Systematic studies on three streptomycete strains isolated from the Egyptian desert

Wael N. Hozzein[1,2]

[1.] Bioproducts Research Chair (BRC), College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia
[2.] Botany Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt
hozzein29@yahoo.com

Abstract: Three streptomycete strains were isolated from desert soil samples collected from Beni-Suef Governorate, Egypt. The isolates were identified taxonomically using the polyphasic approach. The strains, designated WH2104, WH2105 and WH2108 were found to have morphological and chemical properties typical of genus *Streptomyces*. The aerial mycelia differentiated into long spore chains with smooth surface. They contain LL-diaminopimelic acid, no diagnostic sugars, type PII phospholipids, and MK-9(H_6), MK-9(H_2) and MK-9(H_8) as the predominant menaquinones. All of these characters are consistent with their affiliation to the genus *Streptomyces*. The 16S rRNA gene sequence analysis supported the classification of the three isolates in the genus *Streptomyces* and also showed that WH2105 and WH2108 formed separate clades in the *Streptomyces* 16S rRNA gene tree. It was concluded from the phenotypic and phylogenetic data that the three isolates should be formally recognized as members the genus *Streptomyces* and either novel species or not should be confirmed by DNA-DNA pairing.
[Hozzein WN. **Systematic studies on three streptomycete strains isolated from the Egyptian desert**. *Life Sci J* 2013;10(4):1798-1802]. (ISSN:1097-8135). http://www.lifesciencesite.com. 235

Keywords: Streptomycete; polyphasic taxonomy; desert soil; 16S rRNA

1. Introduction

Actinomycetes is still considered as an unexhausted source of bioactive secondary metabolites (Bérdy, 2005). However, due to the decline in the number of newly-discovered active compounds, there is a big demand to discover novel actinomycetes to be introduced to the screening programs. This can be achieved by exploited the poorly studied and/or extreme habitats.

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and classified in the family *Streptomycetaceae* on the basis of morphology and subsequently cell wall chemotype. The taxon currently accommodates aerobic Gram-positive bacteria that have DNA with a high G+C content (69 to 78 mol%), LL-diaminopimelic acid and the absence of characteristic sugars in the cell wall (chemotype I according to Lechevalier & Lechevalier, 1970) and produce extensively branched substrate and aerial mycelia (Williams *et al*., 1989; Embley & Stackebrandt, 1994, Li *et al*., 2002). With more than 600 validly described species and subspecies, the taxon currently contains the largest number of species in the domain *Bacteria* (Hain *et al*., 1997). It is evident that the genus is underspeciated (Sembiring *et al*., 2000; Kim & Goodfellow, 2002) and that the description of *Streptomyces* species need to be based on a combination of genotypic and phenotypic data (Manfio *et al*., 1995, 2003; Atalan *et al*., 2000; Li *et al*., 2002). Members of novel streptomycete species are still in great demand as a source of novel commercially significant bioactive compounds (Kumar & Goodfellow, 2008; Santhanam *et al*., 2012).

In the current study the samples were collected from desert soil in Beni-Suef Governorate as a poorly studied habitat for the isolation of novel streptomycete strains for the screening purpose.

2. Material and Methods

Sampling

Six soil samples were collected from the desert habitats in Beni-Suef, Egypt. Each sample was taken at a depth of 5- 20cm with a collecting spatula. Most of the collected samples were obtained from the rhizosphere of the dominant plants in clean sterilized plastic bags, sealed and transported to the laboratory and then the samples were air dried at room temperature.

Isolation and maintenance of the organisms

Strains WH2104, WH2105 and WH2108 were isolated using MM medium (Hozzein *et al*., 2008). After incubation at 28ºC for 3 weeks, the isolates were picked, tested for purity and maintained on modified Bennett's agar (MBA; Jones, 1949) and preserved as a mixture of hyphae and spores in 20%, v/v glycerol at –20°C.

