Nocardiopsis sinuspersici sp. nov., isolated from sandy rhizospheric soil

Javad Hamed, ¹ Fatemeh Mohammadipanah, ¹ Mathias von Jan, ² Gabriele Pötter, ² Peter Schumann, ² Cathrin Spröer, ² Hans-Peter Klenk ² and Reiner M. Kroppenstedt ²

Correspondence
Javad Hamed
jhamed@ut.ac.ir

¹Microbial Biotechnology Laboratory, Department of Microbiology, School of Biology, College of Science, University of Tehran, 14155-6455, Tehran, Iran

²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Inhoffenstraße 7b, 38124 Braunschweig, Germany

A polyphasic taxonomic study of a halotolerant bacterium, isolated from sandy rhizospheric soil in Sarbandar, Persian Gulf, Iran, revealed that strain HM6^T represents a novel species within the genus *Nocardiopsis*. Results of the 16S rRNA gene sequence comparison revealed that strain HM6^T clustered with strains of the genus *Nocardiopsis*, showing the highest degree of 16S rRNA gene sequence similarity to *Nocardiopsis quinghaiensis* (99.2 %), *Nocardiopsis aegyptia* (98.5 %) and *Nocardiopsis halotolerans* (98.3 %). However, DNA–DNA hybridization studies with these type strains revealed less than 39.6 % similarity. Rather than genotypic differences, there are some phenotypic discrepancies between strain HM6^T and closely related species of the genus *Nocardiopsis*. Main morphological and chemotaxonomical features of strain HM6^T include: (i) growth characteristics, i.e. the formation of a scant light-yellow to white aerial mycelium and the typical zig-zag form of the hyphae, which fragment during ageing into smooth rod-shaped spores; (ii) the presence of meso-diaminopimelic acid and glucose plus ribose in whole-cell hydrolysates; (iii) the presence of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol, together with three unknown *Nocardiopsis*-specific phospholipids (close to diphosphatidylglycerol in position) in polar lipid extracts; (iv) the presence of the major menaquinones MK-10(H₀), MK-10(H₂) and MK-9(H₀) in the non-polar fraction; (v) the presence of iso/anteiso-branched plus 10-methyl-branched fatty acids, showing the diagnostic combination for species of the genus *Nocardiopsis* of iso-16 : 0 (31.1 %), anteiso-17 : 0 (19.2 %), 10-methyl-17 : 0 (5.8 %) and tuberculostearic acid (8.8 %); and (vi) the absence of mycolic acids. Analysis of the 16S rRNA gene sequence revealed that strain HM6^T represents a distinct taxon within the genus *Nocardiopsis*. Based upon genotypic and phenotypic differences from other members of the genus, a novel species, *Nocardiopsis sinuspersici* sp. nov., is proposed. The type strain is HM6^T (=UTMC 00102^T =DSM 45277^T =CCUG 57624^T).

Originally, members of the genus *Nocardiopsis* were isolated from mildewed grain (Brocq-Rousseau, 1904), but the natural habitat of these species is soil (Meyer, 1976, 1989; Mishra *et al.*, 1987a, b). Numerous studies have shown that *Nocardiopsis* strains are ubiquitously distributed in the environment (Kroppenstedt & Evtushenko, 2006). At the time of writing, there were 28 species of the genus *Nocardiopsis* with validly published names. Many of these species prefer moderately alkaline conditions (pH 8.5) (Kroppenstedt, 1992) and some grow better on media supplemented with sodium chloride. *Nocardiopsis*

strains are frequently isolated from alkaline soils with high salt concentrations (Mikami *et al.*, 1982; Al-Tai & Ruan, 1994; Yassin *et al.*, 1993), but they have also been isolated from clinical material (Yassin *et al.*, 1997).

The aim of this study was to classify strain HM6^T, a strain isolated from rhizospheric soil, by morphological, physiological, chemotaxonomic and molecular biological methods, and it is proposed that it represents a novel species of the genus *Nocardiopsis*.

