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A COMPARATIVE STUDY BETWEEN A NUMERICAL AND RAPD-PCR METHODS FOR THE IDENTIFICATION OF SOME STREPTOMYCETE STRAINS

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ABSTRACT

Streptomyces is a gram-positive bacterium that undergoes morphological differentiation. To taxonomy or classify the streptomycetes according to their properties some systems have been proposed. In this study, a comparative study between a numerical method and the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technology for identification of 19 streptomycetes belonging to different series color (gray, 4 strains; red, 8 strains divided into three groups; white, 3 strains; violet, 2 strains and yellow, 2 strains) was carried out. Results showed that units of utilization of carbon sources (UCS) were effective for some *Streptomyces* strains. As expected the violet *Streptomyces* strains (Si-9 and Si-1) of group 5 showed the lowest total units. Similarity differences based on the numerical and RAPD-PCR analyses of the four gray *Streptomyces* strains (group 1: ST10, ST11, ST12 and ST13) were ranged from 1.7 to 9.5%. The phylogenetic tree reflected the relationship between the identified *Streptomyces* strains (group 3: ST1, ST2 and ST3), as the strains were arranged as follows: RF/C-/SM; RF/C+/SM and RA/C+/SM. Interestingly, the similarity between the phylophenetic and phylogenetic trees of the *Streptomyces* strains (ST08, ST14 and ST15) was 100% and strains were organized in a compatible situation with their characters. Data also showed reasonable differences (9.5% and 6.8%) between the applied analysis methods in case of *Streptomyces* strains of groups 5 and 7, respectively. Finally, the phylophentic data of this study provided a basis for reduction of the number of *Streptomyces* species now known to occur in nature.

INTRODUTION:

In 1943, Waksman and Henrici proposed the genus *Streptomyces*, which termed *Actinomyces* (Baldacci, 1939). Between 1940 and 1957 over 1000 *Streptomyces* species were described (Pridham *et al*., 1958). Numerous classifications were devised to accommodate the increasing number of *Streptomyces* species, most of them based on a few subjectively-chosen morphological and pigmentation properties which were rarely studied under standardized growth conditions. Pridham *et al.* (1965) reduced the number of 400 *Streptomyces* species to 8 on the basis of spore ornamentation and spore chain morphology. In the edition of Bergey's Manual of Determinative Bacteriology (Pridham and Tresner, 1974), the classification of the genus *Streptomyces* was based on the International *Streptomyces* Project (ISP) data (Shiriling and Gottlieb, 1966). Therefore, 463 species were classified into 43 groups based on micromorphology and pigmentation; the groups were subdivided by carbon utilization patterns.

Williams *et al.* (1983) suggested a standard numerical classification of 475 strains, of which 394 type cultures *Streptomyces*, and 14 other actinomycete genera. They found that most of *Streptomyces* type cultures fell into one large cluster-group and they were recovered in 19 major and 40 minor clusters, with 18 strains recovered as single member clusters. Their results provided a basis for reduction of the large number of

Streptomyces species, which have been described. In Egypt, Mohamed *et al.* (2005) suggested an arbitrary numerical method for identification of eight halotolerant streptomycete isolates by comparing their phonetic characters with their corresponding strains in the eighth edition of Bergey's Manual of Determinative Bacteriology (Pridham and Tresner, 1974). At the level of molecular studies on *Streptomyces* species, some investigators carried out trials to identify some *Streptomyces* isolates (strains) using the random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) (Mohamed, 1998; Mohamed *et al*., 2001; Mahfouz and Mohamed, 2002; El-Domyati and Mohamed, 2004 and Abdel-Fattah, 2005).

In this work, a comparative study between the suggested numerical method of Mohamed *et al.* (2005) and RAPD-PCR methods was aimed, in a trial to confirm the possibility to use a less expensive and effective method for the classification of unknown *Streptomyces* isolates. The application of such method was also aimed to encourage the idea of Pridham *et al.* (1965).

MATERIALS AND METHODS:

Source of Streptomyces strains: Data in Table -1 show the series, codes and sources of the 19 *Streptomyces* species used in this study.

Table -1: Series, codes and sources of *Streptomyces* species groups under investigation.