Morphological and cultural characteristics

The three isolates were grown on MBA (Jones, 1949) and oatmeal agar (ISP 6; Shirling & Gottlieb, 1966) at 28°C for 14 days for the microscopic observations. Spore chain morphology and spore ornamentation were observed by light and

scanning electron microscopes following the procedure described by O'Donnell *et al*. (1993). The cultural characteristics were studied on ISP media (Shirling & Gottlieb, 1966), MBA (Jones, 1949), and nutrient agar (Waksman, 1961). The aerial spore mass color, substrate mycelium pigmentation and the color of any diffusible pigment were recorded after incubation for 14 days at 28ºC. The colors were determined by comparison with chips from the ISCC-NBS color charts (Kelly, 1964).

Chemotaxonomy

Biomass for the chemical analyses was prepared by growing the strains in shake flasks of modified Bennett's broth (Jones, 1949) at 200rpm and 28ºC for 7 days. The mycelia and cells were harvested by centrifugation and washed three times with distilled water and then freeze-dried. Standard procedures were used for the extraction and analysis of The diagnostic isomer of diaminopimelic acid (Hasegawa *et al*., 1983), whole-organism sugars (Staneck & Roberts, 1974), Polar lipids and menaquinones (Minnikin *et al*., 1984); and fatty acids (Kämpfer & Kroppenstedt, 1996).

Phenotypic tests

The organisms were examined for a range of phenotypic characters using media and methods described by Williams *et al*. (1983). Carbon sources utilization was examined on ISP9 (Shirling & Gottlieb, 1966) as a basal medium supplemented with 1% final concentration of the tested carbon sources except for sodium acetate, sodium citrate, sodium pyruvate and sodium succinate which were used at 0.1%. Similarly, the organisms were examined for acid production from the tested carbon sources with the help of bromocresol purple as a pH indicator. The effect of different temperature degrees and pH values on the growth and tolerance to salt (NaCl 0, 5, 7, 10 and 15%, w/v) were determined using MBA or MM as a basal medium.

16S rRNA sequencing and phylogenetic analysis

Extraction of genomic DNA and PCR amplification and sequencing of a 16S rRNA gene from the isolated strains were achieved using previously described procedures (Kim *et al*. 1999). The resultant gene sequence was aligned manually using the PHYDIT program (Chun, 1995) against all available corresponding sequences of members of the family *Streptomycetaceae* (Kim *et al*., 2003) retrieved from the DDBJ, EMBL and GenBank databases. Unrooted phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated as described by Jukes and Cantor (1969). Tree topologies were evaluated by a bootstrap analysis of the neighbour-joining dataset, based on 1000 resamplings, by using the SEQBOOT and CONSENSE programs from the PHYLIP package.

3. Results and Discussion

The morphological and chemical properties of the three isolates WH2104, WH2105 and WH2108 were found to be consistent with their assignment to the genus *Streptomyces* (Williams *et al*., 1989; Manfio *et al*., 1995). They form extensively branched substrate mycelia, aerial hyphae that differentiate into smooth-surfaced chains of spores (Fig. 1).