Strain HM6^T was isolated from rhizospheric soil (pH 7.7 and salinity 7.5 %) taken at a depth of 10 cm on the seashore of Sarbandar, Persian Gulf, southern Iran. The samples were air-dried (Nolan & Cross, 1988), followed by heat-drying at

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120 °C for 10 min (Nonomura & Ohara, 1971a) and irradiating at 257 nm for 5 min (Galatenko & Trekhova, 1990). The dried and irradiated samples were then treated with phenol (1.5 %) using the method of Hayakawa *et al.* (1991). Enrichment of the micro-organisms was carried out by using chitin as described by Williams *et al.* (1972). Isolation was carried out by the dilution plating method on GAC agar (Nonomura & Ohara 1971b) supplemented with cycloheximide (100 µg ml⁻¹) after 3 weeks of incubation at 28 °C. Strains for comparison of physiological characteristics with HM6^T were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. These strains were maintained on ISP2 agar slants at 4 °C and as 20 % (w/v) glycerol suspensions at -20 °C.

Determination of morphological traits and colours of the aerial and substrate mycelium, as well as of soluble pigments, was performed as described by Shirling & Gottlieb (1966). Mycelium colour was determined by comparing the culture with chips from ISCC–NBS colour charts (Kelly, 1964). The intact arrangement of hyphae, notably aerial hyphae and spore chains, was observed on ISP2 agar after 14 days at 28 °C by using the coverslip technique (Kawato & Shinobu, 1959). Micromorphological characteristics of strain HM6^T were observed by using scanning electron microscopy (CEM902A, Zeiss).

On ISP2 medium, a sparse spore mass on a light yellow background was observed (Table 1). At the end of the hyphae, a zig-zag formation typical of members of the genus *Nocardiopsis* was observed. In older cultures, hyphae of aerial mycelium disintegrated into spore-like structures. During this process, the colour of colonies changed to dirty yellow. No pigments were released into any of the media tested. Vegetative hyphae were long, well-developed and fragmented during ageing. Long spore-chains were borne on the aerial hyphae. Spores (0.4 × 1.5 µm) were rod-shaped and smooth-surfaced (Fig. 1). Strain HM6^T showed the typical macroscopic and microscopic appearance of most species of the genus *Nocardiopsis* (Meyer, 1976, 1989).

Assimilation of carbon sources at a final concentration of 1 % (w/v) was tested by using ISP 9 as the basal medium (Shirling & Gottlieb, 1966). Utilization of nitrogen sources, decomposition of other organic compounds, degradation activity and enzymic activity were determined as described by Williams *et al.* (1983).

The strain was able to grow at 15–37 °C but not at 10 or 40 °C. Optimum growth temperature was 28 °C. Good growth could be obtained with 0 to 15 % NaCl but not in the presence of 17.5 % NaCl. Optimum salt concentration for growth was 2.5 %. Strain HM6^T could grow well from pH 5 to pH 12 but not at pH 4 or 13. Optimum pH for growth was 7.

Biomass for chemical and molecular systematic studies was obtained by cultivation for 6 days in shake flasks (200 r.p.m.) using GYM broth (Shirling & Gottlieb, 1966), pH 7.2 at 28 °C. Cells were harvested by centrifugation and washed twice with deionized water. Analyses of amino acids and sugars were carried out by using the methods of Stanek & Roberts (1974). Polar lipids and menaquinones were obtained from freeze-dried cells by using the integrated extraction method of Minnikin *et al.* (1984). Polar lipids were separated by TLC (Minnikin *et al.*, 1984) and menaquinones were analysed by HPLC (Kroppenstedt, 1982, 1985). Fatty acids were converted into their corresponding methyl esters, extracted and analysed by gas-liquid chromatography according to the method of Sasser (1990).

Whole-cell hydrolysates of strain HM6^T contained meso-diaminopimelic acid and non-diagnostic sugars, including glucose and ribose. The diagnostic sugars arabinose, xylose and madurose were not detected. Therefore HM6^T is classified in cell-wall type III (Lechevalier & Lechevalier, 1980). Strain HM6^T contained menaquinones in the following proportions: 48 % MK-10(H0), 22 % MK-10(H2), 18 % MK-9(H0), 7 % MK-9(H2) and 6 % MK-10(H4). Analysis of the polar lipid extracts by TLC showed the diagnostic phospholipids phosphatidylcholine and phosphatidylethanolamine (phospholipid pattern type III, according to Lechevalier *et al.*, 1977). Three phospholipid spots close to diphosphatidylglycerol, which is diagnostic for members of the genus *Nocardiopsis*, could be detected also, together with the non-diagnostic phosphatidylinositol, phosphatidylglycerol and some glycolipids of unknown structure (Kroppenstedt, 1992).

