Nocardiopsis arabia sp. nov., a halotolerant actinomycete isolated from a sand-dune soil

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The taxonomic status of an unknown actinomycete isolated from a sand-dune soil was established using a polyphasic approach. Isolate S186^T had chemotaxonomic and morphological properties consistent with its classification in the genus *Nocardiopsis*, grew on agar plates at NaCl concentrations of up to 15% (w/v) and formed a distinct phyletic line in the *Nocardiopsis* 16S rRNA gene sequence tree. Its closest phylogenetic neighbours were *Nocardiopsis chromatogenes*, *Nocardiopsis composta*, *Nocardiopsis gilva* and *Nocardiopsis trehalosi*, with sequence similarity to the various type strains of 96.9%, but it was readily distinguished from the type strains of these and related species using a range of phenotypic properties. It is apparent from the genotypic and phenotypic data that strain S186^T belongs to a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis arabia* sp. nov. is proposed. The type strain is S186^T (=CGMCC 4.2057^T =DSM 45083^T).

The genus *Nocardiopsis* was proposed by Meyer (1976) on the basis of chemotaxonomic and morphological characteristics. At the time of writing, the taxon encompasses 24 recognized species (Li *et al.*, 2006), which form a distinct clade within the evolutionary radiation occupied by members of the family *Nocardiopsaceae* (Rainey *et al.*, 1996; Cui *et al.*, 2001; Kroppenstedt & Evtushenko, 2006). *Nocardiopsis* strains are frequently isolated from saline and alkaline soils (Li *et al.*, 2004, 2006; Hozzein *et al.*, 2004), but have also been recovered from an alkaline slag dump (Schippers *et al.*, 2002), clinical material (Yassin *et al.*, 1997; Mordarska *et al.*, 1998), indoor environments (Peltola *et al.*, 2001), the atmosphere of a composting facility (Kämpfer *et al.*, 2002) and a marine sediment (Sabry *et al.*, 2004). In the present polyphasic study, an actinomycete isolated from a sand-dune soil was shown to represent a novel species of the genus *Nocardiopsis*.

Strain S186^T was isolated on an M1 agar plate (Mincer *et al.*, 2002) that had been inoculated with a suspension of a sand-dune soil (with a pH of 7.8), collected from Borg Al-Arab, Egypt, and incubated at 28 °C for 14 days. The isolate was maintained on modified Bennett's (Jones, 1949) and yeast extract-malt extract (ISP medium 2; Shirling & Gottlieb, 1966) agars at room temperature and as glycerol suspensions of hyphae and spores (20%, v/v) at –20 °C. Biomass for chemotaxonomic and molecular systematic studies was derived from a 7-day-old modified Bennett's broth (Jones, 1949) shake culture incubated at 28 °C,

harvested by centrifugation and washed twice with distilled water.

Extraction of chromosomal DNA, PCR amplification and direct sequencing of the 16S rRNA gene from isolate S186^T were achieved using established procedures (Chun & Goodfellow, 1995). The resultant sequence was aligned manually with the corresponding sequences of strains classified within the family *Nocardiopsaceae*, as retrieved from the GenBank/EMBL/DDBJ databases using the pairwise alignment option and 16S rRNA secondary structure information held within the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices were generated for the least-squares and neighbour-joining methods as described by Jukes & Cantor (1969) and the resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset (based on 1000 resamplings) using the CONSENSE and SEQBOOT options from the PHYLIP package. A comparison of the sequence of S186^T with those of representatives of genera classified within the family *Nocardiopsaceae* showed that the isolate fell within the evolutionary radiation occupied by the genus *Nocardiopsis* (data not shown). The 16S rRNA gene sequence similarity between strain S186^T and the type strains of *Nocardiopsis* species ranged from 94.2 to 96.9%.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S186^T is EF095149.

