Nocardiopsis alkaliphila sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt

Wael N. Hozzein,[1] Wen-Jun Li,[2] Mohammed Ibrahim A. Ali,[1] Ola Hammouda,[1] Ahmed S. Mousa,[1] Li-Hua Xu[2] and Cheng-Lin Jiang[2]

[1]Botany Department, Faculty of Science, Cairo University, Beni-Suef, Egypt

[2]Key Laboratory for Microbial Resources of the Ministry of Education, Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, P.R. China

Correspondence
Cheng-Lin Jiang
lihxu@ynu.edu.cn

An alkaliphilic actinomycete strain, designated YIM 80379^T, was isolated from a soil sample collected from the eastern desert of Egypt and subjected to polyphasic taxonomy. The strain produced substrate and aerial mycelia on different media, with an optimum pH for growth of 9·5–10 and scarce or no growth at pH 7. Strain YIM 80379^T contained *meso*-diaminopimelic acid, no diagnostic sugars, type PIII phospholipids and MK-10(H_6) and MK-10(H_8) as the predominant menaquinones. All of these characters assign isolate YIM 80379^T consistently to the genus *Nocardiopsis*. This was confirmed by 16S rDNA analysis. It can be differentiated from all *Nocardiopsis* species with validly published names by phenotypic characteristics, phylogenetic analysis and DNA–DNA hybridization results. On the basis of polyphasic evidence, a novel species, *Nocardiopsis alkaliphila* sp. nov., is proposed. The type strain of the species is YIM 80379^T (=CCTCC AA001031^T=DSM 44657^T).

Although alkaliphilic bacteria have been studied extensively for a long time, work on alkaliphilic actinomycetes is very rare. This is clear from the fact that very few articles about alkaliphilic actinomycetes have been published previously (Miyashita *et al.*, 1984; Groth *et al.*, 1997; Duckworth *et al.*, 1998; Kroppenstedt & Evtushenko, 2002). It is therefore important to pay more attention to this group of extreme actinomycetes, as a possible way to discover novel taxa and, consequently, new secondary metabolites. It was reported that most of the alkaliphilic actinomycetes that have been isolated belong to the genus *Nocardiopsis* (Mikami *et al.*, 1982, 1986; Kroppenstedt & Evtushenko, 2002); most *Nocardiopsis* species are alkaliphilic, as their growth optimum is above pH 8·0, and the genus *Nocardiopsis* contained 16 species with validly published names at the time of writing (Meyer, 1976; Grund & Kroppenstedt, 1990; Yassin *et al.*, 1993, 1997; Al-Tai & Ruan, 1994; Chun *et al.*, 2000; Evtushenko *et al.*, 2000; Peltola *et al.*, 2001; Al-Zarban *et al.*, 2002; Kämpfer *et al.*, 2002; Schippers *et al.*, 2002; Li *et al.*, 2003). In this study, a novel alkaliphilic actinomycete was identified by a polyphasic approach and was found to be a novel species of the genus *Nocardiopsis*. The name *Nocardiopsis alkaliphila* sp. nov. is proposed.

Strain YIM 80379^T was isolated from a soil sample collected from the eastern desert of Egypt by using medium A, which was recommended by Sato *et al.* (1983) for the isolation of alkaliphilic and alkaline-resistant micro-organisms. This medium contained (g l^{-1}): glucose, 10·0; peptone, 5·0; yeast extract, 5·0; $K_2HPO_4.3H_2O$, 1·0; $MgSO_4.7H_2O$, 0·2; Na_2CO_3, 10·0; and agar, 15·0. Sodium carbonate was sterilized separately and then added to the medium. The pH of the medium was 10·0–10·5; $NaHCO_3/Na_2CO_3$ buffer was used to adjust the pH. After incubation at 28 °C for 14 days, a visible colony (designated YIM 80379^T) was picked and subcultured until purification. A preliminary test was carried out to confirm its requirement for alkalinity; it was unable to grow below pH 7·0. The strain was maintained in 20 % glycerol and kept at −20 °C.

