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Screening, Isolation and Characterization of a Novel Antimicrobial Producing Actinomycete, Strain RAF10

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Abstract: A novel actinomycete strain designated RAF10, producing antimicrobial substances was isolated from an Egyptian soil. Morphological and chemical studies indicated that strain RAF10 belonged to the genus *Streptomyces*. The comparison of its physiological characteristics with those of known species of *Streptomyces* showed some differences with the nearest species *Streptomyces enissocaesilis*. Analysis of the 16S rDNA sequence of strain RAF10 showed a similarity level ranging between 97.22 and 98.37% within *Streptomyces* species, with *S. enissocaesilis* the most closely related. However, the phylogenetic analysis indicated that strain RAF10 represent a distinct phyletic line suggesting a new genomic species. This novel strain was active against Gram-positive and Gram-negative Bacteria, yeasts and filamentous fungi. The highest antibiotic formation was obtained when using (ISP 4) broth medium with some modifications, for 120 h at 28°C in New Brunswick Scientific Shaker at 200 rpm.

Key words: Actinomycetes, characterization, antimicrobial activity, screening, *Streptomyces*

INTRODUCTION

The search for new antibiotics continues to be of extreme importance in research programs around the world because of the increase of resistant pathogens and toxicity of some used antibiotics (Berdy, 1989). The history of new drug discovery processes shows that novel skeletons have, in the majority of cases, come from natural sources (Bevan *et al*., 1995). This involves the screening of microorganisms and plant extracts (Shadomy, 1987). Among microorganisms, actinomycetes are one of the most attractive sources of antibiotics and other biologically active substances of high commercial value and from which, *Streptomyces* spp. has been the most fruitful source of all types of bioactive metabolites that have important applications in human medicine as anti-viral and anti-cancer compounds and in agriculture as herbicides, insecticides and antiparasitic compounds (Watve *et al*., 2001). Thus, screening and isolation of promising strains of actinomycetes with potential antibiotics is still a thrust area of search by our group from many years (Hacène and Lefebvre, 1996; Hacène *et al*., 1998, 2000; Forar *et al*., 2006a, b).

In the present study, we describe the isolation of a new *actinomycete* strain from an Egyptian soil having antimicrobial activities against Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi and its identification by conventional and molecular methods as well as the optimal conditions for antimicrobial formation.

MATERIALS AND METHODS

Soil sampling, isolation and screening of *Streptomyces* spp.: Several soil samples were randomly collected from and around Cairo, during 2005-2006, using an open-end soil borer (20 cm depth and 2.5 cm diameter) from a depth of 10-20 cm then air-dried, mixed thoroughly with $CaCO_3$ (10% w/w) and incubated at 28°C for 10 days before use, (El-Nakeeb and Lechevalier, 1963; Tsao *et al*., 1960). Isolation and enumeration of actinomycetes present in the soil sample was performed by serial dilution plate technique using starch casein nitrate agar (El-Nakeeb and Lechevalier, 1963; Kuster and Williams, 1964). Promising isolates were maintained as suspension of spores and mycelia in YEME supplemented with 40% (v/v) glycerol

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(Hopwood *et al*., 1985). This study was conducted in the Laboratory of Microbial Chemistry, NRC-Dokki, Cairo, Egypt.

Morphological and cultural characteristics: The morphological and cultural characteristics of the bacterium were determined by naked eyes examination of 7, 14 and 21 days old cultures grown on various International *Streptomyces* Project (ISP) media recommended by Shirling and Gottlieb (1966). Colors of aerial and substrate mycelia were monitored with the ISCC-NBS centroid color charts (Kenneth, 1958). The spore chains and spore surface ornamentation were examined according to Tresner *et al.* (1961) using Em10 Karl-Zeiss electron microscope. Composition of the cell wall was carried out according to the methods of Becker *et al.* (1964) and Lechevalier and Lechevalier (1970).

Physiological characteristics: Several tests were considered for this study, including the utilization of the carbohydrate compounds evaluated on C1 medium (Pridham and Gottlieb, 1948; Nonomura, 1974), different nitrogen sources, the degradation of many organic compounds such as: milk casein, tyrosin, xanthin (Nitsch and Kutzner, 1969; Goodfellow, 1971), gelatin, starch, esculin and arbutin, the production of melanoid pigments on ISP 6 and ISP 7 media and nitrate reductase (Shirling and Gottlieb, 1966; Marchal *et al.,* 1987). The organism was also tested for its ability to grow on glucose-yeast extract agar (GYEA) medium supplemented with 5 different antibiotics (Athalye *et al*., 1985) and inhibitory compounds including (w/v): sodium azide, 0.001%; sodium chloride, 1, 2, 3, 4, 5, 6 and 7 and to grow at pH 5, pH 9 and at 42°C.