Phospholipid pattern matched quite well with those found in species of the genus *Nocardiopsis* (Kroppenstedt & Evtushenko, 2006). The fatty acid pattern revealed mainly iso/anteiso-branched and 10-methyl-branched fatty acids. The principal fatty acids were iso-16:0 (31.1 %), anteiso-

Table 1. Cultural characteristics of strain HM6^T

ISP, International *Streptomyces* Project (Shirling & Gottlieb, 1966). Colours were taken from ISCC–NBS colour charts (Kelly, 1964).

Medium	Growth	Aerial mycelium	Substrate mycelium
Yeast extract/malt extract agar (ISP 2)	Good	White/ light yellow	Yellow
Oatmeal agar (ISP 3)	Poor	Pale yellow	Pale yellow
Inorganic salts/starch agar (ISP 4)	Poor	White	Light yellow
Glycerol/asparagine agar (ISP 5)	Very poor	Pale yellow	Pale yellow

Fig. 1. Scanning electron micrograph of strain HM6^T after growth on ISP2 medium for 14 days at 28 °C. Bar, 2 µm.

17:0 (19.2%), 10-methyl-18:0 (8.8%), and 10-methyl-17:0 (5.8%). The qualitative and quantitative composition of the fatty acid pattern is diagnostic for members of the genus *Nocardioopsis* (Kroppenstedt, 1985, 1992). All chemotaxonomic properties of strain HM6^T were consistent with its classification in the genus *Nocardioopsis* (Kroppenstedt & Evtushenko, 2006). The DNA G+C base content of strain HM6^T was 71.6 mol% by HPLC (Mesbah *et al.* 1989, Tamaoka & Komagata 1984).

Genomic DNA was extracted by using a DNA extraction kit (JetFlex). PCR-mediated amplification of the 16S rRNA gene was performed by using the primers 10-30F (5'-GAGTTTGATCCTGGCTCA-3') and 1500R (5'-AGAA-AGGAGGTGATCCAGCC-3') as described by Rainey *et al.* 1996. Purification of PCR products was carried out by using a DNA purification kit (Qiagen).

Phylogenies were inferred by using the maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) clustering algorithms from the PHYLIP software package (Felsenstein, 1993). The resultant tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1993) based on 1000 resamplings, using both the neighbour-joining and maximum-parsimony algorithms of the PHYLIP package (Felsenstein, 1993). The root position of the neighbour-joining tree was deduced by using *Nocardioopsis trehalosi* VKM Ac-942 as the outgroup.

Strain HM6^T showed the highest degree of 16S rRNA gene sequence similarity to members of the genus *Nocardioopsis*, especially to *Nocardioopsis quinghaiensis* (Chen *et al.*, 2008) (99.2%), *Nocardioopsis aegyptia* (Sabry *et al.*, 2004) (98.5%) and *Nocardioopsis halotolerans* (Al-Zarban *et al.*, 2002) (98.2%). Fig. 2 shows a phylogenetic dendrogram displaying the relatedness of strain HM6^T to strains of the genus *Nocardioopsis* with the highest degree of sequence similarity (>97.4%, according to Eztaxon), ignoring the less closely related type strains *Nocardioopsis valliformis* HBUM 20028^T, *N. alkaliphila* DSM 44657^T, *N. listeri* DSM 40297^T, *N. composta* KS9^T, *N. halophila* KCTC 9825^T, *N. baichengensis* YIM 90130^T, *N. rosea* YIM 90094^T, *N. gilva* YIM 90087^T, *N. arabia* S186^T, *N. chromatogenes* YIM 90109^T and *N. rhodophaea* YIM 90096^T.

Strain HM6^T can be clearly distinguished from described species of the genus *Nocardioopsis* by the primary structure of the 16S rRNA genes. At this level of 16S rRNA gene relatedness, strain HM6^T and *N. aegyptia* and *N. halotolerans* belong to different DNA–DNA reassociation groups and should not be combined in a single species

Fig. 2. Phylogenetic dendrogram based on 16S rRNA gene sequence analysis, reconstructed from evolutionary distances by using the neighbour-joining method, showing the phylogenetic position of strain HM6^T and the most closely related species. Bootstrap values (>50%; value above branch, neighbour-joining; value below branch, maximum-parsimony) are indicated at the relevant branching points. Bar, 0.01 substitutions per nucleotide position.