The isolate was cultivated on medium A and yeast extract/malt extract agar (ISP 2), both at pH 10·0, and used for microscopic observations of the sporophores, spore-chains and spore surface by using light and scanning electron microscopes (JEOL, JSM-5600LV). Cultural characteristics were studied on ISP media (Shirling & Gottlieb, 1966), medium A (Sato *et al.*, 1983), Czapek's agar (Waksman,

Published online ahead of print on 1 August 2003 as DOI 10.1099/ijs.0.02832-0.

Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PIM, phosphatidylinositol mannosides; PME, phosphatidyl methylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain YIM 80379^T is AY230848.

1967), modified Bennet's medium (Jones, 1949) and nutrient agar (Waksman, 1961). All media were solidified with 2·0 % agar and their pH was adjusted to 9·5–10·0; after incubation for 28 days at 28 °C, the colours of both substrate and aerial mycelia and the production of soluble pigments were determined by comparison with chips from ISCC–NBS colour charts (Kelly, 1964).

For chemotaxonomic studies, strain YIM 80379^T was grown in medium A broth on a shaking incubator at 200 r.p.m. and 28 °C for 7 days. Mycelia and cells were harvested by centrifugation, washed three times with distilled water and then freeze-dried. Amino acid and sugar analyses of whole-cell hydrolysates were performed as described by Hasegawa *et al.* (1983) and Staneck & Roberts (1974), respectively. Polar lipids were extracted and detected by previously described methods (Minnikin *et al.*, 1977; Lechevalier & Lechevalier, 1980). Menaquinones were extracted, purified and identified by HPLC as described by Collins (1985).

All physiological tests were done at 28 °C and pH 9·5–10·0 unless otherwise specified. Production of melanoid pigments was tested on ISP media, as described by Shirling & Gottlieb (1966). Carbon source utilization was examined on ISP 9 as a basal medium (Shirling & Gottlieb, 1966), supplemented with a final concentration of 1 % of the tested carbon sources (except for sodium acetate, sodium citrate and sodium succinate, which were used at a final concentration of 0·1 %). Utilization of different nitrogen sources, catalase production and degradation of tyrosine, hypoxanthine, casein, starch and gelatin were detected in modified Bennett's agar medium (MBA) after 7, 14 and 21 days, as described by Williams *et al.* (1983). Hydrogen sulphide production was detected by the method of Küster & Williams (1964). The effect of different temperatures and pH levels on growth and tolerance to salt (NaCl at 5, 10 and 15 %, w/v) was determined by using MBA or medium A as a basal medium.

DNA was extracted for 16S rDNA analysis by the method described by Orsini & Romano-Spica (2001). PCR-mediated amplification of 16S rDNA, purification of PCR products and sequencing of purified products were done as described previously (Cui *et al.*, 2001). The resultant sequence was aligned manually against bacterial sequences that were available in public databases. A more detailed comparison was performed with members of the genus *Nocardiopsis* and evolutionary distance matrices were calculated by the method of Jukes & Cantor (1969). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

DNA was isolated according to Hopwood *et al.* (1985) and its G+C content was determined by the thermal denaturation method (Mandel & Marmur, 1968) with a Shimadzu UV–visible spectrophotometer (UV1601). DNA–DNA hybridization was carried out spectrophotometrically, as described by De Ley *et al.* (1970).

Alkaliphilic strain YIM 80379^T showed good growth on most agar media used (Table 1). Aerial mycelium was abundant on most media and its colour varied from white to yellowish-white. Substrate mycelium was light yellow to yellowish-brown; no soluble pigments were produced on any medium. Mature aerial mycelium fragmented to branched and straight spore-chains with elongated, irregular and smooth spores (Fig. 1).

