academicJournals

Vol. 12(19), pp. 2558-2564, 8 May, 2013
DOI: 10.5897/AJB2013.11945
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Molecular studies on some soil-*Streptomyces* strains of western region, Kingdom of Saudi Arabia (KSA)

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Accepted 3 May, 2013

Random amplified polymorphic of DNA-polymerase chain reaction (RAPD-PCR) analysis of the DNA extracted from seven *Streptomyces* strains of western region, KSA was the aim of this study. Partial sequence of 16S rRNA gene of *Streptomyces polychromogenes* was also attempted. Results show that a total number of 240 amplified fragments were amplified using 10 RAPD-PCR primers (OPA11, OPB10, OPB15, OPC03, OPC07, OPC18, OPD05, OPE05, OPO14 and OPO17). A total number of 97, 73, 88, 79, 100, 108 and 82 fragments were amplified from the DNA extracts of *S. polychromogenes*, *Streptomyces chattanoogensis*, *Streptomyces lucensis*, *Streptomyces antibioticus*, *Streptomyces violans*, *Streptomyces griseorubiginosus* and *Streptomyces violaceus*, respectively. An obvious variation in the amplified fragments was recorded using the 10 RAPD-PCR primers (31, 27, 17, 19, 23, 29, 24, 26, 23 and 21 fragments for the primers, respectively). The highest similarity (66.7%) was found between *S. lucensis* and *S. chattanoogensis;* lowest similarity (35%) was recorded between the gray *S. chattanoogensis* and the red *S. violaceus.* The 16S rRNA gene was isolated *via* PCR from the DNA of *S. polychromogenes* (1) and sequenced. Fragments of 1003 and 837 nts were amplified using the forward and reverse primers, respectively. On matching, a final sequence of about 1452 nts (GenBank: JQ962978.1) was obtained and compared with five universal *Streptomyces* strains and four bacterial clones. The percent identities between the isolate of this study and the compared bacterial strains was lowest (79.1%) compared to HQ844464.1 and highest (98.3%) compared to EU520331.1. Based on the phenotypic and genotypic (16S rRNA gene) features, the strain could be classified as a new strain of *S. polychromogenes*.

Key words: Identification, *Streptomyces*, RAPD-PCR, 16S rRNA gene, KSA.

INTRODUCTION

Actinomycetes are microscopic soil microorganisms that have characteristics similar to both bacteria and fungi and yet possess sufficient distinctive features to classify them into a separate category. They form aerial mycelia, much smaller than those of fungi and many species produce asexual spores called conidia (Pridham and Tresner, 1974; Mythili and Ayyappa Das, 2011). Actinomycetes are one of the most efficient groups of secondary metabo-

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bolite producers and are very important from an industrial point of view. Among its various genera, *Streptomyces*, *Saccharopolyspora*,*Amycolatopsis*,*Micromonospora* and *Actinoplanes* are the major producers of commercially important bio-molecules (Solanki et al., 2008). Arafa et al. (2010) tested the antimicrobial (anti-bacterial and antifungal) and enzyme production of 14 actinomycetes. One of the most powerful effective iso-lates was *Streptomyces fulvissimus*. Xiao et al. (2002) collected a number of 53 antibiotic-producing *Streptomyces* and evaluated their ability to inhibit plant pathogenic *Phytophthora medicaginis* and *Phytophthora sojae in vitro*.

At KSA, Atta et al. (2011b) showed that one strep-tomycete isolate (symbol 143) out of 28 actinomycetes strains isolated from soil samples collected from Farm Jabbar district, Al-Khurmah governorate, KSA was identified. From the taxonomic features, the actino-mycetes isolate 143 matched with *Streptomyces albidoflavus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces albidoflavus,* 143. Saleh et al. (2011) identified five Basta herbicide-tolerant *Streptomyces* species on the basis of the nucleotide sequence of DNA using random amplification of polymorphic DNA-poly-merase chain reaction (RAPD-PCR) technique.