(Stackebrandt & Goebel, 1994; Stackebrandt & Ebers, 2006). DNA–DNA hybridization analyses were performed between strain HM6^T and the three type strains showing the highest 16S rRNA gene sequence similarity to HM6^T.

DNA was isolated by using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian).

Analysis of strain HM6^T revealed only low DNA–DNA reassociation of 39.6 % with *N. quinghaiensis* DSM 44739^T, 30.7 % with *N. halotolerans* DSM 44410^T and 31.8 % with *N. aegyptia* DSM 44442^T; values were far below the 70 % suggested for strains of the same species (Wayne *et al.*, 1987). Strain HM6^T differs from the type strains of *N. quinghaiensis*, *N. aegyptia* and *N. halotolerans* not only in its genotype but also in the phenotype (Table 2). Strain HM6^T can be differentiated from *N. quinghaiensis* DSM 44739^T by physiological and chemotaxonomic characteristics (Table 2). Strain HM6^T can also be distinguished from *N. quinghaiensis* DSM 44739^T by utilization of D-galactose, melibiose, lactose, L-rhamnose, D-xylose, propionate, L-arabinose, inositol and allantoin. More impor-

Table 2. Diagnostic characteristics of strain HM6^T and species of the genus *Nocardiopsis* with closely related 16S rRNA gene sequences

Strains: 1, HM6^T; 2, *N. quinghaiensis* DSM44739^T; 3, *N. aegyptia* DSM 44442^T; 4, *N. halotolerans* DSM 44410^T; 5, *N. lucentensis* DSM 44048^T (data for strains 1–5 from this study); 6, *N. alba* DSM 43377^T (data from Yassin *et al.*, 1997); 7, *N. dassonvillei* subsp. *dassonvillei* DSM 43111^T (Evtushenko *et al.*, 2000; Kroppenstedt & Evtushenko, 2006); 8, *N. umidischolae* DSM 44362^T (Peltola *et al.*, 2001); 9, *N. dassonvillei* subsp. *albirubida* DSM 40465^T (Evtushenko *et al.*, 2000). +, Positive; –, negative; v, variable; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9
Aerial mycelium	White/ light yellow	White/ yellowish	White/ yellowish	White/ yellow	White	White	Greyish-yellow	White	White
Utilization of:									
D-Galactose	+	–	+	+	–	–	+	+	+
L-Arabinose	–	+	+	–	–	–	+	+	+
Melibiose	+	–	+	+	–	–	–	+	–
Adonitol	–	–	ND	–	–	–	–	–	–
Glycerol	+	+	+	+	+	+	+	ND	+
Inositol	–	+	+	–	+	–	–	–	–
Sucrose	+	+	+	+	+	+	+	+	–
Ribose	–	–	–	–	–	ND	+	+	ND
Lactose	+	–	+	–	–	–	–	+	–
Mannitol	+	+	+	–	+	+	+	+	+
D-Mannose	+	+	+	+	+	–	–	+	+
L-Rhamnose	+	–	+	–	+	–	+	+	+
D-Xylose	+	–	+	–	–	–	+	+	+
Raffinose	–	–	–	–	+	–	–	ND	–
Adenine	+	+	–	–	+	+	+	ND	+
Gluconate	–	–	–	–	+	+	+	+	+
Propionate	+	–	+	–	+	+	–	+	–
Allantoin	–	+	+	–	–	ND	ND	ND	ND
Tyrosine	+	+	+	+	+	+	+	ND	+
Valine	+	+	+	+	–	ND	ND	ND	ND
Growth at:									
10 °C	–	+	+	+	+	–	v	+	–
40 °C	–	–	–	–	–	–	–	ND	+
5 % NaCl	+	+	–	+	+	–	+	+	+
10 % NaCl	+	+	–	+	+	–	+	–	+
15 % NaCl	+	–	–	+	–	–	–	–	ND
pH 12	+	–	+	–	–	ND	ND	ND	ND
Major menaquinones*	10, 10/2, 9, 9/2, 10/4	10, 10/2, 11/2, 11, 9/4	10/6, 10/8	10/0, 10/2	10/6, 10/8	10/4, 10/6	10/4, 10/6	10/6, 10/4, 10/8, 11/8	10/0