The isolate's membership of the genus *Nocardiopsis* was confirmed by cell chemistry. Whole-cell hydrolysates contained *meso*-diaminopimelic acid as the only peptidoglycan diamino acid and ribose and glucose as the only sugars, but no diagnostic sugars such as arabinose, xylose, madurose (Lechevalier *et al.*, 1971) or rhamnose (Labeda *et al.*, 1984). This leads to cell wall type III and sugar pattern C (Lechevalier & Lechevalier, 1970). The polar lipid pattern revealed the presence of phosphatidylcholine

Table 1. Cultural characteristics of strain YIM 80379^T

Medium*	Growth	Aerial mycelium	Substrate mycelium
Tryptone/yeast extract (ISP 1)	Poor	−	Light yellow†
Yeast extract/malt extract (ISP 2)	Moderate	White	Yellowish-brown
Oatmeal agar (ISP 3)	Abundant	Yellowish-white	Yellowish-brown
Inorganic salts/starch agar (ISP 4)	Good	White	Pale yellow
Glycerol/asparagine agar (ISP 5)	Good	White	Pale yellow
Tyrosine agar (ISP 7)	Abundant	Yellowish-white	Yellow
Bennet agar	Moderate	White	Greyish-yellow
Czapek agar	Abundant	Yellowish-white	Soft yellow
Nutrient agar	Good	Yellowish-white	Pale yellow
Sato A	Abundant	Yellowish-white	Deep yellow

*All media were adjusted to pH 9·5–10·0. ISP, International Streptomyces Project (Shirling & Gottlieb, 1966).
†Colours were taken from ISCC–NBS colour charts (Kelly, 1964).

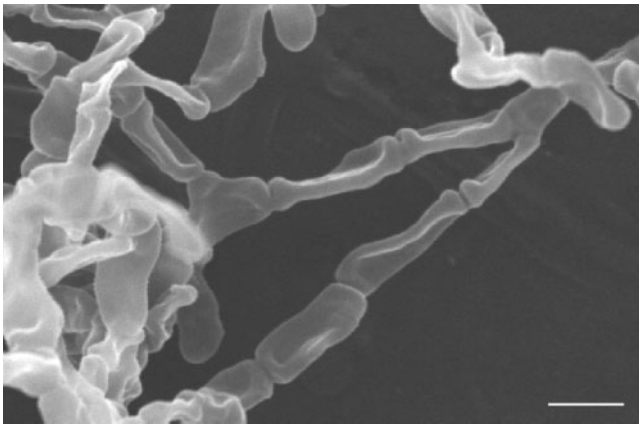

Fig. 1. Scanning electron micrograph of strain YIM 80379^T after growth on medium A for 14 days at 28 °C. Bar, 1 μm.

(PC), phosphatidylglycerol (PG), phosphatidyl methylethanolamine (PME), phosphatidylethanolamine (PE), phosphatidylinositol mannosides (PIM), diphosphatidylglycerol (DPG), an unknown glycolipid and about four unknown phospholipids with high R_f values (above that of DPG). The phospholipid pattern is type PIII according to Lechevalier *et al.* (1977), with PC as the diagnostic phospholipid. This phosopholipid type is found in species of the genera *Nocardiopsis*, *Actinopolyspora*, *Pseudonocardia* and *Saccharopolyspora*. However, *Nocardiopsis* strains are easily differentiated from these other taxa by the presence of PME, high amounts of PG, lack of hydroxy-PE and the detection of unknown phospholipids with high R_f values (above that of DPG). These unknown phospholipids are of diagnostic value and, until now, have only been found in *Nocardiopsis* species (Kroppenstedt, 1992).

Strain YIM 80379^T synthesized a complex pattern of menaquinones with 9, 10 and 11 isoprenoid units in the side chain and a variable degree of saturation. Major menaquinones were MK-10(H_6), MK-10(H_8), MK-11(H_2), MK-9(H_6), MK-9(H_{10}) and MK-10(H_4). Minor menaquinones were MK-11(H_4), MK-9(H_2), MK-9(H_4) and MK-10(H_2). Trace amounts of some other menaquinones were also found. This quinone system, with the predominant menaquinones MK-10(H_6), MK-10(H_8) and other MK-10 menaquinones, is characteristic of species of the genus *Nocardiopsis*. Also, such a complex quinone system, or one even more complicated, was reported in earlier studies (Evtushenko *et al.*, 2000; Al-Zarban *et al.*, 2002; Kämpfer *et al.*, 2002). All these characteristics are typical of the genus *Nocardiopsis* (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992).

