized the nine test microorganisms used in this study and was affected by streptomycin (4 μg ml^{-1}).

Isolate ST86 identification

Data in **Table (3)** show that the *Streptomyces* isolate ST86 belonged to the red series group and the vegetative mycelium was also pigmented with yellow colour. This isolate had RA spore chain with smooth surface. It produced a melanoid pigment, gave also an excellent growth on Czapek's agar medium and only actively utilized D-glucose, D-Xylose, L-Arabinose, L-Rhamnose, D-Fructose, and D-Mannitol as carbon sources for growth. This isolate showed antagonistic activity against the test organisms used. However, no growth was observed in the presence of 4 μg ml^{-1} streptomycin antibiotic in the medium. According to the keys proposed by **Pridham and Tresner (1974)**, the experimental *Streptomyces* isolate ST86 appeared to be related to ***S. umbrinus*** as illustrated in **Table (3)** although there was a slight difference in the utilization of some carbon sources for growth and in the colour of substrate mycelium. Therefore, isolate ST86 could be considered a strain of ***S. umbrinus***.

Table 1. Cultural, morphological and physiological characteristics of streptomycete isolate ST55 compared with those of similar species reported by **Pridham and Tresner (1974)**

Character	Isolate ST55	*S. lincolnensis* **Pridham and Tresner (1974)**
Color of aerial mycelium	Red	Red
Spore-chain	RF	RF
Melanoid pigment	+	+
Spore surface	SM	SM
Growth on Czapek's medium	Good	Excellent
Color of substrate mycelium	Yellow	ND
Diffusable pigments	—	ND
Utilization of Carbon:		
No carbon	—	—
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
Raffinose	+	+
D-Mannitol	—	+
i-Inositol	+	+
Sucrose	—	+
Antagonistic activity	Antibacterial and antifungal*	Produce lincomycins A, B, C and D antibacterial
Sensitivity to Streptomycin	NS	ND
NaCl tolerance	0-7%	≥ 7% but < 10%

+: Growth. -: No growth. RF: Spores in straight (R) or flexuous (F) chains.
ND: Not determined. SM: Smooth. NS: Not sensitive.
* Test organisms used: Refer to materials and methods.

Table 2. Cultural, morphological and physiological characteristics of streptomycete isolate ST71 compared with those of similar species reported by **Pridham and Tresner (1974)**

Character	Isolate ST71	*S. venezuelae* **Pridham and Tresner (1974)**
Color of aerial mycelium	Red	Red
Spore-chain	RF	RF
Melanoid pigment	—	—
Spore surface	SM	SM
Growth on Czapek's medium	Moderate	ND
Color of substrate mycelium	Yellow	ND
Diffusable pigments	—	
Utilization of Carbon:		
No carbon	—	—
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	+	+
Raffinose	+	ND
D-Mannitol	+	—
i-Inositol	+	—
Sucrose	+	ND
Antagonistic activity	Antibacterial and antifungal*	ND
Sensitivity to streptomycin	S	ND
NaCl tolerance	0-7%	ND

+: Growth. -: No growth. RF: Spores in straight (R) or flexuous (F) chains.
SM: Smooth. S: Sensitive. ND: Not determined.
* Test organisms used: Refer to Table 1.

Table 3. Cultural, morphological and physiological characteristics of streptomycete isolate ST86 compared with those of similar species reported **Pridham and Tresner (1974)**

Character	Isolate ST86	S. umbrinus **Pridham and Tresner (1974)**
Color of aerial mycelium	Red	Red
Spore-chain	RA	RF
Melanoid pigment	+	+
Spore surface	SM	SM
Growth on Czapek's medium	Excellent	Excellent
Color of substrate mycelium	Yellow	Purple-brown
Diffusable pigments	—	ND
Utilization of Carbon:		
No carbon	—	—
D-Glucose	+	+
D-Xylose	+	+
L-Arabinose	+	+
L-Rhamnose	+	+
D-Fructose	±	+
Raffinose	—	+
D-Mannitol	+	+
i-Inositol	—	+
Sucrose	—	+
Antagonistic activity	Antibacterial and antifungal*	Antibacterial
Sensitivity to streptomycin	S	S
NaCl tolerance	0-7%	≥ 7% but < 10%

+: Growth. -: No growth. RA: Spore chain in the form of open loops, hooks or greatly extended coils of wide. SM: Smooth. S: Sensitive. ND: Not determined.
* Test organisms used: Refer to Table 1.