DNA isolation: Chromosomal DNAs were isolated by a versatile quick-prep method for genomic DNA of Gram-positive bacteria (Pospiech and Neumann, 1995), with some modifications. Mycelia (5 mL) grown in a LB broth shake culture were centrifuged, rinsed with TE and resuspended in 0.4 mL SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris, pH 7.5). Lysozyme was added to a concentration of 1 mg mL^{-1} and incubated at 37°C for 0.5-1 h. Then 0.1 vols 10% SDS and 0.5 mg Proteinase K mg mL^{-1} were added and incubated at 55°C with occasional inversion for 2 h. One-third volume 5 M NaCl and 1 vol. chloroform were added and incubated at room temperature for 0.5 h with frequent inversion. The mixture was centrifuged at 4500 g for 15 min and the aqueous phase was transferred to a new tube using a blunt-ended pipette tip. Chromosomal DNA was precipitated by the addition of 1 vol. 2-propanol with gentle inversion. The DNA was transferred to a new tube, rinsed with 70% ethanol, dried under vacuum and dissolved in a suitable volume (about 100 μL) of distilled water. The dissolved DNA was treated with 20 μg RNase A mL^{-1} at 37°C for 1 h. Samples were extracted in the same volume of phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with 2.5 vols cold ethanol and 0.1 vols 3 M sodium acetate. The pellets were washed with 70% ethanol, dried and dissolved in TE or distilled water.

PCR amplification: The 16S rDNA gene was amplified using primers fD1 (AGAGTTTGATCCTGGCTCAG) and rP2 (ACGGCTACCTTGTTACGACTT) (Weisburg *et al*., 1991). It was performed in iCycler PCR BIORAD, in a total volume of 50 mL containing 30-50 ng DNA, 100 mM each primer, 10 mM dNTP, 10*X* buffer (100 mM Tris/HCl, pH 8.0, 500 mM KCl, 20 mM MgCl2, 0.1% gelatin) and 1.5 U Taq DNA polymerase (Promega). PCR was performed under the following conditions: 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, 2 min at 72°C and then final extension at 72°C for 7 min, The PCR reaction mix was analyzed by agarose gel electrophoresis and the DNA of the expected size was purified then cloned into pGEM_T Easy vector (Promega).

Cloning and nucleotide sequence determination: PCR products of the 16S rDNA of strain RAF10 were sub-cloned into pGEM-T Easy Vector for nucleotide sequence determination using an automated laser fluorescence sequencer (3100 genetic analyzer ABI PRISM, Applied Biosystem, HITCHI, USA). Sequencing reactions were carried out with the Big Dye termination kit (Applied Biosystems) according to the supplier's instructions. Nucleotide sequence of the 16S rDNA of strain RAF10 was determined and compared for similarity level with the reference species of bacteria contained in genomic database banks, using the NCBI Blast available at the ncbi-nlm-nih.gov Web site.

Phylogenetic analysis: Phylogenetic and molecular evolutionary analyses were conducted using software's included in MEGA version 3.0 (Kumar *et al*., 2004) package. The 16S rDNA sequence of the strain RAF 10 was aligned using the CLUSTAL W program (Thompson *et al*., 1994) against corresponding nucleotide sequences of representatives of the genus *Streptomyces* retrieved from GenBank. Evolutionary distance matrices were generated as described by Jukes and Cantor (1969) and a phylogenetic tree was inferred by the Neighbor joining method (Saitou and Nei, 1987). Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbor joining data set.

Antimicrobial activity: For determining the antimicrobial activity of strain RAF10, the disk paper diffusion method was used (Wu, 1984). Inhibition zones were expressed as diameter and measured after incubation at 37°C for 24 h for bacteria and yeasts and at 28°C for 48 h for filamentous fungi. The used target germs obtained from MIRCEN Cairo, Faculty of Agriculture, Ain-Shams University, Egypt, were (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. pseudotropicalis*, *Rhodotorula minuta*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *Botrytis allii*, *Diplodia oryzae*, *Fusarium oxysporum*, *Helmenthosporium turcicum*, *Machrophomina phaseoli*, *Trichoderma viride*.