Chemosystematic studies were carried out to establish whether isolate S186^T had a chemical profile consistent with its assignment to the genus *Nocardiopsis*. Standard procedures were used to extract and analyse the isomeric forms of diaminopimelic acid (Staneck & Roberts, 1974), the whole-organism sugars (Hasegawa *et al.*, 1983), the muramic acid type (Uchida *et al.*, 1999), the isoprenoid quinones (Minnikin *et al.*, 1984; Collins, 1994), the polar lipids (Minnikin *et al.*, 1984) and the fatty acids (Sutcliffe, 2000). The isolate was characterized by the following: the presence of *meso*-diaminopimelic acid, galactose and glucose in whole-organism hydrolysates (wall chemotype III, according to Lechevalier & Lechevalier, 1970), *N*-acetylated muramic acid, tetrahydrogenated and hexahydrogenated menaquinones with 10 isoprene units as the predominant isoprenologues (menaquinone type 4C2; according to Kroppenstedt, 1985) and the presence of phosphatidylcholine, phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, two uncharacterized glycolipids and two unknown phospholipids with high *R_F* values (above that of diphosphatidylglycerol) as major polar lipids (phospholipid type III, according to Lechevalier *et al.*, 1977). The fatty acid profile was dominated by iso-/anteiso-branched and 10-methyl-branched fatty acids. The principal components were 14-methylpentadecanoic acid (iso-C_{16:0}, 34%), 10-methyl-octadecanoic acid (tuberculostearic acid; 10-methyl C_{18:0}, 17.3%) and 14-methylhexadecanoic acid (anteiso-C_{17:0}, 14.6%), though smaller proportions of iso-heptadecanoic acid (iso-C_{17:0}, 7.4%), iso-octadecanoic acid (iso-C_{18:0}, 4.0%) and 10-methylheptadecanoic acid (10-methyl C_{17:0}, 4.8%) were detected (fatty acid type 3d, according to Kroppenstedt, 1985). All of these chemical properties are consistent with the classification of the isolate in the genus *Nocardiopsis* (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992; Kroppenstedt & Evtushenko, 2006).

It is apparent from Fig. 1 that isolate S186^T forms a distinct phyletic line towards the periphery of the *Nocardiopsis* gene tree, being more closely related to *Nocardiopsis chromatogenes* KCTC 19008^T, *Nocardiopsis composta* DSM 44551^T, *Nocardiopsis gilva* KCTC 19006^T and *Nocardiopsis trehalosi* VKM Ac-942^T (with similarity of 96.9%) than to *Nocardiopsis halophila* DSM 44494^T (96.8%) and *N. baichengensis* KCTC 19009^T (96.7%). DNA–DNA relatedness experiments were not carried out between isolate S186^T and its closest phylogenetic neighbours, as the type strains of most of the latter share higher 16S rRNA gene sequence similarities, but have levels of DNA–DNA relatedness well below the 70% cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987). The type strains of *N. baichengensis* and *N. halophila*, for example, share a 16S rRNA gene sequence similarity of 99.9% but have a DNA–DNA relatedness value of only 55.9% (Li *et al.*, 2006). Similarly, the type strain of *N. composta* shares 16S rRNA gene sequence similarity of 97.5 and 97.4% with the type strains of *N. halophila* and *N.*

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of isolate S186^T within the *Nocardiopsis* gene tree. Asterisks indicate branches of the tree that were also recovered using the least-squares, maximum-likelihood and maximum-parsimony tree-making algorithms. Numbers at nodes are bootstrap percentages (based on 1000 resampled datasets); only values above 50% are given. The sequence of *Actinomadura madurae* DSM 43067^T (GenBank accession no. X97889) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

chromatogenes, respectively, but shows DNA–DNA relatedness of 0% with respect to these strains (Li *et al.*, 2006).

The cultural properties of the isolate, notably the aerial spore-mass colour, the pigmentation of the substrate mycelium and the colour of any diffusible pigments, were recorded on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic salts-starch and glycerol-asparagine agars (ISP media 1 to 5, respectively; Shirling & Gottlieb, 1966) after 2 weeks at 28 °C; colours were recorded using the National Bureau of Standards Color Name Charts (Kelly, 1958; National Bureau of Standards, 1964). The arrangement of undisturbed hyphae, notably aerial hyphae and spore chains, was observed on modified Bennett's agar (Jones, 1949) after 15 days at 28 °C, using the cover-slip technique of Kawato & Shinobu (1959). Growth on the cover-slips was fixed and examined as described by Zhou *et al.* (1998). Additional morphological properties were determined by examining a gold-coated dehydrated preparation with a Cambridge Stereoscan 240 scanning electron microscope, using the procedure described by O'Donnell *et al.* (1993).