*Menaquinones abbreviated e.g. 10/4, MK-10(H4).

tantly, strain HM6^T shows differences in growth at 10 °C, 15% NaCl and pH 12 compared with *N. quinghaiensis* DSM 44739^T, *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T. Also, from a chemotaxonomical point of view, it has differences in whole-cell sugars, phospholipid pattern, major menaquinones and predominant fatty acids (Table 3) compared with *N. quinghaiensis* DSM 44739^T, *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T. Therefore, we consider strain HM6^T to represent a novel species of the genus *Nocardiopsis*, for which we propose the name *Nocardiopsis sinuspersici* sp. nov.

Description of *Nocardiopsis sinuspersici* sp. nov.

Nocardiopsis sinuspersici (si.nus.per'si.ci. L. masc. n. *sinus persicus* the Persian Gulf, *sinuspersici* L. gen. n. from the Persian Gulf, referring to where the type strain was isolated).

Aerobic, Gram-stain-positive actinomycete. Grows well on yeast extract/malt extract agar but only scant growth appears on oatmeal agar and inorganic salts/starch agar. Colour of the spore mass changes from white to dirty-yellow during ageing. No soluble pigments are produced. At the end of the straight substrate and aerial hyphae, a zig-zag formation typical to the genus *Nocardiopsis* is observed. These structures disintegrate into spore-like elements during ageing. Hydrolyses casein and starch but not

adenine, lecithin and pectin. Nitrate is reduced to nitrite. H₂S, melanin and urease are not produced. D-Galactose, lactose, melibiose, glycerol, sucrose, maltose, mannitol, D-mannose, L-rhamnose, D-xylose, inulin, citrate, malonate, pyruvate and propionate are used as sole carbon sources, whereas L-arabinose, fructose, adonitol, inositol, ribose, raffinose, gluconate, sorbitol and allantoin cannot be used. L-Alanine, L-asparagine, L-histidine, L-proline, L-arginine, L-serine, L-valine, L-phenylalanine, L-lysine and L-tyrosine can be utilized as sole nitrogen sources, whereas L-cysteine, L-ornithine and L-tryptophan cannot. Grows at 15–37 °C (optimum 28 °C), pH 5–12 (optimum pH 7) and 0–15% NaCl (optimum 2.5%). The fatty acid profile includes iso-16:0, anteiso-17:0, 10-methyl-18:0, anteiso-15:0, 18:0, 10-methyl-17:0, iso-18:0, cis-9 18:1, iso-14:0, iso-17:0, 17:0 and cis-9 17:1. Whole-cell hydrolysates contain meso-diaminopimelic acid and the non-diagnostic sugars glucose and ribose. The principal menaquinones are MK-10(H₀), MK-10(H₂) and MK-9(H₀); however, MK-9(H₂) and MK-10(H₄) are also present. The DNA G + C content of the type strain is 71.6 mol%. Polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol together with three unknown phospholipids located above diphosphatidylglycerol, which is diagnostic for members of the genus *Nocardiopsis*. Mycolic acids are absent.

The type strain is HM6^T (=UTMC 00102^T =DSM 45277^T =CCUG 57624^T) isolated from rhizospheric soil taken at a depth of 10 cm on the seashore of the Persian Gulf, Sarbandar, southern Iran.

Table 3. Cellular fatty acid composition (%) of strain HM6^T and type strains of closely related species

Strains: 1, HM6^T; 2, *Nocardiopsis quinghaiensis* DSM 44739^T (data from Chen *et al.*, 2008); 3, *Nocardiopsis aegyptia* DSM 44442^T (Sabry *et al.*, 2004); 4, *Nocardiopsis halotolerans* DSM 44410^T (Al-Zarban *et al.*, 2002); 5, *Nocardiopsis lucentensis* DSM 44048^T (Yassin *et al.*, 1993). –, Not present.