Selection of suitable broth medium and correct culture conditions: In favor of optimum formation of antibiotic from the selected organism, a number of broth culture media such as yeast-extract malt-extract glucose broth (ISP_2), Inorganic salts-starch broth (ISP_4), tryptone-yeast extract-glucose broth (TYG), TSB and starch nitrate broth medium were tried. After incubation at 28°C for 144 h in New Brunswick Scientific Shaker at 200 rpm, antibacterial activities were assayed for each culture supernatant. After determination of the better culture broth, effects of various carbon and nitrogen sources and temperatures, on the antibiotic production were also investigated in the same culture conditions described above. Finally and based on the obtained results, the effect of incubation periods (up to 144 h) was also determined. A range of extraction solvents was screened for effectiveness, including petroleum ether, n-hexane, chloroform, diethyl ether, ethyl acetate, butyl acetate, benzene and n-butanol. The organic extracts were evaporated to dryness then recuperated in 1 mL of methanol and tested for their antimicrobial activities using disks of 8mm diameter against *Bacillus cereus* and *Candida albicans*. The solvent which gave the highest inhibition diameter, using respective solvents as control, was then kept for the extraction of antibiotics. The biomass was extracted with ethanol.

RESULTS

Screening of *Streptomyces* isolates: Thirteen out of nineteen isolates of *Streptomyces* spp. obtained showed noticeable antimicrobial activities against Gram positive and Gram negative bacteria, yeasts and filamentous fungi. Three isolates exhibited high activities against all the tested microorganisms and appeared promising (Table 1). The most active one (Table 2) was selected for identification and designated strain RAF10. It exhibited different activities compared to its closest species *Streptomyces enissocaesilis* (Table 3).

Taxonomy: The examination of the strain RAF10 grown on ISP 2 medium at 28°C for 7 days revealed that sporophores are spiral. Transmission electron micrograph showed that spores are numerous, very fine and oval with smooth membranes (Fig. 1). The cultures are brown, earthy-black or gray-black. Strain RAF10 grew well to moderate on the tested organic and synthetic media. The color of the Aerial Mycelium (AM) is lilac to pinkish lilac; it varied depending on the type of used media. The brown substance was produced on synthetic and organic media and stains them. This strain hydrolyzed starch, reduced nitrate, liquefied gelatin and peptonized milk, but it did not produce H_2S. It utilized glucose, arabinose, mannose, maltose, xylose, inositol and sodium citrate, it could not utilize lactose, raffinose, sucrose, galactose, mannitol and sodium acetate. As nitrogen sources, it utilized nitrates well and ammonium salts are either poorly utilized or not utilized at all. Strain RAF10 was not able to grow on Glucose-Yeast Extract Agar (GYEA) medium supplemented with 5 different antibiotics, Chloramphenicol (25 mg L^{-1}), Erythromycin (10 mg L^{-1}), Gentamicin (5 mg L^{-1}), Oxytetracycline (25 mg L^{-1}) and Penicillin (25 mg L^{-1}). It did not grow at 0.001% sodium azide and 6% sodium chloride. Well growth was recorded at a temperature range of 15 to 37°C and at pH range of 6 to 9. The chemotaxonomic study showed the presence of a chemotype I cell wall characterized by (LLDAP) (Lechevalier and Lechevalier, 1970), no diagnostic sugars were detected.

The alignment of the nucleotide sequence (1489 bp) of strain RAF10 (Accession No EF 474464) through matching with 16S rDNA reported genes sequences in the gene bank using the NCBI Blast available at the ncbi-nlm-nih.gov Web site and compared with sequences of the reference species of bacteria contained in genomic database banks exhibited a similarity level ranged from 97.22 to 98.37% with *Streptomyces enissocaesilis* having the closest match. The phylogenetic tree obtained by applying the neighbor-joining method is shown in Fig. 2.

Antimicrobial activity: Results in Table 2 and 3 showed the broad antimicrobial spectrum of strain RAF10 against various target microorganisms. It exhibited a good activity against Gram-positive bacteria such as (*Staphylococcus aureus* and *Bacillus cereus)* and Gram-negative bacteria (*E. coli*), then filamentous fungi (*Aspergillus niger*) and yeasts (*Candida albicans*).