Abdel-Fattah (2005) used RAPD-PCR to determine the fingerprints of three *Streptomyces* strains using 10-decamer oligonucleotides, that is, OP-A02, OP-D01, OP-D02, OP-D05, OP-D06, OP-D07, OP-D08, OP-D11, OP-D18 and OP-D20. Atta et al. (2011b) determined the nucleotide sequence of the 16S rRNA gene (1.5 Kb) of the most potent streptomycetes strain symbol 143 from Farm Jabbar district, Al-Khurmah Governorate, KSA evidenced a 77% similarity with *Streptomyces albidoflavus*. From the taxonomic features, the actinomy-cetes isolate143 matched with *S. albidoflavus* in the morphological, physiological and biochemical cha-racters. Mahasneh et al. (2011) determined the sequence of the 16S rRNA gene and thus to conduct the phylogenic position of the naturally occurring wild type strain of *Streptomyces* QU66C. The full sequence of the 16S rRNA gene of a novel wide type strain of *Streptomyces coelicolor* which had been isolated from the soil of Qatar and thus characterized on the basis of its phenotypic and genotypic features was determined. This study aimed at RAPD-PCR analysis of the DNA extracted from seven *Streptomyces* strains of western region, KSA and PCR-isolation of the 16S rRNA gene of *Streptomyces polychromogenes*.

MATERIALS AND METHODS

Streptomyces strains

Seven *Streptomyves* strains (*S. polychromogenes*, *S. chattanoogensis*, *S. lucensis*, *S. antibioticus*, *S. violans*, *S. griseorubiginosus* and *S. violaceus*) isolated from soil of Jeddah, Makah and Taif area, KSA were used.

DNA preparation

The DNA extracts were prepared from the pulverized strepto-mycetes cells as described by El-Domyati and Mohamed (2004). The concentration and purity of DNA extracts of the *Streptomyces* strains were determined as recommended by Brown (1990).

RAPD-PCR analysis of seven *Strepromyces* strains

To reach such goal, a number of 10 RAPD-PCR primers (OPA11, OPB10, OPB15, OPC03, OPC07, OPC18, OPD05, OPE05, OPO14 and OPO17) were used. Amplification reaction and PCR amplification were carried out in a total volume of 25 µl based on the method of El-Domyati and Mohamed (2004). The amplification products were resolved by electrophoresis (Sambrook et al., 1989) in a 1.2% agarose gel at 60 volts for 2.5 h with 1xTAE buffer. The bands were checked by visualising the gel under the UV light transilluminator. The presence or absence of each band was scored as 1 (amplified) or 0 (not amplified). Bands of the same mobility were scored as monomorphic. The similarity coefficient (F) between isolates was defined by the formula of Nei and Li (1979). A dendrogram was derived from the distance by un-weighted paired-group method (Rohlf, 1990).

PCR-isolation of 16S rRNA gene of *S. polychromogenes* and its sequence analysis

The DNA of *S. polychromogenes* was used as a template for PCR-isolation of 16S rRNA gene using two universal primers (518F: 5'CCA GCA GCC GCG GTA ATA CG3'; 800R: 5'TAC CAG GGT ATC TAA TCC3') (Jacob, 2012). PCR amplification was performed in a GeneAmp 2400 PCR machine using the following program: Denaturation (5 min at 95°C, 1 cycle; 35 cycles, each of denaturation at 95°C for 1 min, annealing for 1 min at 56°C and extension for 2 min at 72°C). The primer extension segment was extended to 5 min at 72°C in the final cycle. The PCR products were resolved by electrophoresis in a 1.2% agarose gel at 80 volts for 1 h with 1xTAE buffer and then stained with ethidium bromide solution for around 10 to 15 min. Amplified fragments were visually examined under UV transilluminator.

The PCR product of 16S rRNA was firmly packed and was delivered to Lab-Technology® (Macrogen agent in Egypt) on crushed ice. Sequencing was performed using the ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits, ABI PRISM 3730XL Analyzer (96 capillary type) sequencer (Applied Biosystems), MJ Research PTC-225 Peltier Thermal Cycler, DNA polymerase (FS enzyme) (Applied Biosystems). Following the protocol supplied by the manufacturer, Single-pass sequencing was performed on each template using the same primer pairs described in the PCR step. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were re-suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer.