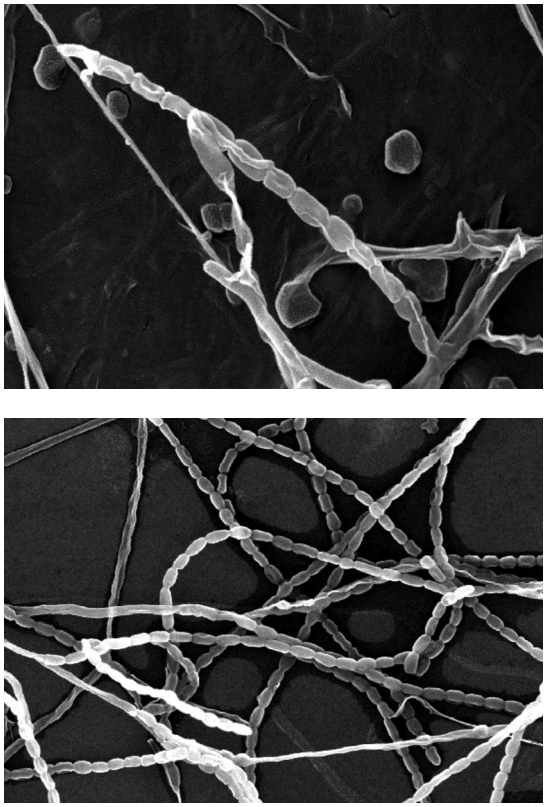

Figure 1. Scanning electron micrographs of strains WH2104 and WH2108 grown on MM for 14 days at 28˚C showing the long spore chains with smooth spores.

The strains showed good growth on most used media and their cultural characteristics and some of their phenotypic characters are summarized in Tables 1 & 2.

Table 1. Cultural characteristics of the three *Streptomyces* isolates.

Medium[a]	Character[b]	WH2104	WH2105	WH2108
ISP1	G	Good	Good	Good
	AM	White[c]	Reddish	White
	SM	Reddish brown	Brown	Yellowish white
ISP2	G	Good	Good	Good
	AM	Pinkish gray	Reddish brown	White
	SM	Reddish brown	Brown	Yellowish white
ISP3	G	Good	Good	Good
	AM	White	Reddish	Yellowish
	SM	Yellowish brown	brown Brown	white Yellow
ISP4	G	Good	Moderate	Moderate
	AM	White	Reddish	Yellowish
	SM	Yellowish brown	brown Brown	white Yellow
ISP5	G	Moderate	Good	Good
	AM	Pinkish gray	Reddish brown	White
	SM	Reddish brown	Brown	Yellowish white
Bennet agar	G	Good	Good	Good
	AM	Pinkish gray	Reddish brown	Yellowish white
	SM	Reddish brown	Brown	Yellow
Nutrient agar	G	Good	Moderate	Good
	AM	White	Brown	Yellowish
	SM	Yellowish brown	Reddish brown	white Yellow

[a] ISP, International Streptomyces Project (Shirling & Gottlieb, 1966). [b] G, Growth; AM, aerial mycelium and SM, substrate mycelium. [c] Colors were taken from ISCC-NBS COLOR CHARTS (Kelly, 1964).

Table 2. Some characteristic phenotypic characters of the three *Streptomyces* isolates.

Character	WH2104	WH2105	WH2108
Utilization of carbon sources: D (-) Arabinose	+	−	+
D (+) Galactose	+	−	−
D (+) Melezitose	−	+	+
D (+) Rafinose	+	±	−
Sodium citrate	−	−	−
Degradation of:			
Adenine	−	−	+
Hypoxanthine	−	+	+
Tyrosine	−	+	+
Growth at:			
45ºC	+	−	−

In addition, they contain the LL-isomer of diaminopimelic acid in the wall peptidoglycan, lack major characteristic whole-organism sugars, contain phosphatidylethanolamine as a diagnostic polar lipid, and hexa- & octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue. Chemotaxonomic characters that confirm their affiliation to genus *Streptomyces*.

The classification of the organisms in the genus *Streptomyces* is also supported by the results of the 16S rRNA gene sequence studies. Comparison of the almost complete 16S rRNA gene sequences of the strains WH2104, WH2105 and WH2108 with all available sequences in the public databases revealed that those strains belong to genus *Streptomyces*. The phylogenetic position of the strains with the closest relatives and representatives of the genus *Streptomyces* is shown in Fig. 2.