Table 1: Antimicrobial activity of nineteen isolates of *Streptomyces* spp. against Gram positive and Gram negative bacteria, yeasts and filamentous fungi

Isolate No.	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
ES1	-	-	-	+	+
ES2	-	-	-	-	-
ES3	+	+	+	-	-
ES4	-	+	+	-	-
ES5	+	+	+	-	-
ES6	-	-	-	-	-
ES7	+	+	+	+	+
ES8	+	+	+	-	-
ES9	-	-	+	-	+
ES10	+	+	+	+	+
ES11	-	-	-	-	-
ES12	-	-	-	-	-
ES13	+	+	+	-	-
ES14	+	+	+	-	-
ES15	+	+	-	+	+
ES16	+	+	+	-	-
ES17	-	-	-	-	-
ES18	-	-	-	-	-
ES19	+	+	+	+	+

+: Antibiosis, -: No effect

Table 2: Antimicrobial activity of the culture broth of the three selected isolates of *Streptomyces* spp. against the same test organisms mentioned above

Isolate No.	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
ES7	18	21	20	15	17
ES10	25*	23	24	22	23
ES19	23	22	22	18	18

*Antimicrobial activity was measured as growth inhibition zone diameter (mm)

Table 3: Antibiosis of strain RAF 10 and *S. enissocaesilis* towards various test organisms

Test organisms	Strain RAF10	*S. enissocaesilis*
Gram + bacteria		
Bacillus cereus	+	ND
B. subtilis	+	ND
Staphylococcus aureus	+	+
Micrococcus luteus	+	ND
Mycobacterium. sp.	+	+
Gram - bacteria		Inactive*
Escherichia coli	+	-
Pseudomonas aeruginosa	+	-
Yeasts		Inactive*
Candida albicans	+	-
C. tropicalis	+	-
C. pseudotropicalis	+	-
Rhodotorula minuta	+	-
Molds		Inactive*
Aspergillus niger	+	-
A. flavus	+	-
A.terreus	+	-
Botrytis allii	+	-
Diplodia oryzae	+	-
Fusarium oxysporum	+	-
Helmenthosporium turcicum	+	-
Trichoderma viride	+	-

ND: Not Determined, + Active, -: Not Active, *: Data from Krassilnikov (1981)

Suitable broth medium and correct culture conditions: Different broth media, carbon and nitrogen sources, temperatures and incubation periods were tested for the best production of active compounds. It was found that, (ISP 4) broth medium using, starch and ammonium sulphate at concentrations of 2.5 and 0.25% (w/v) as carbon and nitrogen sources respectively, for 120 h at 28°C in orbital incubator with shacking at 200 rpm were the most suitable for antibiotic formation. The ethanol extract of the biomass showed no antimicrobial activity. Petroleum ether, n-hexane, chloroform, diethyl ether and benzene were negative for antibiotics extraction. While ethyl acetate and butyl acetate were poor solvents for extraction. Because, n-butanol was good for extraction of active compounds, it was then kept for antibiotics extraction.

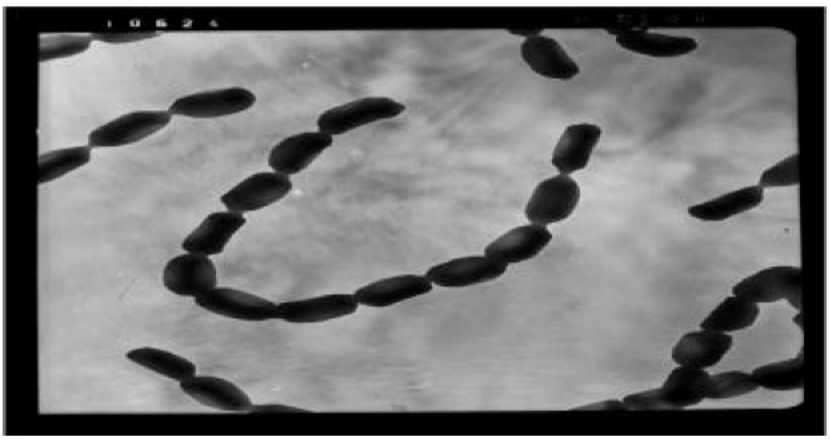

Fig. 1: Transmission electron micrograph of strain RAF10, showing smooth surface of spores (X10000)