The DNA sequences of the PCR product of the 16S rRNA gene of *Streptomyces* sp. (Isolate 1) were aligned with the nucleotide sequences of the universal isolates (*S. polychromogenes* strain JSHS31 (HQ844476.1), *S. polychromogenes* strain GXSS21 (HQ844464.1), *S. polychromogenes* strain NRRL B-3032 (AY999923.1), *S. polychromogenes* strain HBUM175164 (FJ547116.1), Bacterium DE5 (EU520331.1), uncultured compost bacterium, clone FS1599 (FN667151.1), uncultured bacterium

Table 1. Total amplified fragments (TAFs) and unique fragments (UFs), produced by RAPD-PCR analysis of the seven *Streptomyces* strains using 10 RAPD-PCR primers.

Primer		Number of Fragment	*Streptomyces* strain						
Name	Fragment		1	2	3	4	5	6	7
OPA11	TAFs	31	13	10	12	9	12	16	8
	UFs	15	3	1	3	1	4	3	0
OPB10	TAFs	27	13	10	12	14	18	13	8
	UFs	2	0	0	0	0	2	0	0
OPB15	TAFs	17	11	9	11	9	11	10	8
	UFs	12	2	0	0	1	2	2	5
OPC03	TAFs	19	6	2	1	2	5	15	3
	UFs	12	2	0	0	0	2	8	0
OPC07	TAFs	23	6	9	10	4	10	9	10
	UFs	6	0	0	0	0	1	2	3
OPC18	TAFs	29	11	6	7	9	7	8	7
	UFs	13	3	0	1	1	3	3	2
OPD05	TAFs	24	8	5	8	11	9	7	12
	UFs	14	2	1	0	2	3	2	4
OPE05	TAFs	26	11	7	8	6	13	14	10
	UFs	9	1	0	2	0	1	3	2
OPO14	TAFs	23	9	10	11	7	9	9	7
	UFs	6	2	2	1	0	1	0	0
OPO17	TAFs	21	9	5	8	8	6	7	9
	UFs	10	3	0	1	0	2	1	3
Total	TAFs	240	97	73	88	79	100	108	82
	UFs	99	18	4	8	5	21	24	19

1, *S. polychromogenes*; 2, *S. chattanoogensis*; 3, *S. lucensis*; 4, *S. antibioticus*; 5, *S. violans*; 6, *S. griseorubiginosus*; 7, *S. violaceus*.

clone 8-sw-su5-3 (DQ981820.1), uncultured bacterium clone 8-sw-su5-2 (DQ981819.1) and uncultured *Streptomyces* sp., clone AMPF4 (AM935121.1) collected from http://www.ncbi.nlm.nih.gov/http://www.ncbi.nlm.nih.gov/, using the DNA Star Software Package–Lasergene (Expert Sequence Analysis Software, USA) and MegaAlign program.

RESULTS AND DISCUSSION

RAPD-PCR technique was used in some studies to determine the DNA fingerprinting of actinomycetes (Mohamed et al., 2001; Mahfouz and Mohamed, 2002; Abdel-Fattah, 2005; Saleh et al., 2011; Shash, 2011; Mohamed et al., 2012; Shori et al., 2012; Mohamed et al., 2013). Highly purified DNA extracts of the seven *Streptomyces* strains were used as templates for RAPD-PCR analysis. Data revealed that no amplified fragments were observed in any of the negative controls (PCR mixture without any DNA templates). Data in Table 1 and Figures 1, 2 and 3 shows that the number of amplified fragments differed with different primers, which is expected. On the other hand, the number and sizes of amplified fragments differed from one strain to another for the same primer. A total number of 240 amplified fragments were obtained using the ten RAPD-PCR primers. Out of the 240 fragments, 97, 73, 88, 79, 100, 108 and 82 fragments were amplified from the DNA extracts of the seven *Streptomyces* strains (*S. polychromogenes*, *S. chattanoogensis*, *S. lucensis*, *S. antibioticus*, *S. violans*, *S. griseorubiginosus* and *S. violaceus*, respectively. The streptomycetes isolates showed a number of 99 unique fragments distributed as follows: 18, 4, 8, 5, 21, 24 and 19 for the *Streptomyces* strains (*S. polychromogenes*, *S. chattanoogensis*, *S. lucensis*, *S. violaceus, S. violans*, *S. griseorubiginosus* and *S. antibioticus*), respectively. Also, the ten RAPD-PCR primers showed an obvious variation in the amplified fragments, as 31, 27, 17, 19, 23, 29, 24, 26, 23 and 21 fragments were recorded using the following primers, OPA11, OPB10, OPB15, OPC03, OPC07, OPC18, OPD05, OPE05, OPO14, and OPO17, respect-tively.