Streptomyces series	Group (G)	Streptomycetes		References
		Code	Species	
Gray	G1	ST10	*S. echinatus*	Mohamed *et al*. (2005)
		ST11	*S. griscochromogenes*	
		ST12	*S. echinatus*	
		ST13	*S. antibioticus*	
Red	G2	Si-4	*S. tuirus*	Mahfouz and Mohamed (2002)
		Si-6	*S. lateritius*	
		Si-11	*S. melanogenes*	
	G3	ST1	*S. lincolnensis*	Abdel-Fattah (2005)
		ST2	*S. venezuelae*	
		ST3	*S. umbrinus*	
	G4	BB24	*S. roseolus*	Mohamed (1998)
		W86	*S. aureomonopodiales*	
Violet	G5	Si-1	*Streptomyces* sp.	Mahfouz and Mohamed (2002)
		Si-9	*S. muavecolor*	
White	G6	ST08	*S. longisporus*	Mohamed *et al*. (2005)
		ST14	*S. baarnensis*	
		ST15	*S. albolongus*	
Yellow	G7	Si-8	*S. hawaiiensis*	Mohamed *et al.* (2000)
		Is-10	*S. alboflavus*	

Numerical method: The proposed key of Pridham and Tresner (1974) divided the classification characters of *Streptomyces* into two categories. The major category includes four taxonomical characters, i.e., color of aerial mycelium or series group (SG), spore-chain (SC), melanoid pigments (MP) and spore surface (SS). While, the minor category includes: growth on Czapek's medium (GC), utilization of carbon compounds (UCS) and

antagonistic activities (AA) (anti-bacterial or antifungal). In addition, color of substrate mycelium, diffusible pigments, sensitivity to streptomycin, antiviral activity and NaCl tolerance characters were also considered. The suggested numerical method of Mohamed *et al.* (2005) was arbitrary scored the taxonomical characters in 58 units as follows: SG, 22 units; SC, 4 units; MP, 1 units; SS, 5 units; GC, 5 units; UCS, 17 units and AA, 4 units (antibacterial, 2 units and antifungal 2 units. It is of importance to mention that each character was internally disturbed according to frequency distribution of sub-characters. For example, gray, red, white, blue, yellow, green, and violet serial groups were given 22, 16, 13, 10, 7, 4 and 1 units, respectively.

The numerical scoring of the characters of the 19 *Streptomyces* strain under investigation is shown in the results and discussion. The similarity matrix between the identified species 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 Database™ Version 2.0 from Bio-Rad. The numerical analysis of eight halotolerant *Streptomyces* strains that described by Mohamed *et al*. (2005) was also used.

RAPD-PCR analysis: DNA was extracted from the two *Streptomyces* strains (*S. alboflavus* Is-10 and *S. hawaiienis* Si-8) of Mohamed *et al.* (2000) and these were described by El-Domyati and Mohamed (2004). These DNA extracts were then used as templates for PCR using five random primers (OPA01, OPA10, OPA12, OPB07 and OPB07) in a volume of 50 µl. The mixture, PCR program, electrophoresis and RAPD-PCR analysis was done as reported by El-Domyati and Mohamed (2004). On the other hand, the RAPD-PCR results of the other applied *Streptomyces* strains [ST1, ST2, ST3, Abdel-Fattah (2005); Si-1, Si-4, Si-6, Si-9, Si-11, Mahfouz and Mohamed (2002); BB24 and W86, Mohamed (1998); IS-10 and Si-8, Mohamed *et al.* (2000) while ST08, ST10, ST11, ST12, ST13, ST14 and ST15, El-Domyati and Mohamed (2004)] were directly compared to the numerical results of the same strains. The detailed data of RAPD-PCR analysis of the 19 *Streptomyces* species under investigation is illustrated in Table (2).

Table -2: Data of RAPD-PCR analysis of the 19 *Streptomyces* species under investigation.

Streptomyces series	Group (G)	Primers used	CSS	AF	References
Gray	G1	OPA10, OPA15, OPD06, OPD14, OPE03, OPE05, OPO04, OPO07, OPO11, OPO12, OPO15, OPO18	ST10	63	El-Domyati and Mohamed (2004)
			ST11	62	
			ST12	59	
			ST13	65	
Red series	G2	OPA13, OPA20, OPB02, OPB19, OPD07, OPD08, OPD20, OPE01, OPE02, OPE06, OPE11, OPE20, OPO10, OPO11, OPZ01, OPZ18	Si-4	133	Mahfouz and Mohamed (2002)
			Si-6	128	
			Si-11	126	
	G3	OPA02, OPD01, OPD02, OPD05, OPD06, OPD07, OPD08, OPD11, OPD18, OPD20	ST1	69	Abdel-Fattah (2005)
			ST2	73	
			ST3	76	