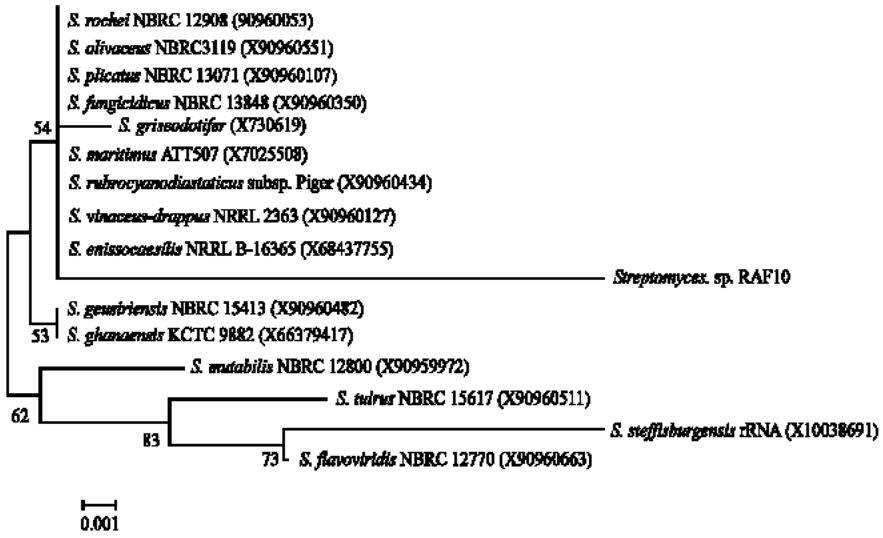

Fig. 2: 16S rDNA tree showing the phylogenitic relationship neighbor-joining method between strain RAF 10 and other known sequences of *Streptomyces* sp.

DISCUSSION

On the basis of its morphological and chemical properties, the strain RAF10 was classified in the genus *Streptomyces*. The characterization of *Streptomyces* species is mainly based on, the color of aerial and substrate mycelia and of soluble pigment, the shape and ornamentation of spore surface because of its stability. Furthermore and for adequate identification, some physiological characters such as temperature range growth, degradation of starch, gelatin, inositol and rhamnose and reduction of nitrates, some additional tests relative to the use of arabinose, glycerol, galactose and mannitol are also considered to ascertain species classification of new isolates strains as recommended by Shirling and Gottlieb (1972) and Holt *et al*. (1989). Comparison of cultural and physiological characteristics of the strain RAF10 with those of *Streptomyces* known species indicated that *Streptomyces enissocaesilis* was the nearest species. This species was identified as *Actinomyces enissocaesilis* INMI 40-31, when it was first isolated and described by Krassilnikov in 1970 (Gauze *et al*., 1983). The two strains have the same aerial and substrate mycelia colors, spore shape and physiological characters with some differences between them.

Modern *Streptomyces* identification systems are based on 16S rDNA sequence data, which have provided invaluable information about Streptomycetes systematic and then have been used to identify several newly isolated *Streptomyces* (Pineau *et al*., 2003; Lee *et al*., 2005; Forar *et al*., 2006a; Hyo *et al*., 2006). The 16S rDNA sequence of strain RAF10 was compared with those of other *Streptomyces* species, it showed the highest sequence similarity of 98.37% with *S. enissocaesilis* the most closely related species. However, it is clear from phylogenetic analysis that, strain RAF10 did cluster with neither S. *enissocaesilis* nor any of *Streptomyces* species and represented a distinct phyletic line suggesting a new genomic species. This may suggest the novelty of this strain. The use of phylogenetic technique gives a better resolution in the species level identification, (Stackebrandt and Woese, 1981; Dyson and Schrempf, 1987).

On the other hand, the antagonism of *S. enissocaesilis* is manifested poorly towards individual species of Gram-positive bacteria (*Staphylococci* and *Mycobacteria*), they do not suppress the growth of Gram-negative bacteria, fungi and yeasts (Krassilnikov, 1981), while strain RAF10 showed greater potency against Gram-positive and Gram- negative bacteria, yeasts and fungi.