RAPD-PCR analysis

The RAPD technique developed by **Williams *et al* (1990)** provides a faster and easier approach for exploring genetic polymorphism, and also requires very small amounts of DNA. RAPD markers have been successfully used in isolate analysis in some streptomycetes **(Nava *et al* 2001; Rintala *et al* 2002; Tolba *et al* 2002; Wirth and Ulrich, 2002; Cook and Meyers, 2003; Raad *et al* 2003** and **Sony *et al* 2004).**

This study aimed to use the RAPD-PCR technology to obtain DNA markers for identification of some identified species from the genus *Streptomyces*. RAPD analysis resolved that 125 DNA fragments were amplified from the DNA of the four *Streptomyces* strains using ten 10-nt random primers **Table (4**) and **Figure (5).** The strains were varied in the number of PCR products as 69, 73, 76 and 68 fragments were amplified from the DNA of *S. lincolnensis*, *S. venezuelae*, *S. umbrinus* and *S. tuirus* Si-4, respectively, (**Table 5**). Results in **Table** (**7**) show that the similarity between the four species ranged from 30.6% to 59.2%.

In addition, 74 scorable markers from the ten primers were obtained (**Table 5**). Primers produced between 1 and 10 amplification products as markers (present or absent). The *S. venezuelae* showed the highest number (32) of these markers followed by *S. umbrinus* (26), *S. lincolnensis* (9) and *S. tuirus* Si-4 (7). Results in **Figure (6)** show that two major clusters of *Streptomyces* strains were found in the dendrogram. The first cluster included the *S. lincolnensis* and *S. venezuelae*.
On the other hand, the *S. umbrinus* and *S. tuirus* Si-4 gave the second cluster.

Our results recommended the utilization of the DNA markers as a powerful tool for variety identification in *Streptomyces*. This is in agreement with those found by **Kearley *et al* (1994); Mehling *et al* (1995); Weigler *et al* (1996); Klein *et al* (1998); Malkawi *et al* (1999); Mohamed *et al* (2001); Nava *et al* (2001); Tolba *et al* (2002); Wirth and Ulrich (2002); Raad *et al* (2003)** and **Sony *et al* (2004).**

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Table 4. RAPD-PCR polymorphism of the three applied *Streptomyces* isolates using 10-decamer oligonucleotides

Amplified fragments	*Streptomyces* strains			
	S. lincolnensis	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
OPA-0201	0	0	1	0
OPA-0202	0	0	1	0
OPA-0203	1	1	0	1
OPA-0204	1	1	1	1
OPA-0205	1	1	0	1
OPA-0206	0	0	1	0
OPA-0207	1	1	0	0
OPA-0208	1	1	1	1
OPA-0209	1	1	0	1
OPA-0210	1	1	1	1
OPA-0211	0	0	1	0
OPA-0212	1	1	0	1
OPA-0213	1	1	0	1
OPA-0214	0	1	1	1
OPA-0215	0	0	1	0
OPD-0101	0	1	0	1
OPD-0102	1	0	1	1
OPD-0103	1	1	1	1
OPD-0104	1	1	0	1
OPD-0105	1	0	1	0
OPD-0106	0	0	0	1
OPD-0107	0	1	0	0
OPD-0108	1	0	1	1
OPD-0201	0	0	1	0
OPD-0202	0	1	1	1
OPD-0203	1	1	1	1
OPD-0204	0	1	0	0
OPD-0205	0	0	1	1
OPD-0206	1	0	1	1
OPD-0207	0	1	0	0
OPD-0208	1	1	0	0
OPD-0501	1	1	0	1
OPD-0502	1	1	1	1

Table 4. Cont.

