Nocardiopsis flavescens sp. nov., an actinomycete isolated from marine sediment

Caiyuan Fang,¹ Jianli Zhang,¹ Huancheng Pang,² Yuyi Li,² Yuhua Xin³ and Yabo Zhang¹

Correspondence

Jianli Zhang

zhangjianli@bit.edu.cn

¹School of Life Science, Beijing Institute of Technology, Beijing 100081, PR China

²Institute of Agri-resources and Regional Planning, CAAS, Beijing 100081, PR China

³China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

A Gram-positive, aerobic bacterium, strain SA6^T, was isolated from marine sediment taken at a depth of 20 cm on the seashore of Lianyungang, Jiangsu Province, China. Strain SA6^T contained *meso*-diaminopimelic acid, no diagnostic sugars, type PIII phospholipids, and MK-10(H₂) and MK-10(H₄) as the predominant menaquinones. The organism showed a range of chemical and morphological properties consistent with its classification in the genus *Nocardiopsis*. The almost-complete 16S rRNA gene sequence of strain SA6^T was aligned with corresponding sequences of representatives of the genus *Nocardiopsis* and related taxa by using two tree-making algorithms. Strain SA6^T formed a distinct phyletic line within the evolutionary radiation occupied by the genus *Nocardiopsis* and was related most closely to the type strain of *Nocardiopsis lucentensis*. Strain SA6^T could be distinguished from its nearest phylogenetic relatives in the genus *Nocardiopsis* based on DNA–DNA relatedness data and a combination of phenotypic properties. Strain SA6^T should therefore be assigned to the genus *Nocardiopsis* as a representative of a novel species, for which the name *Nocardiopsis flavescens* sp. nov. is proposed. The type strain is SA6^T (=CGMCC 4.5723^T =JCM 17424^T).

The genus *Nocardiopsis* was proposed by Meyer (1976) and, at the time of writing, comprises 33 recognized species. *Nocardiopsis* strains are distributed ubiquitously in the environment (Kämpfer *et al.*, 2002; Kroppenstedt & Evtushenko, 2002; Hozzein *et al.*, 2004). They are frequently isolated from habitats with moderate to high salt concentrations such as saline soil or marine sediments (Al-Zarban *et al.*, 2002; Al-Tai & Ruan, 1994; Evtushenko *et al.*, 2000) and salterns (Chun *et al.*, 2000). The genus *Nocardiopsis* comprises actinomycetes that possess *meso*-diaminopimelic acid as the cell-wall diamino acid, have no characteristic sugar in whole-cell hydrolysates and no mycolic acids in whole-cell methanolysates (cell-wall chemotype III), have phosphatidylcholine as a characteristic phospholipid (phospholipid type III), have fatty acid profiles which comprise saturated, unsaturated, iso-branched, anteiso-branched and tuberculostearic acids, contain menaquinones with 10 isoprene units with a high degree of hydrogenation as major components, and have DNA G + C contents of 64–71 mol% (Grund & Kroppenstedt, 1990). The aim of this

study was to classify a novel actinomycete (designated strain SA6^T) isolated from marine sediment by using morphological, physiological, chemotaxonomic and molecular biological methods.

Strain SA6^T was isolated on glucose-yeast extract-malt extract [GYM; International *Streptomyces* Project (ISP) 2 medium; Shirling & Gottlieb, 1966] agar plates, which had been seeded with a sea mud suspension and incubated at 35 °C for 2 weeks. The marine sediment was taken at a depth of 20 cm on the seashore of Lianyungang, Jiangsu Province, China. Strain SA6^T was maintained on GYM agar at 4 °C and as suspensions of mycelial fragments in 20% (v/v) glycerol at –20 °C. Biomass for chemotaxonomic and molecular systematic studies was prepared as described by Zhang *et al.* (2002).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product from isolate SA6^T were carried out as described by Rainey *et al.* (1996). The PCR product was sequenced directly following the procedure described by Lu *et al.* (2001). Sequence gel electrophoresis was performed and the nucleotide sequences were obtained automatically by using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. Identification of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SA6^T is GU997639.