Data in Table 2 show that the highest similarity (66.7%)

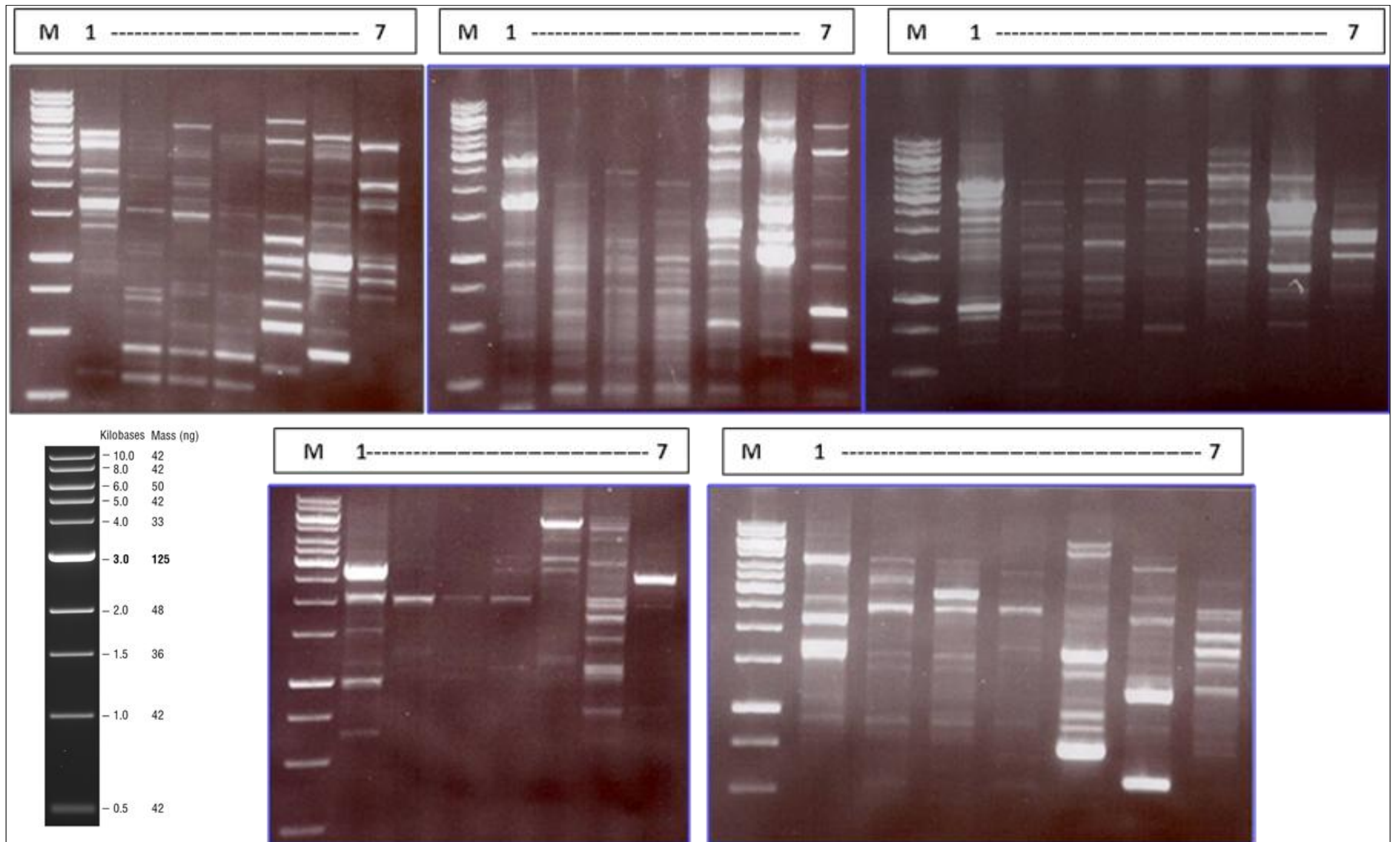

Figure 1. Agarose gel electrophoresis (1.2%) stained with ethidium bromide shows RAPD-PCR polymorphisms of DNA of the seven *Streptomyces* strains (1, *S. polychromogenes*; 2, *S. chattanoogensis*; 3, *S. lucensis*; 4, *S. antibioticus*; *5, S. violans*; 6, *S. griseorubiginosus*; 7, *S. violaceus*) using five (OPA11, OPB10, OPB15, OPC03 and OPC07) RAPD-PCR primers. M: DNA marker (1 Kb DNA Ladder).

was found between *S. lucensis* (belonging to gray series) and *S. chattanoogensis* (belonging to gray series)*;* lowest similarity (35%) was between *S. chattanoogensis* (belonging to gray series) and *S. violaceus* (belonging to red series).The phylogenetic tree (Figure 3) of the seven *Streptomyces* strains showed that the first cluster included isolates 1, 2, 3, 4 and 5, while the second cluster included isolates 6 and 7. RAPD-PCR technique was used in some studies to determine the DNA finger-printing of actinomycetes (Mohamed et al., 2001; Mahfouz and Mohamed, 2002; Abdel-Fattah, 2005; Saleh et al., 2011; Shash, 2011, Mohamed et al., 2012; Shori et al., 2012).

PCR isolation and sequencing the 16S rRNA gene of *S. polychromogenes*