Amplified fragments	*Streptomyces* strains			
	S. lincolnensis	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
OPD-0503	1	1	1	1
OPD-0504	1	0	1	0
OPD-0505	0	1	1	1
OPD-0506	0	1	0	0
OPD-0507	1	1	1	1
OPD-0514	1	0	0	0
OPD-0601	0	1	0	0
OPD-0602	1	0	1	0
OPD-0603	0	1	0	1
OPD-0604	0	1	1	1
OPD-0605	1	0	1	0
OPD-0606	0	1	0	1
OPD-0607	1	0	1	0
OPD-0608	1	1	1	1
OPD-0609	1	1	1	1
OPD-0610	1	0	1	1
OPD-0701	0	1	0	0
OPD-0702	0	0	0	1
OPD-0703	0	1	0	0
OPD-0704	1	0	0	1
OPD-0705	0	1	0	0
OPD-0706	1	0	1	1
OPD-0707	1	1	1	1
OPD-0708	1	0	1	1
OPD-0709	1	0	1	0
OPD-0710	1	1	1	1
OPD-0711	0	0	1	1
OPD-0712	1	1	1	1
OPD-0713	0	1	0	0
OPD-0714	1	1	1	1
OPD-0715	0	0	1	0
OPD-0716	0	1	0	0
OPD-0717	1	0	1	1
OPD-0718	1	0	0	0

Table 4. Cont.

Amplified fragments	*Streptomyces* strains			
	S. lincolnensis	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
OPD-0719	0	1	1	0
OPD-0801	0	0	1	1
OPD-0802	0	0	1	1
OPD-0803	0	0	1	1
OPD-0804	0	0	1	1
OPD-0805	1	1	0	1
OPD-0806	0	0	1	0
OPD-0807	0	1	0	0
OPD-0808	1	0	1	1
OPD-0809	1	0	1	1
OPD-0810	1	1	0	0
OPD-0811	1	1	1	1
OPD-0812	0	0	1	1
OPD-0813	1	1	0	1
OPD-0814	0	0	1	0
OPD-0815	0	1	1	1
OPD-0816	1	0	1	0
OPD-0817	1	1	0	0
OPD-0818	1	0	0	1
OPD-1101	0	0	1	0
OPD-1102	0	1	0	0
OPD-1103	0	0	1	0
OPD-1104	1	0	1	1
OPD-1105	1	1	1	1
OPD-1106	1	1	1	1
OPD-1107	0	1	0	0
OPD-1108	1	0	0	1
OPD-1109	1	1	1	0
OPD-1110	0	1	0	0
OPD-1111	0	0	1	0
OPD-1112	1	0	0	0
OPD-1113	0	1	0	0
OPD-1801	0	0	1	0
OPD-1802	0	1	0	0

Table 4. Cont.

Amplified fragments	*Streptomyces* strains			
	S. lincolnensis	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
OPD-1803	1	0	1	1
OPD-1804	1	1	0	0
OPD-1805	1	1	1	0
OPD-1806	1	1	0	1
OPD-1807	0	1	1	0
OPD-1808	0	1	1	1
OPD-1809	1	0	1	1
OPD-1810	1	1	1	1
OPD-1811	1	1	0	1
OPD-2001	0	0	0	1
OPD-2002	0	0	1	0
OPD-2003	0	0	1	0
OPD-2004	0	1	0	0
OPD-2005	1	1	1	1
OPD-2006	0	1	1	0
OPD-2007	1	1	1	0
OPD-2008	1	1	1	1
OPD-2009	1	0	1	1

0 = Absent. 1= Present.

Table 5. Total amplified fragment (TAF) produced *via* RAPD-PCR using 10-decamers primers from the DNA genome of four *Streptomyces* strains

Primers	TAF	*Streptomyces* strains			
		S. lincolnensis	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
OPA-02	15	10	10	09	09
OPD-01	08	05	04	04	06
OPD-02	08	03	05	05	04
OPD-05	14	09	11	09	09
OPD-06	10	06	06	07	06
OPD-07	19	10	10	11	10
OPD-08	18	09	07	10	10
OPD-11	13	06	07	07	04
OPD-18	11	07	08	07	06
OPD-20	09	04	05	07	04
Total	125	69	73	76	68

Table 6. Unique amplified fragments produced *via* RAPD-PCR using 10-decamer primers from the DNA genome of four *Streptomyces* strains