Two supplementary tables are available with the online version of this paper.

phylogenetic neighbours and calculation of levels of pairwise 16S rRNA gene sequence similarity were achieved by using MEGA, version 4.1 (Tamura *et al.*, 2007). The resultant 16S rRNA gene sequence of strain SA6^T was aligned manually with corresponding sequences of representatives of genera classified in the suborder *Streptosporangineae* (Zhi *et al.*, 2009) retrieved from the DDBJ/EMBL/GenBank databases via CLUSTAL X 1.8 software (Thompson *et al.*, 1997). Evolutionary distances were calculated by using distance options according to Kimura's two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) algorithms in MEGA, version 4.1 (Tamura *et al.*, 2007). Topologies of the resultant unrooted trees were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining dataset, based on 1000 resamplings.

The almost-complete 16S rRNA gene sequence of strain SA6^T was determined (1401 nt). It is apparent from the neighbour-joining tree shown in Fig. 1 that strain SA6^T forms a monophyletic clade with the type strain of *Nocardiopsis lucentensis*. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain SA6^T were *N. lucentensis* DSM 44048^T (99.0 % 16S rRNA gene sequence similarity, Yassin *et al.*, 1993), *Nocardiopsis aegyptia* DSM 44442^T (98.6 %, Sabry *et al.*, 2004), *Nocardiopsis synnemataformans* IMMIB D-1215^T (98.6 %, Yassin *et al.*,

1997) and *Nocardiopsis dassonvillei* subsp. *albirubida* DSM 40465^T (98.6 %, Evtushenko *et al.*, 2000). These similarity values are lower than those found for some other individual pairs of species of the genus *Nocardiopsis*, for example between *Nocardiopsis exhalans* DSM 44407^T and *Nocardiopsis prasina* DSM 43845^T (99.4 %, Peltola *et al.*, 2001), between *Nocardiopsis metallicus* DSM 44598^T and *N. exhalans* DSM 44407^T (99.4 %), between *N. metallicus* DSM 44598^T and *N. prasina* DSM 43845^T (99.3 %, Schippers *et al.*, 2002), between *Nocardiopsis litoralis* DSM 45168^T and *Nocardiopsis kunsanensis* DSM 44524^T (99.6 %, Chen *et al.*, 2009), or between *Nocardiopsis valliformis* DSM 45023^T and *N. exhalans* DSM 44407^T (99.93 %, Yang *et al.*, 2008). It is also significant that the 16S rRNA gene sequence of strain SA6^T includes signature nucleotides that are characteristic of members of the genus *Nocardiopsis* (Meyer, 1994).

The new isolate was examined for a range of chemotaxonomic markers to determine whether its chemical profile was typical of members of the genus *Nocardiopsis* (Meyer, 1994). Standard procedures were used for the extraction and analysis of the diagnostic isomers of diaminopimelic acid (Hasegawa *et al.*, 1983) and whole-organism sugars (Lechevalier & Lechevalier, 1980), menaquinones (Collins *et al.*, 1987; Wu *et al.*, 1989), mycolic acids (Minnikin *et al.*, 1975) and polar lipids (Minnikin *et al.*, 1984), by using appropriate controls. Fatty acids were extracted, purified, methylated and quantified by GC with the standard

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain SA6^T and representatives of recognized species of the genus *Nocardiopsis*. The sequence of *Actinomadura madurae* DSM 43067^T was used as an outgroup. Asterisks at nodes indicate branches that were also obtained in the maximum-parsimony tree. Numbers at nodes indicate percentages of bootstrap support, based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0.01 substitutions per nucleotide position.

Microbial Identification System (TSBA40, MIDI version 6; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The base composition of the genomic DNA of the isolate was determined by using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as the control. The organism contained *meso*-diaminopimelic acid as the cell-wall diamino acid in the peptidoglycan and no diagnostic sugars in whole-cell hydrolysates (wall chemotype III/C *sensu* Lechevalier & Lechevalier, 1970). The predominant menaquinones were MK-10(H₂) and MK-10(H₄), with MK-10, MK-10(H₆), MK-9(H₂), MK-9(H₄) and MK-9(H₆) present as minor components. The phospholipid type was type PIII *sensu* Lechevalier *et al.* (1977), and phosphatidylcholine was the characteristic phospholipid. Other phospholipids, such as phosphatidylmethylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, were also detected. This pattern matched quite well with those found in recognized species of the genus *Nocardiosis* (Kroppenstedt & Evtushenko, 2002). The fatty acid pattern of strain SA6^T was mainly composed of iso- and anteiso-branched components together with

10-methyl branched-chain fatty acids with 17 and 18 carbon atoms, straight-chain saturated (C_{16:0}, C_{17:0}, C_{18:0}) and unsaturated (C_{17:1}, C_{18:1}) components (see Supplementary Table S1 available in IJSEM Online); no hydroxy fatty acids were detected. Strain SA6^T lacked mycolic acids. The G + C content of the DNA of strain SA6^T was 68.6 mol%. These properties were consistent with the classification of the isolate in the genus *Nocardiosis* (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992).