Various parameters were tested for their suitability to increase antibiotics production by strain RAF10. It was found that, (ISP 4) broth medium using, starch and ammonium sulphate at concentrations of 2.5 and 0.25% (w/v) as carbon and nitrogen sources respectively, for 120 h at 28°C in orbital incubator with shacking at 200 rpm, were the most suitable. In fact, it has been shown that the nature of carbon and nitrogen sources, temperature, pH and incubation period, strongly affect active metabolite production in different organisms, (Vilshes *et al*., 1990; Holmalahti *et al*., 1998). Results obtained match with what was reported by Chattopadhyay and Sen (1997). The active compounds were extracted by n-butanol from the culture supernatant, whereas the ethanol extract of the biomass showed no antimicrobial activity. This shows the extracellular nature of active substances. Mostly antibiotics are extracellular (Hacene *et al*., 2000; Augustine *et al*., 2005). The investigation of these molecules is now in progress.

In conclusion, in view of all the previous characteristics of RAF10, it could be stated that, RAF10 is suggested of being a new variety of *Streptomuyces enissocaesilis*. Thus it is designated as *Streptomuyces enissocaesilis* RAF10. It is a potential source of active compounds. Results obtained from the present work are promising and hence merit further studies.

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REFERENCES

Athalye, M., M. Goodfellow, J. Lacey and R.P. White, 1985. Numerical classification of Actinomadura and Nocardiopsis. Int. J. Syst. Bacteriol., 35: 86-98.

Augustine, S.K., S.P. Bhavsar and B.P. Kapadnis, 2005. Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK 39. Indian J. Med. Res., 121: 164-170.

Becker, B., M.P. Lechevalier, R.E. Gordon and H. A. Lechevalier, 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Applied Microbiol., 12: 421-423.

Berdy, J., 1989. The Discovery of New Bioactive Microbial Metabolites: Screening and Identification. In: Bioactive Metabolites from Microorganisms. Bushell, M.E. and U. Grafe (Eds.), Amsterdam: Elsevier Science Publishers, pp: 3-25.

Bevan, P., H. Ryder and I. Shaw, 1995. Identifying small-molecule lead compounds: The screening approach to drug discovery. Trends Biotechnol., 113: 115-121.

Chattopadhyay, D. and S.K. Sen, 1997. Optimization of cultural conditions for antifungal antibiotic accumulation by *Streptomyces rochei* G164. Hindustan Antibiot. Bull., 39: 64-71.

Dyson, P. and H. Schrempf, 1987. Genetic instability and DNA amplification in *Streptomyces lividans* 66. J. Bacteriol., 169: 4796-4803.

El-Nakeeb, M.A. and H.A. Lechevalier, 1963. Selective isolation of aerobic actinomycetes. Applied Microbiol., 11: 75-77.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution., 39: 783-791.

Forar, L.R., K. Amany, E. Ali and Ch. Bengraa, 2006a. Taxonomy, identification and biological activities of a novel isolate of *Streptomyces tendae*. Arab J. Biotech., 9: 427-436.

Forar, L.R., A. Norrya and Ch. Bengraa, 2006b. Screening, isolation and characterization of antifungal producing actinomycete, *Streptomyces* strain RN+ 8. Afr. J. Biol. Sci., 2: 73-82.

Gauze, G.F., T.P. Preobrazhenskaya, M.A. Sveshnikova., L.P. Terekhova and T.S. Maximova, 1983. A guide for the determination of actinomyctes Genera *Streptomyces*, *Streptoverticillium* and *Chaina*. Nauka, Moscow (In Russian), pp: 137-138.

Goodfellow, M., 1971. Numerical taxonomy of some nocardioform bacteria. J. Gen. Micrbiol., 69: 33-90.

Hacene, H. and G. Lefebvre, 1996. HP17, a new pigment-like antibiotic produced by a new strain of *spirillospora*. J. Applied Bact., 89: 565-569.

Hacene, H., F. Boudjellal and G. Lefebvre, 1998. AH7, a new nonpolyenic antibiotic produced by a new strain of *Streptosporangium roseum*. Microbios, 96: 103-109.

Hacene, H., F. Daoudi-Hamdad, T. Bhatnagar, J.C. Baratti and G. Lefebvre, 2000. H107 a new aminoglycoside anti-*Pseudomonas* antibiotic produced by a new strain of *spirillospora*. Microbios, 102: 69-77.

Holmalahti, J., O. Raatikainen, A. Wright, H. Laatsch, A. Spohr, O.K. Lyngberg and J. Neilson, 1998. Production of dihydroabikoviromycin by *Streptomyces analatus*. Production parameters and chemical characterization of genotoxicity. J. Applied Microbiol., 85: 61-68.

Holt, J.G., M.E. Sharpe and S.T. Williams, 1989. Bergey's Manual of Systematic Bacteriology. Williams and Williams. Baltimore, London.