Primers	TAF	*Streptomyces* strains			
		S. lincolnensis	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
OPA-02	15	1	0	10	0
OPD-01	08	0	3	1	1
OPD-02	08	1	2	1	0
OPD-05	14	2	3	1	0
OPD-06	10	1	3	0	0
OPD-07	19	1	8	1	1
OPD-08	18	1	3	4	0
OPD-11	13	1	5	3	1
OPD-18	11	1	3	3	0
OPD-20	09	0	2	2	2
Total	125	9	32	26	7

Table 7. Similarity between four *Streptomyces* strains based on RAPD-PCR analysis using 10-decamer primers

Streptomyces strains	*S. venezuelae*	*S. umbrinus*	*S. tuirus* Si-4
S. lincolnensis	41.0	33.9	30.6
S. venezuelae	*	39.4	42.0
S. umbrinus		*	59.2

Figure 1. Agarose gel electrophoresis of RAPD-PCR polymorphism of DNA of four *Streptomyces* strains (*S. lincolnensis* (A), *S. venezuelae* (B), *S. umbrinus* (C) and *S. tuirus* Si-4 (D)) using OP-A02 (Right) and OP-OD11 (Left). M: DNA marker (1 Kb DNA Ladder).

Figure 2. Agarose gel electrophoresis of RAPD-PCR polymorphism of DNA of four *Streptomyces* strains (*S. lincolnensis* (A), *S. venezuelae* (B), *S. umbrinus* (C) and *S. tuirus* Si-4 (D)) using OP-D18 (Right) and OP-OD20 (Left). M: DNA marker (1 Kb DNA Ladder).

Figure 3. Agarose gel electrophoresis of RAPD-PCR polymorphism of DNA of four *Streptomyces* strains (*S. lincolnensis* (A), *S. venezuelae* (B), *S. umbrinus* (C) and *S. tuirus* Si-4 (D)) using using OP-D01 (Right) and OP-D02 (Middle) and OP-D05 (Left). M: DNA marker (1 Kb DNA Ladder).

Figure 4. Agarose gel electrophoresis of RAPD-PCR polymorphism of DNA of four *Streptomyces* strains (*S. lincolnensis* (A), *S. venezuelae* (B), *S. umbrinus* (C) and *S. tuirus* Si-4 (D)) using OP-D06 (Right), OP-D07 (Middle) and OP-D08 (Left). M: DNA marker (1 Kb DNA Ladder).

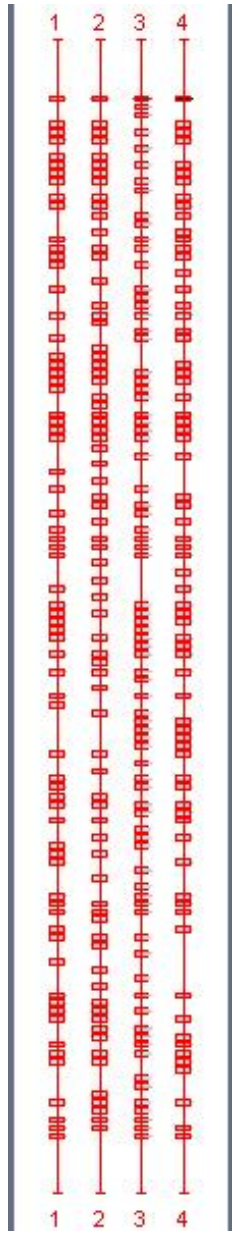

Figure 5. DNA polymorphisms of four *Streptomyces* strains (*S. lincolnensis* (1), *S. venezuelae* (2), *S. umbrinus* (3) and *S. tuirus* Si-4 (4)) using based on RAPD-PCR analysis.