Clarridge (2004) reported that the traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Comparison of the bacterial 16S rRNA gene sequence has emerged as a preferred genetic technique. 16S rRNA gene sequence analysis can better identify poorly described, rarely isolated, or phenotypically aberrant strains, can be routinely used for identification of mycobacteria, and can lead to the recognition of novel pathogens and non-cultured bacteria.

In this study, the 16S rRNA gene was isolated via PCR from the DNA of *S. polychromogenes* and sequenced. Fragments of 1003 and 837 nts were amplified using the forward and reverse primers, respectively. On matching, a final sequence of about 1452 nts (GenBank: JQ962978.1) was obtained and compared with five universal *Streptomyces* strains and four bacterial clones (Table 3). The percent identities between the isolate of this study and the compared bacterial strains was lowest (79.1%) compared to HQ844464.1 and highest (98.3%) compared to EU520331.1.

Results in Figure 4 show that the isolate under investigation fall into the same cluster with the uncultured bacterium clone 8-sw-su5-2, DQ981819.1 and a bacterium DE5; EU520331.1. Based on the phenotypic (Shori et al., 2012) and genotypic (16S rRNA gene) features, the strain could be classified as a new strain of *S. polychromogenes*. This result is in harmony with that found by Al-Askar et al. (2011) who identified an