No melanoid pigments were produced. Arabinose, xylose, maltose, cellobiose, raffinose and sucrose were utilized as good carbon sources, but weak utilization was observed with glucose, galactose, lactose, rhamnose, xylitol, sorbitol, inositol, dulcitol, sodium citrate and sodium succinate. Ribose, fructose, mannose, mannitol and sodium acetate were not utilized. Growth on potassium nitrate, asparagine, phenylalanine and serine as nitrogen sources was recorded, but histidine, methionine, valine, threonine, cysteine and glycine were utilized weakly, whereas growth on arginine and hydroxyproline was not observed. Strain YIM 80379^T could degrade tyrosine, hypoxanthine, casein, gelatin, starch and tributrin; it also produced catalase, but not H_2S. Temperature range for growth was 10–45 °C; it showed optimum growth at 28–30 °C. It grew only on alkaline media. No growth was observed below pH 7·0 and the optimum pH for growth was 9·5–10·0. The maximum pH for growth was 12·0. Good growth was shown at NaCl concentrations up to 10·0 %.

The almost-complete 16S rDNA sequence of strain YIM 80379^T, which consisted of 1490 bp, was determined. Preliminary comparison of the sequence against those in GenBank indicated that members of the genus *Nocardiopsis* were the closest phylogenetic neighbours. Binary similarity values of this strain and other species of the genus *Nocardiopsis* ranged between 95·4 % (*Nocardiopsis halophila* DSM 44494^T) and 98·5 % (*Nocardiopsis prasina* DSM 43845^T). Pairwise similarity values >97 % were also found for *Nocardiopsis listeri* DSM 40297^T (98·4 %), *Nocardiopsis metallicus* DSM 44598^T (98·2 %), *Nocardiopsis exhalans* DSM 44407^T (98·1 %), *Nocardiopsis alba* DSM 43377^T (97·8 %), *Nocardiopsis lucentensis* DSM 44048^T (97·7 %), *Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111^T (97·7 %), *Nocardiopsis umidischolae* DSM 43662^T (97·1 %) and *Nocardiopsis synnemataformans* DSM 44143^T (97·2 %). These 16S rDNA sequence similarity values are approximately the same or less than the similarity values between closely related *Nocardiopsis* species, such as *N. dassonvillei* and *N. synnemataformans* (99·3 %), *N. metallicus* and *N. exhalans* (99·4 %), *N. alba* and *N. prasina* (99·0 %), *N. halotolerans* and *N. dassonvillei* (98·4 %) and *N. listeri* and *N. prasina* (98·8 %). A phylogenetic tree of *Nocardiopsis* species is shown in Fig. 2. The closest phylogenetic neighbours of strain YIM 80379^T are *N. listeri* DSM 40297^T, *N. prasina* DSM 43845^T, *N. metallicus* DSM 44598^T and *N. exhalans* DSM 44407^T. These data indicate that strain YIM 80379^T probably belongs to a novel species. However, sequence similarity values of ⩾97 % was reported to be of limited usefulness in species differentiation, and DNA pairing studies need to be performed to confirm the species affiliation (Stackebrandt & Goebel, 1994).

DNA of strain YIM 80379^T was hybridized against that of *N. prasina* DSM 43845^T, *N. listeri* DSM 40297^T, *N. metallicus* DSM 44598^T and *N. exhalans* DSM 44407^T, which were the closest phylogenetic neighbours in the same subclade. DNA–DNA relatedness between strain YIM 80379^T and the latter four strains was 42, 58, 18 and 35 %, respectively. These values are below the value of 70 % that was recommended by Wayne *et al.* (1987) for strains of the same species. The DNA G+C content of strain YIM 80379^T was