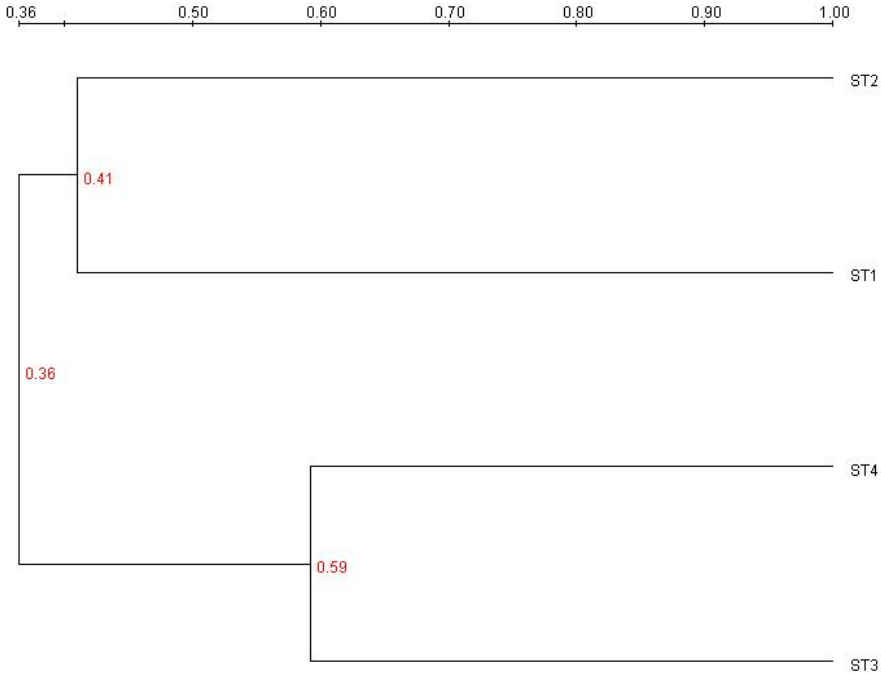

Figure 6. Dendrogram showing molecular relationship between four *Streptomyces* strains (*S. lincolnensis* (ST1), *S. venezuelae* (ST2), *S. umbrinus* (ST3) and *S. tuirus* Si-4 (ST4)) using 10-decamer primers based on RAPD-PCR analysis.

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دراسات مزرعية ومورفولوجية وفسيولوجية وجزيئية علي بعض عزلات الأستربتوميسيسات

[15]

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في هذه الدراسة تم تعريف ثلاثة عزلات من الأستربتوميسيسات تابعة للمجموعة الحمراء والمعزولة من التربة في محافظة الجيزة بمصر وذلك بناء علي خواصها المزرعية والمورفولوجية والفسيولوجية. وبناء علي التقسيم المقترح بواسطة **بريدهام وترسنر عام 1974** فإن العزلات ST55 ، ST71، ST86 اتضح أنها سلالات من *S. lincolnensis, S. venezuelae* و *S. umbrinus* علي التوالي. وبالإضافة الي ذلك تم استخدام تكنيك RAPD-PCR لدراسة جينوم الثلاثة عزلات المستخدمة لتقدير البصمة الوراثية الكل منهم. وقد تم تنفيذ ذلك باستخدام عشرة من البادئات هي: OP-A02, OP-D01, OP-D02, OP-D05, OP-D06, OP-D07, OP-OP-D20, D08, OP-D11, OP-D18 . وقد تم الكشف عن منتجات الـ PCR بواسطة الأليكتروفوريسيس لآجاروز جل 1.5% وإيضاحها بالصبغ بواسطة الأيثيديم بروميد والأشعة فوق البنفسجية. وقد أظهرت النتائج أن بنفس البادىء تم الحصول علي قطع من الـ DNA مختلفة من عزلة لأخري وبأحجام مختلفة. كما تم الحصول علي قطع من النوع Monomorphic وPolymorphic مما يؤكد العلاقة والتقارب علي المستوي الجزيئي للعزلات الأربعة المستخدمة من الأستربتوميسيتات. وبناء علي التحليل الأحصائي للبوليمرفيزم المتحصل عليه ثبت وجود درجة من التشابه تتراوح ما بين 33.9 الي 59.2 % للأنواع الأربعة لنفس الجنس. والأكثر من ذلك ثبت وجود قطع من الـ DNA مميزة تم استخدامها كماركرز جزيئية والتي يمكن استخدامها للتميز بين العزلات. وهذه النتيجة تعضد الفكرة لأستخدام هذه التقنية للمساهمة في تأكيد تقسيم الأستربتومايسيسات.

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