	G4	OPG01, OPG02, OPG03, OPG04, OPG05 OPG06 OPG07 OPG08 OPG09 OPG10	BB24	41	Mohamed (1998)
			W86	53	
Violet	G5	OPA13, OPA20, OPB02, OPB19, OPD07, OPD08, OPD20, OPE01, OPE02, OPE06, OPE11, OPE20, OPO10, OPO11, OPZ01, OPZ18	Si-1	136	Mahfouz and Mohamed (2002)
			Si-9	133	
White	G6	OPA10, OPA15, OPD06, OPD14, OPE03, OPE05, OPO04, OPO07, OPO11, OPO12, OPO15, OPO18	ST08	73	El-Domyati and Mohamed (2004)
			ST14	65	
			ST15	61	
Yellow	G7	OPA01, OPA06, OPB02, OPC07, OPZ02	Si-8	25	This study
			IS-10	24	

AF: Amplified fragments. CSS: Code of *Streptomyces* species.

RESULTS AND DISCUSSION:

Streptomycetes are gram-positive bacterium that undergoes morphological differentiation. To taxonomy or classify the streptomycetes according to their properties some systems have been proposed, i.e., Shirling and Gottlieb (1966), Shirling and Gottlieb (1968), Küster (1972), Pridham and Tresner (1974) and Williams *et al*. (1983).

In this study, a comparative study between the numerical method of Mohamed *et al*. (2005) and the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) method for the identification of a number of streptomycete strains was carried out.

Data in Table (3) show the scoring of the characters of the *Streptomyces* strains belonging to the series groups under investigation. Results of scoring showed that the total units of the *Streptomyces* strains inside each group were varied. Units of UCS were found to be effective as some *Streptomyces* strains such as Si-6 (7 units) and BB24 (8 units) strains which belonging to red series were differed from the other red strains in groups 2, 3 and 4 which represented with units ranged from 11 to 16 units out of the 17 units of UCS. As expected, the violet *Streptomyces* strains (Si-9 and Si-1) of group 5 showed the lowest total units, i.e., 25 and 29 out of 58 units, respectively. While, the yellow *Streptomyces* strains (Si-8 and Is-10) in group 7 showed total units (33 and 42, respectively) similar or higher than that of some *Streptomyces* strains belonging to different groups, i.e., Si-6 (33/G2); ST10 (41/G1); BB24 (41/G4) and the three red *Streptomyces* strains (ST08; ST14 and ST15: 40, 41 and 40, respectively) of group 6.

Results in Table (4) as illustrated by Figure (1) showed that the similarity differences based on the numerical and RAPD-PCR analyses of the four gray *Streptomyces* strains (G1: ST10, ST11, ST12 and ST13) ranged from 1.7 to 9.5%. In addition, the phylophenetic (Figure 2) and phylogenetic (Figure 3) trees of the same *Streptomyces* strains were very close. As interesting results, the phylogenetic tree was too close to the realty, as two clusters were obtained and the three *Streptomyces* strains characterized with G/RA/C+/SPY were fell in one cluster, while, the fourth *Streptomyces* strain that characterized with G/RF/C+/SM fell in a separate cluster.

Table-3: Numerical scoring of the characters of the 19 *Streptomyces* strains used in this study.

Code	Streptomyces species	Main characters	SG	SC	MP	SS	GC	UCS	AA	Total Units
			22	4	1	5	5	17	4	
Group 1										
ST10	S. echinatus	G/RA/C+/SPY	22	2	1	3	2	11	0	41
ST11	S. griscochromogenes	G/RA/C+/SPY	22	3	1	3	5	14	2	50
ST12	S. echinatus	G/RA-S/C+/SPY	22	2	1	3	3	11	2	44
ST13	S. antibioticus	G/RF/C+/SM	22	4	1	4	5	12	4	52
Group 2										
Si-4	S. tuirus	R/S/C+/SM	16	2	1	5	3	16	4	47
Si-6	S. lateritius	R/RA/C+/WTY	16	2	1	1	2	7	4	33
Si-11	S. melanogenes	R/RF/C+/SM	16	4	1	5	4	14	4	48
Group 3										
ST1	S. lincolnensis	R/RF/C+/SM	16	4	1	5	4	11	4	45
ST2	S. venezuelae	R/RF/C-/SM	16	4	0	5	3	16	4	48
ST3	S. umbrinus	R/RA/C+/SM	16	4	1	5	5	16	2	49
Group 4										
BB24	S. roseolus	R/RF/C-/SM	16	4	0	5	4	8	4	41
W86	S. aureomonopodiales	R/RF/C-/SM	16	4	0	5	4	14	4	47
Group 5										
Si-1	Streptomyces sp.	V/RA/C+/WTY	1	2	1	1	4	16	4	29
Si-9	S. muavecolor	V/RF/C+/SPY	1	4	1	3	2	10	4	25
Group 6										
ST08	S. longisporus	W/RA/C+/SPY	13	2	1	3	5	16	0	40
ST14	S. baarnensis	W/RF/C-/SM	13	4	0	5	5	12	2	41
ST15	S. albolongus	W/RA/C+/SM	13	4	1	5	4	11	2	40
Group 7										
Si-8	S. hawaiiensis	Y/S/C+/SPY	7	2	1	3	4	12	4	33
Is-10	S. alboflavus	Y/RF/C+/SM	7	4	1	5	5	16	4	42