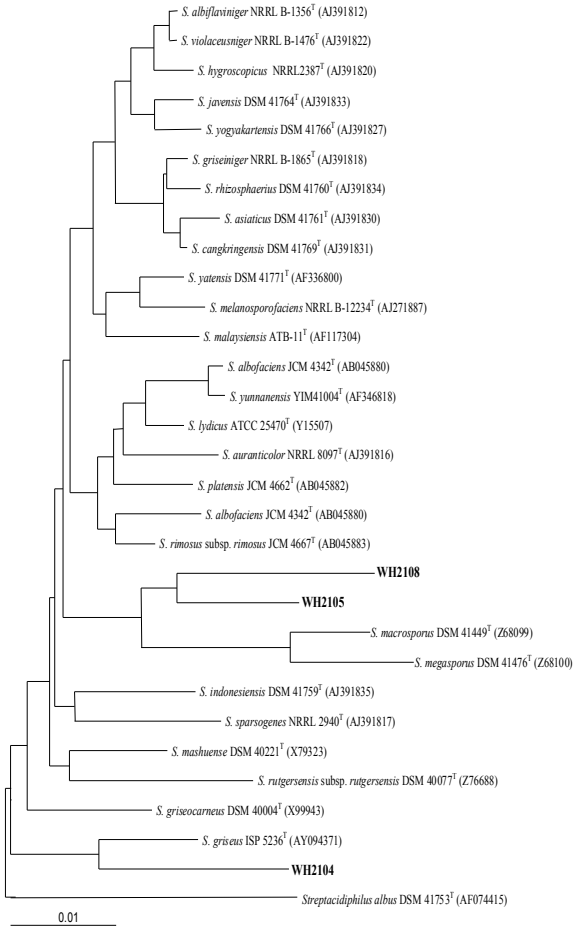

Figure 2. Phylogenetic tree based on 16S rRNA sequence analysis reconstructed from evolutionary distances using the neighbor-joining method, showing the phylogenetic positions of WH2104, WH2105 and WH2108.

Comparison of the almost complete 16S rRNA nucleotide (nt) gene sequence of strain WH2104 with corresponding streptomycete sequences shows that the organism forms a separate clade with *Streptomyces grieus* in the 16S rRNA *Streptomyces* gene tree. The two strains shared a 16S rRNA gene sequence similarity of 97.3%, irrespective of the treeing algorithms used.

Phylogenetic analysis of the other two strains, WH2105 and WH2108, revealed that they are closer to each other than with other validly described *Streptomyces* species and they form a distinct clade. Binary similarity values of WH2105 and other species of the genus *Streptomyces* showed that WH2108, *Streptomyces hygroscopicus* NRRL 2387^T and *Streptomyces albofaciens* JCM 4342^T are the most closely related neighbors (97%). On the other hand, WH2105 (97%), *Streptomyces violaceusniger* NRRL B-1476^T (96.7%), *Streptomyces hygroscopicus* NRRL 2387^T (96.6%) and *Streptomyces albiflaviniger* NRRL B-1356^T (96.6%) are the most closely related neighbors to WH2108.

These similarity values are less than the similarity values between many validly described species belonging to genus *Streptomyces*, such as *S. avermitilis* and *S. griseochromogenes* (99%; Kim & Goodfellow, 2002), *S. thermocarboxydovorans* and *S. thermoviolaceus* (98.8%; Kim *et al*., 1998), and several other streptomycete species (Sembiring *et al*., 2000; Saintpierre *et al*., 2002).

DNA:DNA relatedness studies need to be carried out to confirm that the relatedness values are below the 80% cut-off point recommended for the recognition of genomic species of *Streptomyces* (Labeda & Lyons, 1992; Labeda, 1993, 1998).

Minimal standards for the delineation of *Streptomyces* species need to be based on a complementary set of genotypic and phenotypic data (Manfio *et al*.,1995; Kim *et al*., 1999, 2003). The aim of the present study was to determine the taxonomic status of some streptomycete isolates using genotypic and phenotypic procedures. The resultant data indicate that the three isolated organisms WH2104, WH2105 and WH2108 are members of the genus *Streptomyces* and some of them could be formally recognized as novel species.

Acknowledgements:

The author extends his appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-205.