The strain grew well on all of the ISP media, producing a pale-yellow to deep greyish-yellow substrate mycelium that

Table 1. Phenotypic characteristics that serve to differentiate strain S186^T and representatives of closely related *Nocardiopsis* species

Strains: 1, S186^T; 2, *N. baichengensis* KCTC 19009^T; 3, *N. chromatogenes* KCTC 19008^T; 4, *N. composta* DSM 44551^T; 5, *N. gilva* KCTC 19006^T; 6, *N. halophila* DSM 44494^T; 7, *N. rhodophaea* KCTC 19049^T; 8, *N. rosea* KCTC 19007^T; 9, *N. trehalosi* VKM Ac-942^T. Data for reference type strains were taken from Al-Tai & Ruan (1994), Evtushenko *et al.* (2000), Kämpfer *et al.* (2002) and Li *et al.* (2006). +, Positive; –, negative; w, weak; ND, not determined/no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Spore chains	Long	Long	Long	Long	Short	Long	Short	Short	Long
Growth on ISP 5 medium*									
Aerial mycelium	YW	WH	WH	WH	PY	WH	PP	PP	WH
Substrate mycelium	GY	OY	SB	YB	MB	ND	RB	RP	YB
Soluble pigments	None	None	YP	None	None	ND	None	None	ND
Growth on sole carbon sources									
Cellobiose	+	+	+	ND	+	ND	–	–	ND
D-Fructose	+	+	+	+	+	+	–	+	+
D-Galactose	+	+	+	+	+	+	–	–	+
D-Glucose	w	+	+	+	+	+	+	+	+
Maltose	+	+	+	–	–	+	–	+	+
D-Mannitol	+	+	+	ND	+	+	–	–	+
D-Mannose	+	+	+	+	–	+	–	–	+
Raffinose	–	–	+	ND	+	+	–	–	ND
D-Ribose	–	+	+	+	–	+	+	+	–
D-Sorbitol	+	–	–	ND	+	ND	–	–	–
Sucrose	+	+	+	–	+	+	–	+	–
D-Xylose	–	–	+	–	+	+	–	–	+
Acetate	–	+	+	+	+	–	+	+	+
Citrate	–	+	+	+	+	ND	–	–	+
Temperature for growth (°C)									
Range	10–40	20–50	20–60	15–50	10–40	15–36	20–60	20–60	18–45
Optimum	28–30	37–40	37–40	37	28–30	30	37–40	37–40	28–37
NaCl concentration for growth (%)									
Range	0–15	0–18	0–18	0–10	0–18	3–20	0–18	0–18	0–5
Optimum	5	5–8	5–8	5–10	5–8	5–15	5–8	5–8	0–5
Major menaquinones	10(H ₄), 10(H ₆)	9(H ₄), 10(H ₂), 10(H ₄), 10(H ₆)	10, 10(H ₂), 10(H ₄)	10(H ₈), 11(H ₈), 10(H ₆), 12	11(H ₄), 11(H ₆), 11(H ₈)	10(H ₆), 10(H ₈)	11(H ₆), 11(H ₈)	11, 11(H ₂), 11(H ₄)	10(H ₄), 10(H ₆)

*GY, Greyish yellow; MB, moderately brown; OY, orange–yellow; PP, pale pink; PY, pale yellow; RB, reddish brown; RP, reddish pink; SB, soft brown; WH, white; YB, yellow–brown; YP, yellowish pink; yw, yellowish white.

carried white aerial hyphae; diffusible pigments were not produced. Well-developed, branched substrate hyphae that fragmented into coccoid and rod-shaped elements were produced. Long, straight spore chains (with 10–20 spores per chain) were borne on aerial hyphae. The spores were rod-shaped, of variable length (0.7–1.5 µm) and had smooth surfaces.

Isolate S186^T was examined for a range of phenotypic markers, using standard procedures (Gordon & Mihm, 1962; Williams *et al.*, 1983). Gram and Ziehl–Neelsen's stains were applied to cells grown on modified Bennett's agar (Jones, 1949) for 7 days at 28 °C. The hanging drop method was used to test for motility. The results are given in Table 1 and in the species description. It is evident from Table 1 that strain S186^T can be distinguished from the type strains of its closest phylogenetic neighbours using a combination of phenotypic features.