AA: Antagonistic (bacterial and fungal) activities. C+: Melaniod pigment (Produced). C-: Melaniod pigment (Not produced). G: Gray series. GC: Growth on Czapek's medium. MP: Melaniod pigment. R: Red series. 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: Spiral chain spore. SC: Spore chain. SG: Series group. SM: Smooth spore surface. SPY: Spiny spore surface SS: Spore surface. UCS: Utilization of carbon sources. V: Violet series. W: White series. WTY: Warty spore surface. Y: Yellow series.

Table-4: Similarities between four *Streptomyces* species (G1: ST10, ST11, ST12 and ST13) belonging to gray series based on numerical and RAPD-PCR methods.

Streptomyces strains	Analysis Method	ST10	ST11	ST12
ST11	Numerical	87.0		
	RAPD-PCR	90.8	100	
ST12	Numerical	92.0	90.5	
	RAPD-PCR	87.0	92.2	100
ST13	Numerical	87.9	90.9	89.4
	RAPD-PCR	85.4	81.4	81.6

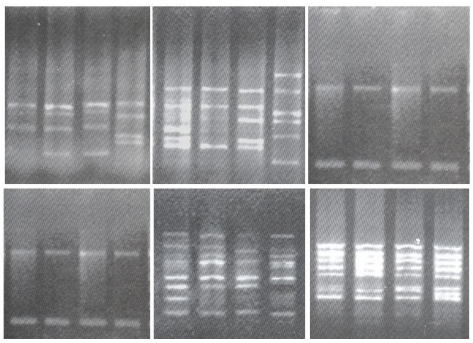


Figure -1: Agarose gel electrophoresis shows DNA polymorphisms of RAPD-PCR analysis of *Streptomyces* strains (G1: ST10, ST11, and ST13, from left to right, respectively) belonging to gray series using 6 primers (El-Domyati and Mohamed, 2004).

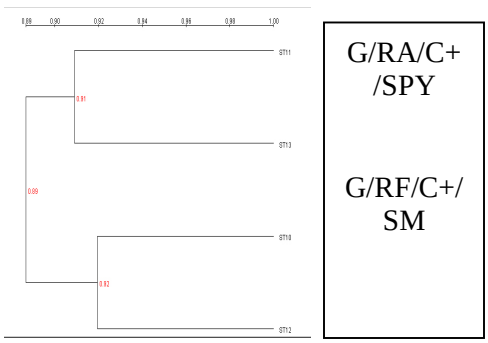


Figure -2: Phylophenetic tree of *Streptomyces* strains(G1: ST10, ST11, ST12 and ST13) belonging to ST12 gray series based on analysis of their selected characters.

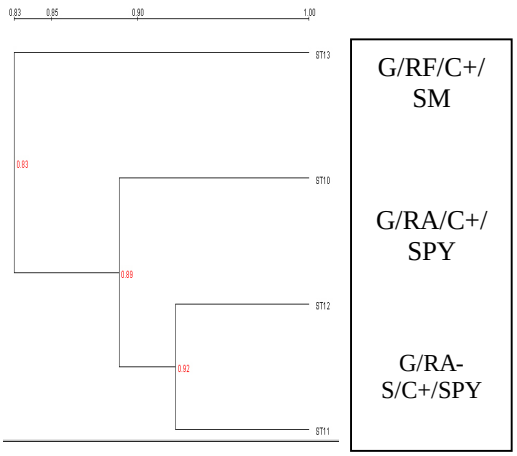


Figure -3: Phylogenetic tree of *Streptomyces* strains (G1: ST10, ST11, ST12 and ST13) belonging to gray series based on RAPD-PCR analysis.