The organism was grown on GYM agar, trypticase soy agar, modified Sauton's agar (Mordarska *et al.*, 1972) and standard ISP media (Shirling & Gottlieb, 1966) for 3–14 days at 35 °C, and was examined for pigmentation, aerial mycelium and other morphological features. The colours of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with those of Kelly (1964). Growth over various temperature, pH and NaCl ranges was determined on GYM agar plates incubated for up to 14 days. Additional physiological and biochemical properties were determined by using standard media and

Table 1. Differential phenotypic characteristics between strain SA6^T and the type strains of closely related species of the genus *Nocardiosis*

Strains: 1, SA6^T; 2, *N. lucentensis* DSM 44048^T; 3, *N. aegyptia* DSM 44442^T; 4, *N. synnemataformans* IMMIB D-1215^T; 5, *N. dassonvillei* subsp. *albirubida* DSM 40465^T; 6, *N. dassonvillei* subsp. *dassonvillei* DSM 43111^T; 7, *N. prasina* DSM 43845^T; 8, *N. listeri* DSM 40297^T; 9, *N. alba* DSM 43377^T; 10, *N. alkaliphila* DSM 44657^T. Data were taken from this study. w, Weak; v, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colour of aerial mycelium	White	White	White/yellowish grey	White	White	Greyish yellow	White	—	Yellow or colourless	White/yellowish white
Utilization of:										
D-Fructose	+	+	ND	+	—	+	—	—	—	—
D-Galactose	+	—	+	+	+	+	—	+	—	w
Glycerol	+	+	+	ND	+	+	+	—	+	ND
myo-Inositol	+	+	+	—	—	—	—	w	—	w
Lactose	+	—	+	—	—	—	—	+	—	w
D-Mannitol	+	+	+	+	+	+	+	—	+	—
D-Mannose	+	+	+	+	+	—	—	—	—	—
Melibiose	+	—	+	—	—	—	—	—	—	ND
α-L-Rhamnose	+	+	—	+	+	+	—	+	—	w
D-Ribose	+	—	—	—	+	+	+	+	+	—
Sucrose	+	+	+	—	—	+	+	—	+	+
D-Xylose	+	—	—	+	+	+	+	+	—	+
Acetate	+	—	ND	—	—	—	+	—	+	—
Growth at/with:										
10 °C	—	+	+	—	—	v	—	+	—	+
42 °C	—	—	—	—	+	+	+	—	+	+
5 % NaCl	+	+	+	+	+	+	+	+	—	+
10 % NaCl	+	+	—	+	+	+	+	—	—	+
Major menaquinone(s)*	10/2, 10/4	10/6, 10/8	10/6, 10/8	10/0, 10/2	10/0	10/4, 10/6	10/4, 10/6	10/0, 10/2	10/4, 10/6	10/6, 10/8
Urease activity	—	—	ND	+	—	—	—	—	+	ND
Nitrate reductase	+	+	ND	+	+	—	—	+	—	+

*For example, 10/4=MK-10(H₄).

methods (Shirling & Gottlieb, 1966; Gordon *et al.*, 1974; Kroppenstedt & Evtushenko, 2006; Zhang *et al.*, 2003). The results are given in the species description below and in Table 1 and Supplementary Table S2. DNA–DNA relatedness studies were carried out by using thermal denaturation (De Ley *et al.*, 1970; Huß *et al.*, 1983) with a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermbath and standard software (Jahnke, 1992); the results were expressed as the mean of two determinations $\pm$ SD.