Corresponding Author:
Prof. Wael N. Hozzein
Bioproducts Research Chair (BRC), College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia
E-mail: hozzein29@yahoo.com

References

1. Atalan, E., Manfio, G.P., Ward, A.C., Kroppenstedt, R.M. & Goodfellow, M. (2000). Biosystematic studies on novel streptomycetes from soil. *Antonie van Leeuwenhoek* 77, 337-353.
2. Bérdy, J. (2005). Bioactive microbial metabolites. *J Antibiot* 58, 1- 26.
3. Chun, J. (1995). *Computer –assisted Classification and Identification of Actinomycetes.* Ph.D. thesis, University of Newcastle, Newcastle upon Tyne, UK.
4. Dieter, A., Hamm, A., Fiedler, H-P., Goodfellow, M., Müller, W.E.G., Brun, R., Beil, W. & Bringmann, G. (2003). Pyrocoll, an antibiotic, antiparasitic and antitumor compound produced by a novel alkaliphilic *Streptomyces* strain. *J Antibiotics* 56, 639-646.
5. Embley, T.M. & Stackebrandt, E. (1994). The molecular phylogeny and systematics of the actinomycetes. Ann. Rev. Microbiol. 48, 257- 289.
6. Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package), version 3.5c Department of Genetics, University of Washington, Seattle, USA.
7. Fitch, W.M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome c sequences is of general applicability. *Science* 1551, 279-284.
8. Hain, T., Ward-Rainey, N., Kroppenstedt, R.M., Stackebrandt, E., & Rainey, F.A. (1997). Discrimination of *Streptomyces albidoflavus* strains based on the size and number of 16S-23S ribosomal DNA intergenic spacers. *Int J Syst Bacteriol* 47, 202-206.
9. Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* 29, 319- 322.
10. Hozzein, W.N., Ali, M.I.A. & Rabie, W. (2008). A new preferential medium for enumeration and isolation of desert actinomycetes. *World J Microbiol Biotechnol* 24, 1547–1552.
11. Jones, K.L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenus aerial mycelia is a fluctuating characteristic. *J Bacteriol* 57, 141- 145.
12. Jukes, T.H. & Cantor, C.R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21- 132. Edited by H.N. Munro. New York: Academic Press.
13. Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Canadian J. Microbiol.* 42, 989-1005.
14. Kelly, K. L. (1964). Inter-society color council-national bureau of standards color name charts illustrated with centroid colors published in US.
15. Kim, S.B., & Goodfellow, M. (2002). *Streptomyces avermitilis* sp. nov., nom. rev., a taxonomic home for the avermectin-producing streptomycetes. Int. J. Syst. Evol. Microbiol. 52: 2011- 2014.
16. Kim, S.B., Brown, R., Oldfield, C., Gilbert, S.C. & Goodfellow, M. (1999). *Gordonia desulfuricans* sp. nov., a benzothiophene-desulphurizing actinomycete. *Int J Syst Bacteriol* 49, 1845-1851.
17. Kim, S.B., Falconer, C., Williams, E., and Goodfellow, M. (1998). *Streptomyces thermocarboxydovorans* sp. nov., and *Streptomyces thermocarboxydus* sp. nov., two moderately