Similarly, a difference range of 2.6-4.9% was found between the numerical and RAPD-PCR analyses of the *Streptomyces* strains of G2 (Table 5) and (Figure 4). Also, the phylophenetic (Figure 5) and phylogenetic (Figure 6) trees of these *Streptomyces* strains were very close to each other, as the *Streptomyces* strain characterized with WTY spore surface and RA spore chain was reported after those characterized with RF or S spore chain and SM spore surface.

Streptomyces strains	Analysis method	Si-4	Si-6
Si-6	Numerical	86.4	
	RAPD-PCR	89.7	100
Si-11	Numerical	92.5	80.5
	RAPD-PCR	87.6	83.1

Table–5: Similarities between three *Streptomyces species* (G2: Si-4, Si-6 and Si-11) belonging to red series based on numerical and RAPD-PCR methods.

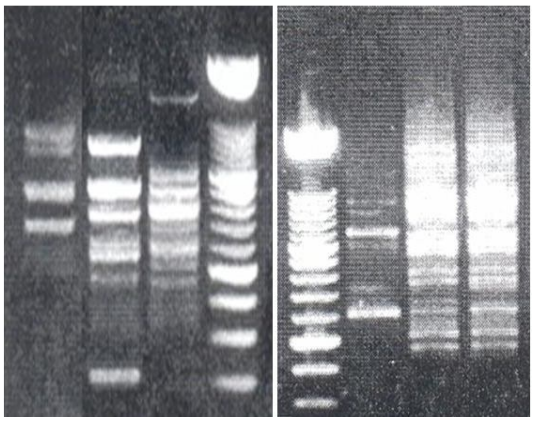
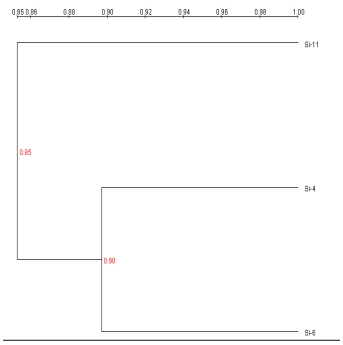


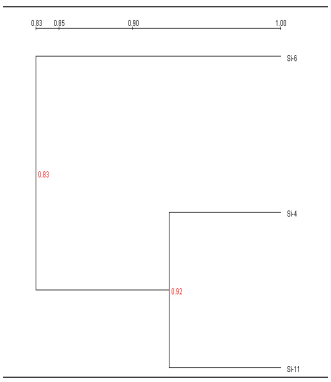
Figure (4): Agarose gel electrophoresis shows DNA polymorphisms of RAPD-PCR analysis of *Streptomyces* strains (G2: Si-6, Si-4 and Si-11, from left to right, respectively) belonging to red series using two primers (Mahfouz and Mohamed, 2002).



R/RF/C+ /SM

R/S/C+/ SM

R/RA/C +/WTY



R/RA/C +/WTY

R/S/C+/ SM

R/RF/C +/SM

Figure -5: Phylophenetic tree of *Streptomyces* strains (G2: Si-4, Si-6 and Si-11) belonging to red series based on analysis of their selected characters.

Figure -6: Phylogenetic tree of *Streptomyces* strains (G2: Si-4, Si-6 and Si-11) belonging to red series based on RAPD-PCR analysis.

Concerning the numerical and RAPD-PCR analyses of the *Streptomyces* strains of group 3 (ST1, ST2 and ST3), a high difference between the two applied analyses were noted, and ranged from 30.9 to 35.7% (Table 6). In spite of that, the phylophenetic (Figure 7) and phylogenetic (Figure 8) trees showed very high similarity as the *Streptomyces* strains characterized with RF/C-/SM was represented in a single cluster. Furthermore, the phylo-genetic tree reflected the relationship between the identified *Streptomycs* strains, as the

strains were arranged as follows: RF/C-/SM; RF/C+/S78 M and RA/C+/SM.

Table-6: Similarities between three *Streptomyces* species (G3: ST1, ST2 and ST3) belonging to red series based on numerical and RAPD-PCR methods.