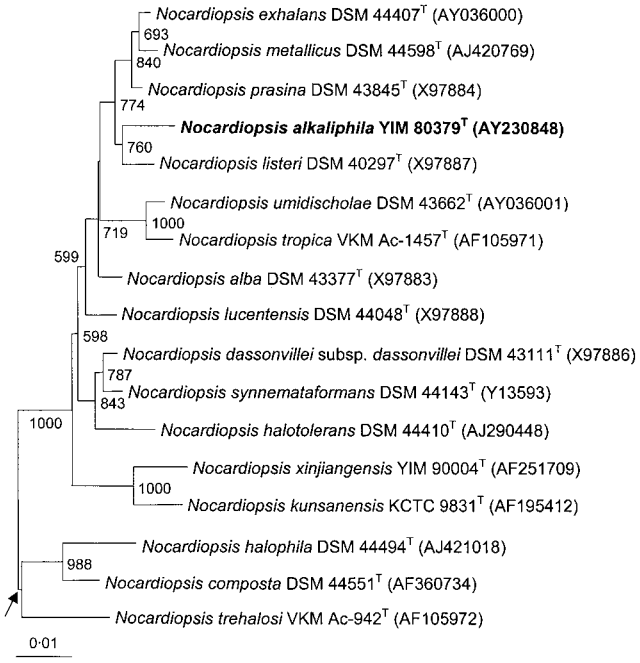

Fig. 2. Phylogenetic dendrogram based on 16S rDNA sequence analysis, reconstructed from evolutionary distances by using the neighbour-joining method, showing the phylogenetic position of strain YIM 80379^T within the genus *Nocardiopsis*. The sequence of *Actinomadura madurae* DSM 43067^T (GenBank no. X97889) was used as the outgroup (not shown). Arrow indicates the position of the root. Bar, one inferred nucleotide substitution per 100 nt.

65·8 mol%, which lies within the range for the genus *Nocardiopsis* (Grund & Kroppenstedt, 1990).

Morphological and phylogenetic analyses and chemotaxonomic features clearly provided evidence that strain YIM 80379^T belongs to the genus *Nocardiopsis*. The phylogenetic position of this strain is within a cluster that contains *N. listeri*, *N. prasina*, *N. metallicus* and *N. exhalans*. However, strain YIM 80379^T can be differentiated from all these species by its ability to grow at 45 °C and pH 12·0, and by a combination of morphological, physiological and chemotaxonomic characteristics (Table 2). Also, it can be differentiated easily from *N. listeri* and *N. prasina*, its closest phylogenetic neighbours, by chemotaxonomy: the predominant menaquinones in *N. listeri* are MK-10(H_0–H_2) and those in *N. prasina* are MK-10(H_4–H_6), whilst those in YIM 80379^T are MK-10(H_6–H_8). It can also be distinguished by morphology from *N. listeri*, which does not produce a well-developed aerial mycelium. Furthermore, DNA–DNA reassociation values determined for strain YIM 80379^T with *N. listeri* and *N. prasina* were only 42 and 58 %, which reinforces the genomic differences between them.

Based on the above phenotypic and genotypic results, it is concluded that isolate YIM 80379^T merits species status in the genus *Nocardiopsis*; the name *Nocardiopsis alkaliphila* sp. nov. is proposed for this isolate, with the type strain YIM 80379^T (=CCTCC AA001031^T=DSM 44657^T).

Description of *Nocardiopsis alkaliphila* sp. nov.

Nocardiopsis alkaliphila (al.ka.li′phi.la. N.L. n. *alkali* from Arabic *al-qaliy* the ashes of saltwort; Gr. adj. *philos* friendly, loving; N.L. fem. adj. *alkaliphila* loving alkaline environments).

Table 2. Characteristics that differentiate strain YIM 80379^T from the phylogenetically most closely related *Nocardiopsis* species

Taxa: 1, YIM 80379^T; 2, *N. listeri*; 3, *N. prasina*; 4, *N. exhalans*; 5, *N. metallicus*. Data are from Yassin *et al.* (1997), Peltola *et al.* (2001), Schippers *et al.* (2002) and the present study.

Characteristic	1	2	3	4	5
Aerial mycelium	+	−	+	+	+
Utilization of:					
D-Galactose	±	−	−	−	+
D-Fructose	−	+	−	+	ND
D-Sucrose	+	+	−	+	+
L-Rhamnose	±	+	−	+	+
Acetate	−	−	+	+	+
L-Serine	+	+	+	+	−
Degradation of hypoxanthine	+	+	−	ND	ND
Growth at:					
10 °C	+	+	−	+	±
45 °C	+	−	−	−	−
pH 12·0	+	ND	−	ND	−
Major menaquinones*	10/6, 10/8	10/0, 10/2	10/4, 10/6	10/6, 10/8	ND

*10/6, MK-10 (H_6) and so on.