Strain SA6^T exhibited phenotypic properties typical of members of the genus *Nocardiopsis* (Meyer, 1994). It was an aerobic, Gram-positive, non-motile actinomycete and formed long, well-developed and branched substrate mycelium which fragmented into non-motile elements. Zigzag-shaped aerial mycelium was observed at the beginning of sporulation. The aerial mycelium subsequently subdivided into spores of varying lengths, and elongated spores were observed. These morphological characteristics are consistent with those described for recognized species of the genus *Nocardiopsis* (Kroppenstedt, 1992). Mean levels of DNA–DNA relatedness between strain SA6^T and the type strains of *N. lucentensis*, *N. aegyptia*, *N. synnemataformans* and *N. dassonvillei* subsp. *albirubida* were 13.1 ± 1.9 , 13.0 ± 2.1 , 12.6 ± 1.9 and 11.8 ± 1.7 %, respectively, values well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for the delineation of genomic species. The new isolate could also be distinguished from recognized species of the genus *Nocardiopsis* by using a combination of phenotypic properties (Table 1). In particular, it could be differentiated from its closest phylogenetic neighbour, *N. lucentensis* DSM 44048^T, in that it was positive for utilization of D-galactose, lactose, melibiose, D-ribose, D-xylose and L-lactate as sole carbon and energy source, and was unable to grow at 10 °C.

It is evident from the genotypic and phenotypic data that strain SA6^T represents a novel species of the genus *Nocardiopsis*, for which we propose the name *Nocardiopsis flavescens* sp. nov.

Description of *Nocardiopsis flavescens* sp. nov.

Nocardiopsis flavescens [fla'ves.cens. L. part. adj. *flavescens* (from L. v. *flavesco*), becoming golden yellow in colour].

Aerobic, Gram-positive, non-motile actinomycete. A pale yellow to yellowish brown substrate mycelium is formed on GYM agar, ISP media 3, 4 and 5, trypticase soy agar and modified Sauton's agar. The aerial mycelium is white on most media tested. Soluble pigments are not produced. Colony elevation is convex to irregular and colony margins are filamentous. Substrate hyphae are long, well developed and fragment with age. Aerial hyphae are long, and at the beginning of sporulation are more or less zigzag-shaped. The zigzag-shaped hyphae subdivide into smaller spores. Spores are rod-shaped, smooth and non-motile. Optimum growth occurs at 35 °C, at pH 7.2–7.5 and in the presence of 0–3 % (w/v) NaCl. Grows at 23–40 °C, at pH 5.5–11.0 and in the presence of 0–10 % (w/v) NaCl. Hydrolyses

aesculin, arbutin and gelatin, but not urea. Nitrate is reduced. Degrades casein, elastin, starch and L-tyrosine. Positive for catalase, phosphatase, β -galactosidase and β -glucosidase. Acid is formed from D-glucose, lactose (weakly), *myo*-inositol, D-mannitol, α -L-rhamnose, D-sorbitol (weakly) and D-xylose, but not from adonitol, dulcitol, *meso*-erythritol or methyl α -D-glucoside. L-Arabinose (weakly), D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, α -L-rhamnose, D-ribose, sucrose, trehalose, D-xylose, acetate, citrate, L-lactate, L-malate, propionate and succinate are used as sole carbon and energy sources, whereas raffinose and oxalate are not. Resistant to lincomycin (128 μ g ml⁻¹), gentamicin (64 μ g ml⁻¹), penicillin G (128 μ g ml⁻¹), streptomycin (64 μ g ml⁻¹) and neomycin (4 μ g ml⁻¹) but not to rifampicin (128 μ g ml⁻¹). Chemotaxonomic properties are typical of the genus *Nocardiopsis*. Whole-cell hydrolysates contain *meso*-diaminopimelic acid but no diagnostic sugars. The predominant menaquinones are MK-10(H₂) and MK-10(H₄). Polar lipids comprise phosphatidylcholine, phosphatidylmethylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are anteiso-C_{17:0}, iso-C_{16:0} and C_{18:0}; C_{18:1} ω 9c, iso-C_{18:0}, anteiso-C_{15:0}, C_{17:0}, C_{16:0}, 10-methyl C_{18:0}, C_{18:1} ω 7c, C_{14:0}, iso-C_{17:0}, 10-methyl C_{17:0}, anteiso-C_{19:0} and C_{17:1} ω 8c are present as minor components. Mycolic acids are absent. The DNA G + C content of the type strain is 68.6 mol%.

The type strain, SA6^T (=CGMCC 4.5723^T =JCM 17424^T), was isolated from marine sediment at a depth of 20 cm on the seashore of Lianyungang, Jiangsu Province, China. The species description is based on a single strain.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NSFC, grant numbers 30970009, 31070002), by the Special Fund for Public Welfare Industrial (Agriculture) Research of China (grant number 200903001) and by the Basic Research Foundation of Beijing Institute of Technology (grant number 20091642006). We are grateful to Dr T. Kudo (JCM) for the type strains of the reference species of the genus *Nocardiopsis*, and to Dr Yafang Tan for carrying out the quantitative fatty acid analysis on isolate SA6^T.