***Streptomyces* strains**	**Analysis Method**	**ST1**	**ST2**
ST2	Numerical	93.6	
	RAPD-PCR	62.7	100
ST3	Numerical	94.4	87.9
	RAPD-PCR	66.7	52.2

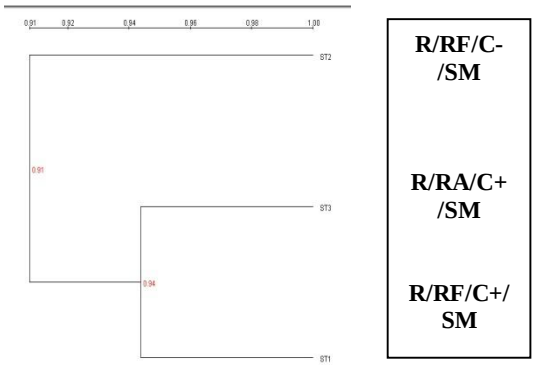


Figure-7: Phylophenetic tree of *Streptomyces* strains (G3: ST1, ST2 and ST3) belonging red series based on analysis of their selected characters.

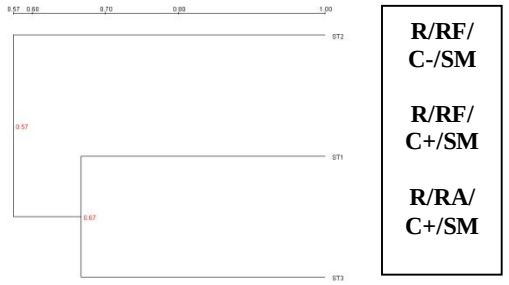


Figure-8:Phylogenetic tree of *Streptomyces* strains (G3: ST1, ST2 and ST3) belonging to to red series based on RAPD-PCR analysis.

Regarding the *Streptomyces* strains (ST08, ST14 and ST15) in group 6, a difference range of 21-22.1% was reported (Table 7) when the numerical and RAPD-PCR (Figure 9) analyses were applied. Interestingly, the phylophenetic (Figure 10) and phylogenetic (Figure 11) trees were 100% similar as the *Streptomyces* strains were organized in a compatible situation with their characters as follows: W/RA/C+/SPY, W/RF/C-/SM and W/RA/C+/SM.

Table-7: Similarities between three *Streptomyces* species (G6: ST08, ST14 and ST15) belonging to white series based on numerical and RAPD-PCR methods.

***Streptomyces* strains**	**Analysis Method**	**ST08**	**ST14**
ST14	Numerical	86.4	
	RAPD-PCR	64.6	100
ST15	Numerical	85.0	91.4
	RAPD-PCR	64.0	69.3

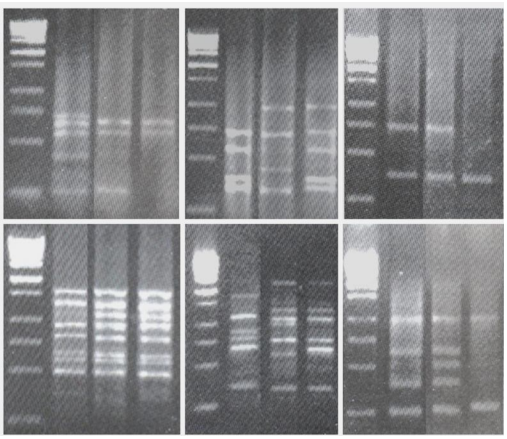


Figure –9: Agarose gel electrophoresis shows DNA polymorphisms of RAPD-PCR analysis of *Streptomyces* strains (G6: ST08, ST14 and ST15, from left to right, respectively) belonging to white series using 6 primers (El-Domyati and Mohamed, 2004).

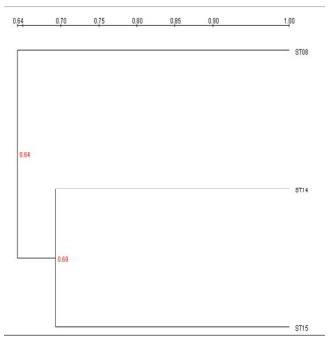


Figure-10: Phylophenetic tree of *Streptomyces* strains (G6: ST08, ST14 and ST15) belonging to white series based on analysis of their selected characters.

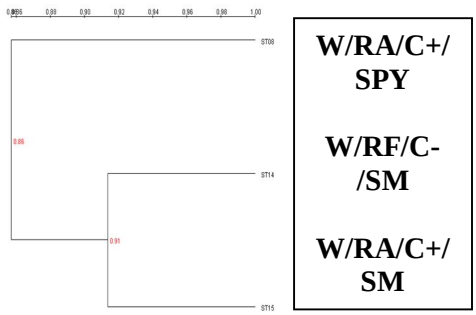


Figure-11: Phylogenetic tree of *Streptomyces* strains (G6: ST08, ST14 and ST15) belonging to white series based on RAPD-PCR analysis.