It can be concluded from the genotypic and phenotypic data that isolate S186^T is phylogenetically distant from its neighbours and distinguishable from them on the basis of its phenotypic properties. Therefore S186^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis arabia* sp. nov. is proposed.

Description of *Nocardiopsis arabia* sp. nov.

Nocardiopsis arabia (a.ra'bi.a. L. fem. adj. *arabia* pertaining to Arabia, referring to the isolation of the type strain in Egypt).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete. Forms a branched substrate mycelium that fragments into coccoid and rod-shaped elements. Aerial hyphae differentiate into straight to flexuous chains of rod-shaped spores (0.7–1.5 µm) with smooth surfaces. Pale-yellow to deep greyish-yellow substrate mycelium that carries white aerial hyphae is formed on ISP media 1–5. Diffusible pigments are not produced. Grows between 10 and 40 °C (optimally around 28 °C). Hydrolyses aesculin but not arbutin. Degrades casein and hypoxanthine, but not adenine, guanine, tyrosine, uric acid or xanthine. D-Arabinose, *meso*-erythritol, D-salicin and trehalose (all at 1 %, w/v) are used as sole carbon sources for energy and growth, but L-arabitol, melezitose and xylitol (all at 0.1 %, w/v) are not. Similarly, sodium pyruvate and sodium succinate (all at 0.1 %, w/v) are used as sole carbon sources, but sodium malonate (at 0.1 %, w/v) is not. Growth occurs in the presence of NaCl at 0–15 %, w/v (optimally around 5 %). Additional phenotypic properties are shown in Table 1. Whole-organism hydrolysates contain *meso*-diaminopimelic acid and the sugars galactose and glucose. The major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, two unknown glycolipids and two unknown phospholipids with high *R_F* values (above that of diphosphatidylglycerol). The major menaquinones are MK-10(H₄) and MK-10(H₆); minor amounts of MK-11(H₆) and

MK-9(H₄) are present. The major fatty acids are iso-C_{16:0}, tuberculostearic acid (10-methyl C_{18:0}) and anteiso-C_{17:0}; iso-C_{17:0}, iso-C_{18:0} and 10-methyl C_{17:0} are present as minor fatty acids.

The type (and only) strain, S186^T (=CGMCC 4.2057^T =DSM 45083^T), was isolated from a sand-dune soil collected at Borg El-Arab, Egypt.

References

- Al-Tai, A. M. & Ruan, J. S. (1994). *Nocardiopsis halophila* sp. nov., a new halophilic actinomycete isolated from soil. *Int J Syst Bacteriol* **44**, 474–478.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Collins, M. D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265–309. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Cui, X.-L., Mao, P. H., Zeng, M., Li, W. J., Zhang, L. P., Xu, L. H. & Jiang, C.-L. (2001). *Streptomonospora salina* gen. nov., sp. nov., a new member of the family Nocardiopsaceae. *Int J Syst Evol Microbiol* **51**, 357–363.
- Evtushenko, L. I., Taran, V. V., Akimov, V. N., Kroppenstedt, R. M., Tiedje, J. M. & Stackebrandt, E. (2000). *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 73–81.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA. <http://evolution.gs.washington.edu/phylip.html>
- 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* **155**, 279–284.
- Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.
- Grund, E. & Kroppenstedt, R. M. (1990). Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5–11.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Hozzein, W. N., Li, W.-J., Ali, M. I. A., Hammouda, O., Mousa, A. S., Xu, L.-H. & Jiang, C.-L. (2004). *Nocardiopsis alkaliphila* sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt. *Int J Syst Evol Microbiol* **54**, 247–252.
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