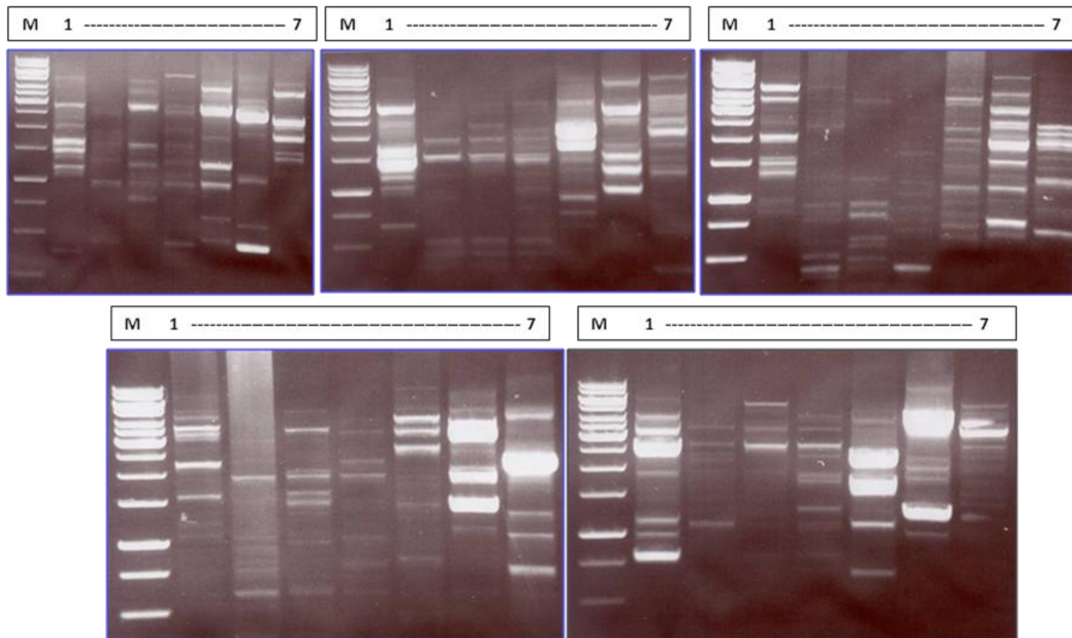

Figure 2. Agarose gel electrophoresis (1.2%) stained with ethidium bromide shows RAPD-PCR polymorphisms of DNA of the seven *Streptomyces* strains (1, *S. polychromogenes*; 2, *S. chattanoogensis*; 3, *S. lucensis*; 4, *S. antibioticus*; *5, S. violans*; 6, *S. griseorubiginosus*; 7, *S. violaceus*) using five (OPC18, OPD05, OPE05, OPO14 and OPO17) RAPD-PCR primers. M: DNA marker (1 Kb DNA Ladder).

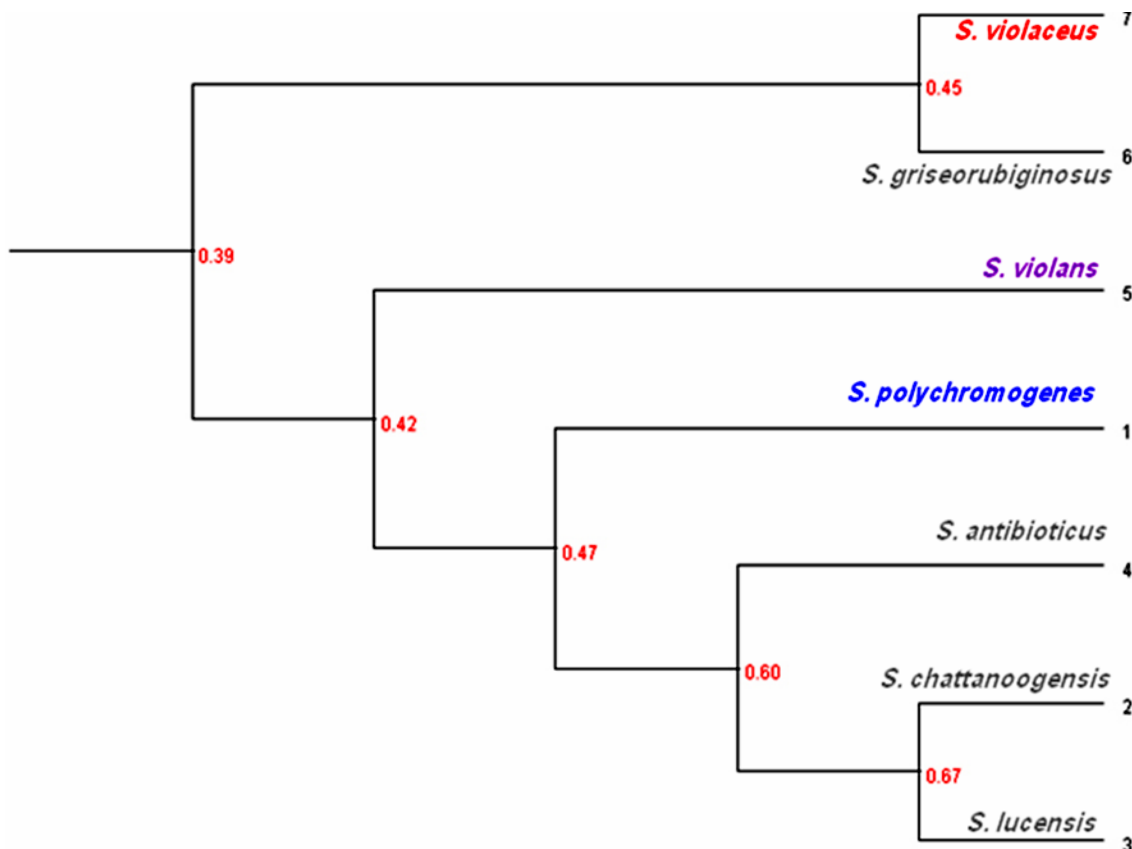

Figure 3. Dendrogram based on RAPD-PCR analysis of the seven *Streptomyces* strains using 10 RAPD-PCR primers.