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.
- Al-Zarban, S. S., Abbas, I., Al-Musallam, A. A., Steiner, U., Stackebrandt, E. & Kroppenstedt, R. M. (2002). *Nocardiopsis halotolerans* sp. nov., isolated from salt marsh soil in Kuwait. *Int J Syst Evol Microbiol* **52**, 525–529.
- Chen, Y.-G., Wang, Y.-X., Zhang, Y.-Q., Tang, S.-K., Liu, Z.-X., Xiao, H.-D., Xu, L.-H., Cui, X.-L. & Li, W.-J. (2009). *Nocardiopsis litoralis* sp. nov., a halophilic marine actinomycete isolated from a sea anemone. *Int J Syst Evol Microbiol* **59**, 2708–2713.
- Chun, J., Bae, K. S., Moon, E. Y., Jung, S.-O., Lee, H. K. & Kim, S.-J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic

- actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50**, 1909–1913.
- Collins, M. D., Howarth, O. W., Grund, E. & Kroppenstedt, R. M. (1987). Isolation and structural determination of new members of the vitamin K₂ series in *Nocardia brasiliensis*. *FEMS Microbiol Lett* **41**, 35–39.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- 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. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- 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.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Busse, H.-J. & Rainey, F. A. (2002). *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. *Int J Syst Evol Microbiol* **52**, 621–627.
- Kelly, K. L. (1964). *Inter-Society Color Council–National Bureau of Standards Color-Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kluge, A. G. & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- 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. (2002). The family *Nocardiopsaceae*. In *The Prokaryotes: an Evolving Electronic Database for the Microbial Community*, 3rd edn (release 3.11). Edited by M. Dworkin, K.-H. Schleifer & E. Stackebrandt. 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, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy* (Special Publication no. 6), pp. 227–291. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society for Industrial Microbiology.
- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Lu, Z., Liu, Z., Wang, L., Zhang, Y., Qi, W. & Goodfellow, M. (2001). *Saccharopolyspora flava* sp. nov. and *Saccharopolyspora thermophila* sp. nov., novel actinomycetes from soil. *Int J Syst Evol Microbiol* **51**, 319–325.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Meyer, J. (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.
- Meyer, J. (1994). The genus *Nocardiopsis* Meyer 1976. In *Bergey's Manual of Determinative Bacteriology*, 9th edn, pp. 2562–2568. Edited by J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley & S. T. Williams. Baltimore: Williams & Wilkins.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolytates. *J Gen Microbiol* **88**, 200–204.
- 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.
- Mordarska, H., Mordarski, M. & Goodfellow, M. (1972). Chemotaxonomic characters and classification of some nocardioform bacteria. *J Gen Microbiol* **71**, 77–86.
- 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.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, Technical Note 101. Newark, DE: MIDI Inc.
- 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.

- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- 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.
- Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* [English translation of *Microbiology (Beijing)*] **16**, 176–178.
- Yang, R., Zhang, L. P., Guo, L. G., Shi, N., Lu, Z. & Zhang, X. (2008). *Nocardiopsis valliformis* sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. *Int J Syst Evol Microbiol* **58**, 1542–1546.
- Yassin, A. F., Galinski, E. A., Wohlfarth, A., Jahnke, K.-D., Schaal, K. P. & Trüper, H. G. (1993). A new actinomycete species, *Nocardiopsis lucentensis* sp. nov. *Int J Syst Bacteriol* **43**, 266–271.
- Yassin, A. F., Rainey, F. A., Burghardt, J., Gierth, D., Ungerechts, J., Lux, I., Seifert, P., Bal, C. & Schaal, 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.
- Zhang, J., Zhang, Y., Xiao, C., Liu, Z. & Goodfellow, M. (2002). *Rhodococcus maanshanensis* sp. nov., a novel actinomycete from soil. *Int J Syst Evol Microbiol* **52**, 2121–2126.
- Zhang, J., Liu, Z. & Goodfellow, M. (2003). *Nocardia caishijiensis* sp. nov., a novel soil actinomycete. *Int J Syst Evol Microbiol* **53**, 999–1004.
- Zhi, X.-Y., Li, W.-J. & Stackebrandt, E. (2009). An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* **59**, 589–608.