- Kämpfer, P., Busse, H. & Rainey, F. A. (2002). *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. *Int J Syst Evol Microbiol* **52**, 621–627.
- Kawato, M. & Shinobu, R. (1959). On *Streptomyces herbaricolor* sp. nov., supplement: a simple technique for microscopical observation. *Mem Osaka Univ Lib Arts Educ B Nat Sci* **8**, 114–119.
- Kelly, K. L. (1958). Centroid notations for the revised ISCC-NBS color name blocks. *J Res Nat Bur Standards U S A* **61**, 427.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Kroppenstedt, R. M. (1992). The genus *Nocardiopsis*. In *The Prokaryotes*, 2nd edn, pp. 1139–1156. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Kroppenstedt, R. M. & Evtushenko, L. I. (2006). The family *Nocardiopsaceae*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 3, pp. 754–795. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Lechevalier, H. A. & Lechevalier, M. P. (1970). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: VEB Gustav Fischer.
- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Li, W.-J., Park, D.-J., Tang, S.-K., Wang, D., Lee, J.-C., Xu, L.-H., Kim, C.-J. & Jiang, C.-L. (2004). *Nocardiopsis salina* sp. nov., a novel halophilic actinomycete isolated from saline soil in China. *Int J Syst Evol Microbiol* **54**, 1805–1809.
- Li, W. J., Kroppenstedt, R. M., Wang, D., Tang, S. K., Lee, J. C., Park, D. J., Kim, C. J., Xu, L. H. & Jiang, C. L. (2006). Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis salina* Li et al. 2004. *Int J Syst Evol Microbiol* **56**, 1089–1096.
- Meyer, J. (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.
- 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 Environ Microbiol* **68**, 5005–5011.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaali, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Mordarska, H., Zakrzewska-Czerwinska, J., Pasciak, M., Szponar, M. & Rowinski, S. (1998). Rare suppurative pulmonary infection caused by *Nocardiopsis dassonvillei* recognized by glycolipid markers. *FEMS Immunol Med Microbiol* **21**, 47–55.
- National Bureau of Standards (1964). *ISCC-NBS Color Manual Charts Illustrated with Centroid Colors*. Supplement to NBS Circular 553. Washington, DC: US Government Printing Office.
- O'Donnell, A. G., Falconer, C., Goodfellow, M., Ward, A. C. & Williams, E. (1993). Biosystematics and diversity amongst novel carboxydophilic actinomycetes. *Antonie Van Leeuwenhoek* **64**, 325–340.
- Peltola, J. S. P., Andersson, M. A., Kämpfer, P., Auling, G., Kroppenstedt, R. M., Busse, H.-J., Salkinoja-Salonen, M. S. & Rainey, F. A. (2001). Isolation of toxigenic *Nocardiopsis* strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov. *Appl Environ Microbiol* **67**, 4293–4304.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Sabry, S. A., Ghanem, N. B., Abu-Ella, G. A., Schumann, P., Stackebrandt, E. & Kroppenstedt, R. M. (2004). *Nocardiopsis aegyptia* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **54**, 453–456.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schippers, A., Bosecker, K., Willscher, S., Spröer, C., Schumann, P. & Kroppenstedt, R. M. (2002). *Nocardiopsis metallicus* sp. nov., a metal-leaching actinomycete isolated from an alkaline slag dump. *Int J Syst Evol Microbiol* **52**, 2291–2295.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Sutcliffe, I. C. (2000). Characterisation of a lipomannan lipoglycan from the mycolic acid containing actinomycete *Dietzia maris*. *Antonie Van Leeuwenhoek* **78**, 195–201.
- Uchida, K., Kudo, T., Suzuki, K. & Nakase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- 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 Microbiol* **129**, 1743–1813.
- Yassin, A. F., Rainey, F. A., Burghardt, J., Gierth, D., Ungerechts, J., Lux, I., Seifert, P., Bal, C. & Schaali, K. P. (1997). Description of *Nocardiopsis synnemataformans* sp. nov., elevation of *Nocardiopsis alba* subsp. *prasina* to *Nocardiopsis prasina* comb. nov., and designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as later subjective synonyms of *Nocardiopsis dassonvillei*. *Int J Syst Bacteriol* **47**, 983–988.
- Zhou, Z.-H., Liu, Z.-H., Qian, Y.-D., Kim, S. B. & Goodfellow, M. (1998). *Saccharopolyspora spinosporotrichia* sp. nov., a novel actinomycete from soil. *Int J Syst Bacteriol* **48**, 53–58.