Fatty acids	1	2	3	4	5
iso-14:0	2.5	5.6	12.1	2.4	1.0
iso-15:0	0.7	2.6	1.7	3.4	5.2
anteiso-15:0	6.7	14.6	6.5	16.7	12.6
15:0	—	—	—	0.7	1.2
iso-16:0	31.1	30.0	19.1	23.3	18.7
16:0	0.6	3.7	4.2	3.2	3.8
10-Methyl-16:0	0.7	1.8	1.5	—	1.2
16:1	0.3	—	1.1	1.3	1.5
iso-16:1	—	7.5	—	—	—
iso-17:0	2.1	2.2	5.1	4.2	30.5
anteiso-17:0	19.2	10.0	17.9	18.7	14.5
17:0	2.0	2.8	1.3	3.1	3.3
17:1	1.6	2.7	1.7	6.8	2.6
anteiso-17:1	0.4	—	1.4	—	—
10-Methyl-17:0	5.8	2.8	2.6	6.6	2.8
iso-18:0	4.2	1.4	3.2	1.3	1.4
18:0	6.0	4.5	7.3	3.5	2.5
18:1	3.3	4.6	7.8	8.5	1.7
10-Methyl-18:0	8.8	4.5	12.2	1.3	1.5

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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.
- Brocq-Rousseau, D. (1904). Sur un *Streptothrix*. *Ref Gen Botanique* **16**, 219–230.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chen, Y.-G., Cui, X.-L., Kroppenstedt, R. M., Stackebrandt, E., Wen, M.-L., Xu, L.-H. & Jiang, C.-L. (2008). *Nocardiopsis quinghaiensis* sp. nov., isolated from saline soil in China. *Int J Syst Evol Microbiol* **58**, 699–705.
- 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. (1993). PHYLIP (phylogeny inference package) version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1972). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Galatenko, O. A. & Trekhova, L. P. (1990). Isolation of antibiotic producing actinomycetes from soil samples exposed to UV light. *Antibiot Khimioter* **35**, 6–8. (in Russian)
- Hayakawa, M., Kajiura, T. & Nonomura, H. (1991). New Methods for the highly selective isolation of *Streptosporangium* and *Dactylosporangium* from soil. *J Ferment Bioeng* **72**, 327–333.
- 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.
- Kawato, N. & Shinobu, R. (1959). On *Streptomyces herbaricolor* sp. nov., supplement: a single technique for microscopical observation. *Mem Osaka Univ Lib Arts Educ B* **8**, 114–119.
- 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.
- Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* **5**, 2359–2367.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: 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.
- 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.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Meyer, J. (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.
- Meyer, J. (1989). Genus *Nocardiopsis* Meyer 1976, 487^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2562–2569. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Mikami, Y., Miyashita, K. & Arai, T. (1982). Diaminopimelic acid profiles of alkalophilic and alkaline-resistant strains of actinomycetes. *J Gen Microbiol* **128**, 1709–1712.
- 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.
- Mishra, S. K., Keller, J. E., Miller, J. R., Heisey, R. M., Nair, M. G. & Putnam, A. R. (1987a). Insecticidal and nematocidal properties of microbial metabolites. *J Ind Microbiol* **2**, 267–276.
- Mishra, S. K., Traft, W. H., Putnam, A. R. & Ries, S. K. (1987b). Plant growth regulatory metabolites from novel actinomycetes. *J Plant Growth Regul* **6**, 75–84.
- Nolan, R. D. & Cross, T. (1988). Isolation and screening of actinomycetes. In *Actinomycetes in Biotechnology*, pp. 2–8. Edited by M. Goodfellow, S. T. Williams & M. Mordarski. London: Academic Press.
- Nonomura, H. & Ohara, Y. (1971a). Distribution of actinomycetes in soil. X. New genus and species of monosporic actinomycetes. *J Ferment Technol* **49**, 895–903.
- Nonomura, H. & Ohara, Y. (1971b). Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier *et al.* *J Ferment Technol* **49**, 904–912.
- Peltola, J. S. P., Anderson, 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 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*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to the identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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., Shameemullah, M., Watson, E. T. & Mayfield, C. I. (1972). Studies on the ecology of actinomycetes in soil. VI. The influence of moisture tension on growth and survival. *Soil Biol Biochem* **4**, 215–225.

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 taxa. *J Gen Microbiol* **129**, 1743–1813.

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.