On the other hand, a high difference (38.8%) between the two analyses was found when used for the identification of W86 (R/RF/C-/SM) and BB24 (R/RF/C-/SM) *Streptomyces* strains of group 4 (Table 8). Data also showed reasonable differences (9.5% and 6.8%) between the applied analysis methods in case of *Streptomyces* strains of groups 5 and 7, respectively. Data in Figure (12) showed the DNA polymerphism produced when RAPD-PCR analysis was used for the determination of the DNA fingerprints of the two *Streptomyces* strains belonging to yellow series. Some mono and poly morphic DNA fragments were observed.

Table 8: Similarity between some *Streptomyces* strains belonging to different color series based on numerical and RAPD-PCR methods.

Streptomyces strains	Analysis methods	Streptomyces strains
Group 4 (Red series)		
		W86 (R/RF/C-/SM)
BB24 (R/RF/C-/SM)	Numerical	93.2
	RAPD-PCR	54.4
Group 5 (Violet series)		
Streptomyces strains		Si-1 (V/RA/C+/WTY)
Si-9 (V/RF/C+/SPY)	Numerical	68.0
	RAPD-PCR	58.5
Group 7 (Yellow series)		
Streptomyces strains		Is-10 (Y/RF/C+/SM)
Si-8 (Y/S/C+/SPY)	Numerical	83.3
	RAPD-PCR	76.5

Finally, the numerical method for identification of Streptomyces strains was done using the eight red *Streptomyces* strains (Si-4, Si-6 and Si-11, ST1, ST2, ST3 and BB24, W86) under investigation. Results in Table (9) showed that the similarities were varied from 76.3% (between BB24 and Si-6 strains) to 97.9% (between Si-11 and W86 strains). The phylophenetic tree showed that the number of be reduced to three main species except for 8 species, as three major clusters were observed. The first cluster included only one *Streptomyces* strain characterized with WTY spore surface. The second cluster represented by three *Streptomyces* strains (BB24, ST3 and ST1). The third cluster contained four *Streptomyces* strains (Si-4, ST2, W86 and Si-11).

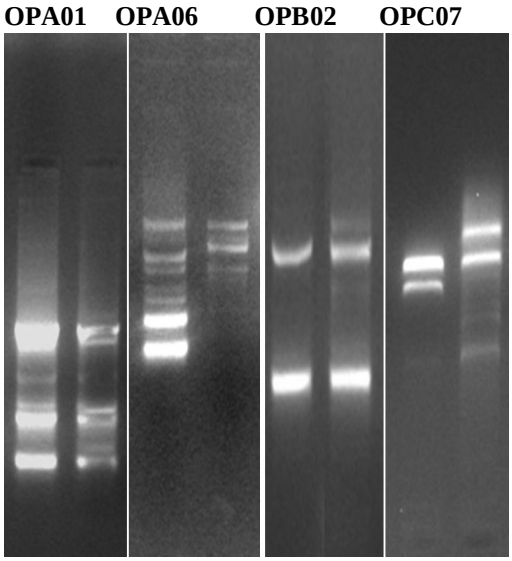


Figure-12: Agarose gel (1.2%) shows DNA polymorphisms of RAPD-PCR analysis of two *Streptomyces* strains (G7: Is-10 and Si-8) belonging to yellow series. Results of four primers (OPA01, OPA06, OPB02, and OPC07) are shown.

Table 9: Similarities between the red *Streptomyces* strains (Si-4, Si-6 and Si-11, ST1, ST2, ST3 and BB24, W86) based on analysis of their selected characters according to the suggested numerical method.