Table 2. Similarity matrix between the seven *Streptomyces* strains using 10 RAPD-PCR primers.

***Streptomyces* strain**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
S. polychromogenes	100						
S. chattanoogensis	46.8	100					
S. lucensis	50.9	66.7	100				
S. antibioticus	42.0	62.9	57.0	100			
S. violans	43.0	41.0	46.2	39.3	100		
S. griseorubiginosus	41.5	36.5	37.6	45.7	42.4	100	
S. violaceus	39.0	35.0	35.4	40.6	37.4	44.7	100

1, *S. polychromogenes*; 2, *S. chattanoogensis*; 3, *S. lucensis*; 4, *S. antibioticus*; 5, *S. violans*; 6, *S. griseorubiginosus*; 7, *S. violaceus*.

Table 3. Percent identities between 16S rRNA gene amplifed from the DNA of *Streptomyces* sp. (isolate 08) and nine universal bacterial strains.

Accession	**Description**	**nts**	**Query coverage (%)**	**Identity (%)**
HQ844476.1	*Streptomyces polychromogenes* strain JSHS31	1405	85	79.2
HQ844464.1	*Streptomyces polychromogenes* strain GXSS21	1407	84	79.1
AY999923.1	*Streptomyces polychromogenes* strain NRRL B-3032	1299	84	79.7
FJ547116.1	*Streptomyces polychromogenes* strain HBUM175164	1419	88	79.2
EU520331.1	Bacterium DE5	1435	91	98.3
FN667151.1	Uncultured compost bacterium partial	1443	91	95.6
DQ981820.1	Uncultured bacterium clone 8-sw-su5-3	1445	91	96.4
DQ981819.1	Uncultured bacterium clone 8-sw-su5-2	1448	91	96.0
AM935121.1	Uncultured *Streptomyces* sp.	1333	87	79.3

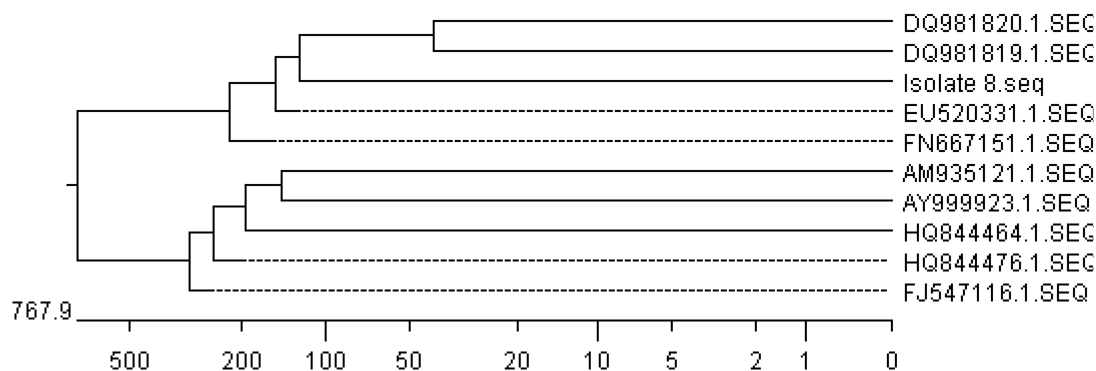

Figure 4. Phylogenetic tree of the nucleotide sequences of the PCR product of 16S rRNA gene amplifed from the DNA of *S. polychromogenes* and nine universal bacterial strains.

actinomycetes isolate from Riyadh, KSA as *Streptomyces sporoaveus* RDS28 according to analysis of 16S rRNA gene sequence.

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