thermophilic carboxydotrophic species from soil. Int. J. Syst. Bacteriol. 48: 59- 68.
18. Kim, S.B., Lonsdale, J., Seong, C-N. & Goodfellow, M. (2003). *Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici (1943) ^{AL} emend Rainey (1997). *Antonie van Leeuwenhoek* 83, 107-116.
19. Kumar, Y. & Goodfellow, M. (2008). Five new members of the *Streptomyces violaceusniger* 16S rRNA gene clade: *Streptomyces castelarensis* sp. nov., comb. nov., *Streptomyces himastatinicus* sp. nov., *Streptomyces mordarskii* sp. nov., *Streptomyces rapamycinicus* sp. nov. and *Streptomyces ruanii* sp. nov. *Int J Syst Evol Microbiol* 58, 1369–1378.
20. Labeda, D.P. & Lyons, A.J. (1992). DNA relatedness among strains of the sweet potato pathogen *Streptomyces ipomoea* (Person and Martin 1949). Waksman and Henrici 1948. *Appl Environ Microbiol* 58, 532-535.
21. Labeda, D.P. (1993). DNA relatedness among strains of the *Streptomyces lavendulae* phenotypic cluster group. *Int J Syst Bacteriol*. 43, 822-825.
22. Labeda, D.P. (1998). DNA relatedness among the *Streptomyces fulvissimus* and *Streptomyces griseoviridis* phenotypic cluster groups. *Int J Syst Bacteriol* 48, 829-832.
23. Lechevalier, M.P. & Lechevalier, H.A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 20, 435- 443.
24. Li, W., Lanoot, B., Zhang, Y., Vancanneyt, M., Swings, J. & Liu, Z. (2002). *Streptomyces scopiformis* sp. nov., a novel streptomycete with fastigiate spore chains. *Int J Syst Evol Microbiol* 52, 1629-1633.
25. Manfio, G.P., Atalan, E., Zakrzewska-Czerwinska, J., Mordarski, M., Rodriguez, C., Collins, M.D. & Goodfellow, M. (2003). Classification of novel soil streptomycetes as *Streptomyces aureus* sp. nov., *Streptomyces laceyi* sp. nov. and *Streptomyces sanglieri* sp. nov. *Antonie van Leeuwenhoek* 83, 245-255.
26. Manfio, G.P., Zakrzewska-Czerwinska, J., Atalan, E. & Goodfellow, M. (1995). Towards minimal standards for the description of *Streptomyces* species. *Biotechnologia* 7-8, 242-253.
27. Mincer, T. J., Gensen, P. R., Kauffman, C. A. & Fenical, W. (2002). Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl. Environmental Microbiol.* 68, 5005- 5011.
28. Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J.H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2, 233-241.
29. O'Donnell, A.G., Falconer, C., Goodfellow, M., Ward, A.C. & Williams, E. (1993). Biosystematics and diversity amongst novel carboxydotrophic actinomycetes. *Antonie van Leeuwenhoek* 64, 325-340.
30. Saintpierre, D., Amir, H., Pineau, R., Sembiring, L., and Goodfellow, M. (2002). *Streptomyces yatensis* sp. nov., a novel bioactive streptomycete isolated from a New-Caledonian ultramafic soil. Antoni van Leeuwenhoek (in press).
31. Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406- 425.
32. Santhanam, R., Okoro, C.K., Rong, X., Huang, Y., Bull, A.T., Andrews, B.A., Asenjo, J.A., Weon, H.Y. & Goodfellow, M. (2012). *Streptomyces deserti* sp. nov., isolated from hyper-arid Atacama Desert soil. *Antonie Van Leeuwenhoek* 101(3), 575-581.
33. Sembiring, L., Ward, A.C. & Goodfellow, M. (2000). Selective isolation and characterisation of members of the *Streptomyces violaceusniger* clade associated with roots of *Paraserianthes falcataria*. *Antonie van Leeuwenhoek* 78, 353-366.
34. Sembiring, L., Ward, A.C., and Goodfellow, M. (2000). Selective isolation and characterization of members of the *Streptomyces violaceusniger* clade associated with the roots of *Paraserianthes falcataria*. Antoni van Leeuwenhoek 78: 353- 366.
35. Shirling, E.B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16, 313- 340.
36. Staneck, J.L. & Roberts, G.D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 28, 226-231.
37. Waksman, S.A. & Henrici, A.T. (1943). The nomenclature and classification of the actinomycetes. *J Bacteriol* 46, 337-341.
38. Waksman, S.A. (1961). The actinomycetes, vol. 2. Classification, identification and descriptions of genera and species. The Williams & Wilkins Co., Baltimore.
39. Williams, S.T., Goodfellow, M. & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici, 1943, 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, Volume 4, pp. 2452-2492. Edited by S.T. Williams, M.E. Sharpe and J.G. Holt. Baltimore, Williams and Wilkins.
40. Williams, S.T., Goodfellow, M., Alderson, G., Wellington, E.M.H., Sneath, P.H.A. & Sackin, M.J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Micrbiol* 129, 1743- 1813.