Streptomyces strains	Streptomyces strains						
	BB24	Si-4	Si-6	Si-11	ST1	ST2	ST3
Si-4	87.4	100					
Si-6	76.3	86.4	100				
Si-11	90.9	92.5	80.5	100			
ST1	92.0	93.5	84.0	92.5	100		
ST2	89.9	95.7	81.9	94.7	93.6	100	
ST3	90.5	87.6	76.9	88.9	94.4	87.9	100
W86	93.2	92.5	78.0	97.9	92.5	96.8	88.9

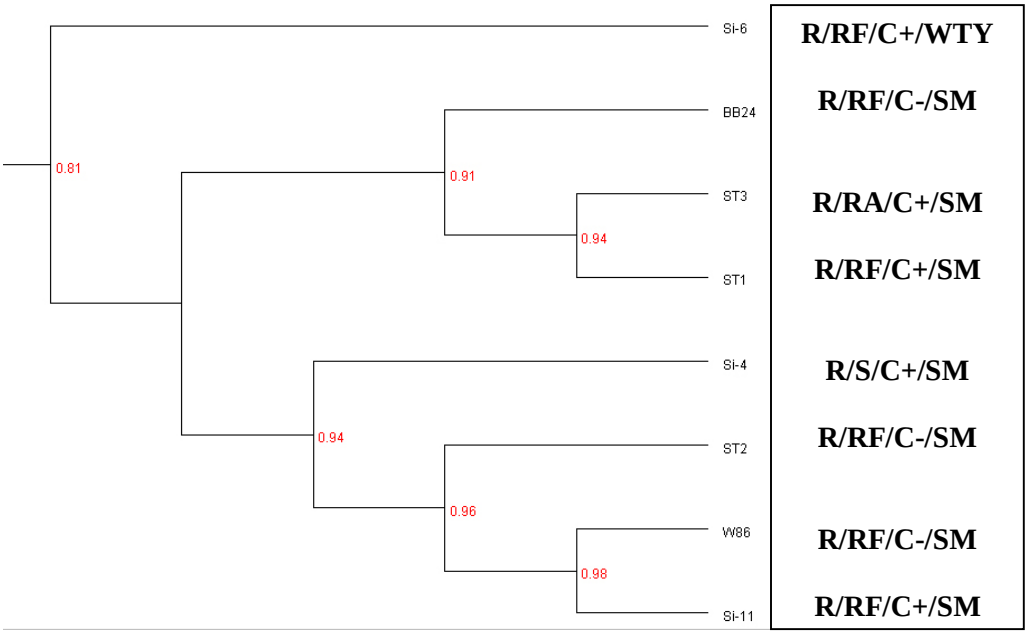


Figure -13: Phylophenetic tree of eight *Streptomyces* strains belonging to red series (Si-4, Si-6 and Si-11, ST1, ST2, ST3 and BB24, W86) based on analysis of their selected characters according to the suggested numerical method.

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REFERENCES:

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

Baldacci, E., Contributo alla sistematica degli Attinomiceti. V-VIII. Atti. Ist. Botan. Pavia 11: 191-231 (1939)

El-Domyati, F.M. and Sonya H. Mohamed., Molecular genetic characterization of some Streptomyces isolates exhibiting different levels of resistance to the herbicide BASTA. Egyptian Journal of Genetics and Cytology **33**: 249-286 (2004)

Küster, E., Simple working key for the classification and identification of named taxa included in the International *Streptomyces* Project. International Journal of Systematic Bacteriology **22:** 139-148 (1972)

Mahfouz H.T. and Sonya H. Mohamed, Physiological, antagonistic and fingerprinting studies on some haloterant *Streptomyces* strains. Arab Journal of Biotechnology **5**(1): 103-120 (2002).

Mohamed, H. Sonya, Role of actinomycetes in the biodegradation of some pesticides, p.151. Ph.D. Thesis, Agric. Microbiol., Dept. Agric. Microbiol., Faculty of Agric., Ain Shams University (1998)

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

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

Mohamed, H. Sonya, E.A. Saleh and M.M. Zaki, Identification of eight halotolerant streptomycete isolates using a suggested numerical taxonomy. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo **13**(3): 641-668 (2005)

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

Pridham, T.G., C.W. Hesseltine and R.G.A. Benedict, A guide for the classification of streptomycetes according to selected groups. Appl. Microbiol. **6**: 52-79 (1958)

Pridham, T.G., A.J. Lyons and H.L. Seckinger, Comparison of some dried holotype and neotype specimens with their living counterparts. International Bulletin of Bacteriological Nomenclature and Taxonom. **15**: 191-237 (1965).

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

Shirling, E.B. and D. Gottlieb, Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology **16**(3): 313-340 (1966)

Shirling, E.B. and D. Gottlieb, Cooperative description of type cultures of Streptomyces. II. Species description from first study. International Journal of Systematic Bacteriology **18**: 69 - 189 (1968)

Waksman, S.A. and A.T. Henrici, The nomenclature and classification of the actinomycetes. Journal of Bacteriol. **46**: 337-341 (1943)

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