Hopwood, D.A., M.J. Bibb and K.F. Chater *et al.*, 1985. Genetic Manipulation of *Streptomyces*. A Laboratory Manual. Norwish: The John Innes Founqation.

Hyo, J.K., C.L. Sung and K.H. Byung, 2006. *Streptomyces cheonanensis* sp. nov., a novel streptomycete with antifungal activity. Int. J. Syst. Evol. Microbiol., 56: 471-475.

Jukes, T.H. and C.R. Cantor, 1969. Evolution of Protein Molecules. In: Mammalian Protein Metabolism. Munro, H.N. (Ed.), Vol. 3. Academic Press, New York, pp: 21-132.

Kenneth, L.K., 1958. Prepared research paper RP 2911, Central Natations for the Revised ISCC-NBS color name blocks. J. Res. NBS, 16: 427.

Krassilnikov, N.A., 1981. Ray fungi. Higher forms (Translated from Luchistye griby. Vysshie). Amerind Publishing Co. Pvt. Ltd., New Delhi, pp: 1001-1002.

Kumar, S., K. Tamura and M. Nei, 2004. MEGA3: Integrated software for Molecular Evolutionary Genetic Analysis and sequence alignment. Briefings Bioinformatics, 5: 150-163.

Kuster, E. and S. Williams, 1964. Selection of media for isolation of streptomycetes. Nature, 202: 928-929.

Lechevalier, M.P. and H.A. Lechevalier, 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol., 20: 435-443.

Lee, J.Y., H.W. Jung and B.K. Hwang, 2005. *Streptomyces koyangensis* sp. nov., a novel actinomycete that produces 4-phenyl-3-butenoic. Int. J. Syst. Evol. Microbiol., 55: 257-262.

Marchal, N., J.L. Bourdon and Cl. Richard, I987. The culture media for isolation and biochemical identification of bacteria. Doin, Paris.

Nitsch, B. and H.J. Kutzner, 1969. Egg-yolk as a diagnostic medium for streptomycetes. Experientia, 25: 113-116.

Nonomura, H., 1974. Key for classification and identification of 458 species of the streptomycetes included in ISP. J. Ferment. Technol., 52: 87-92.

Pineau, R., L. Sembiring and M. Godfellow, 2003. *Streptomyces yatensis* sp. nov.; a novel bioactive streptomycete. Antonie van Leewenhoek, 83: 21-26.

Pospiech, A. and B. Neumann, 1995. A versatile quick-prep of genomic DNA from Gram-positive bacteria. Trends Genet., 11: 217-218.

Pridham, T.G. and D. Gottlieb, 1948. The utilization of carbon compounds by some actinomycetales as an aid for species determination. J. Bacteriol., 56: 107-114.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Shadomy, S., 1987. Preclinical evaluation of antifungal agents. In: Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents. New Jersey: Prous Sci., pp: 8-14.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* sp. Int. J. Syst. Bacteriol., 16: 313-340.

Shirling, E.B. and D. Gottlieb, 1972. Cooperative description of type cultures of *Streptomyces*. V. additional description. Int. J. Syst. Bacteriol., 22: 265-394.

Stackebrandt, E. and C.R. Woese, 1981. Towards a phylogeny of the actinomycetes and related organisms. Cyrr. Microbiol., 5: 197-202.

Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position specific gap penalties and weight matrix choice. Nucl. Acids Res., 22: 4673-4680.

Tresner, H.D., M.C. Davies and E.J. Backus, 1961. Electron microscopy of *Streptomyces* spores morphology and its role in species differentiation. J. Bacteriol., 81: 70-80.

Tsao, P.H., C. Liben and G.W. Kitt, 1960. An enrichment for isolating actinomycetes that produce diffusable antifungal antibiotics. Phytopathology, 50: 88-89.

Vilshes, C., C. Mendez, C. Hardisson and J.A. Salas, 1990. Biosynthesis of oleandomycin by Influence of nutritional conditions and development of resistance. J. Gen. Microbiol., 136: 1447-1454.

Watve, M.G., R. Tickoo, M.M. Jog and B.D. Bhole, 2001. How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol., 176: 386-390.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol., 173: 697-703.

Wu, R.Y., 1984. Studies on the *Streptomyces* SC4. II-Taxonomical and biological characteristics of *Streptomyces* strain SC4. Bot. Bull. Acad. Sci., 25: 111-123.