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is white to yellowish-white. Substrate mycelium is yellow to yellowish-brown. Diffusible pigments are not produced. Mature aerial mycelium fragments to branched and straight spore-chains with elongated, irregular and smooth spores. Whole-cell hydrolysates contain *meso*-diaminopimelic acid and the sugars glucose and ribose. Polar lipid pattern is composed of PC, PG, PME, PE, PIM, DPG, an unknown glycolipid and about four unknown phospholipids with high R_f values. Major menaquinones are MK-10(H_6), MK-10(H_8), MK-11(H_2), MK-9(H_6), MK-9(H_{10}) and MK-10(H_4); minor menaquinones MK-11(H_4), MK-9(H_2), MK-9(H_4) and MK-10(H_2) are also detected. Melanin is not produced. Arabinose, xylose, maltose, cellobiose, raffinose and sucrose are utilized as good carbon sources, but weak utilization of glucose, galactose, lactose, rhamnose, xylitol, sorbitol, inositol, dulcitol, sodium citrate and sodium succinate is observed. Ribose, fructose, mannose, mannitol and sodium acetate are not utilized. Growth on potassium nitrate, asparagine, phenylalanine and serine as nitrogen sources is recorded, but histidine, methionine, valine, threonine, cysteine and glycine are utilized only weakly, whereas growth on arginine and hydroxyproline is not observed. Able to degrade hypoxanthine, tyrosine, casein, gelatin, starch and tributrin. Catalase is produced, but H_2S is not. Growth occurs at 10–45 °C, pH 7·0–12·0 and 0–10·0 % NaCl, with optimum growth at 28–30 °C, pH 9·5–10·0 and 2·5 % NaCl. DNA G+C content is 65·8 mol%.

The type strain is YIM 80379^T (=CCTCC AA001031^T=DSM 44657^T). Isolated from desert soil in Egypt.

Acknowledgements

The authors are grateful to R. M. Kroppenstedt for kindly providing type strains of the genus *Nocardipsis*. This research was supported by the Ministry of Science and Technology, P.R. China (project no. 2001CCC00600), the National Natural Science Foundation of China (project no. 30270004), Yunnan Provincial Natural Science Foundation (project no. 20001C001Q) and Yunnan Education Foundation (project nos 01111134 and 02QJ077).

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 march soil in Kuwait. *Int J Syst Evol Microbiol* **52**, 525–529.

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. (1985). Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Cui, X.-L., Mao, P.-H., Zeng, M., Li, W.-J., Zhang, L.-P., Xu, L.-H. & Jiang, C.-L. (2001). *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **51**, 357–363.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Duckworth, A. W., Grant, S., Grant, W. D., Jones, B. E. & Meijer, D. (1998). *Dietzia natronolimnaios* sp. nov., a new member of the genus *Dietzia* isolated from an east African soda lake. *Extremophiles* **2**, 359–366.

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.

Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Bogoriella caseilytica* gen. nov., sp. nov., a new alkaliphilic actinomycete from a soda lake in Africa. *Int J Syst Bacteriol* **47**, 788–794.

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.

Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P. & Ward, J. M. (1985). Preparation of chromosomal, plasmid and phage DNA. In *Genetic Manipulation of Streptomyces – a Laboratory Manual*, pp. 79–80. Norwich, UK: F. Crowe & Sons.

Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–146.

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.

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.

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. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Küster, E. & Williams, S. T. (1964). Production of hydrogen sulfide by streptomycetes and methods for its detection. *Appl Microbiol* **12**, 46–52.

Labeda, D. P., Testa, R. T., Lechevalier, M. P. & Lechevalier, H. A. (1984). *Saccharothrix*: a new genus of the *Actinomycetales* related to *Nocardiopsis*. *Int J Syst Bacteriol* **34**, 426–431.

Lechevalier, M. P. & Lechevalier, H. (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*, pp. 227–291. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society For Industrial Microbiology.

Lechevalier, H. A., Lechevalier, M. P. & Gerber, N. N. (1971). Chemical composition as a criterion in the classification of actinomycetes. *Adv Appl Microbiol* **14**, 47–72.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Li, M.-G., Li, W.-J., Xu, P., Cui, X.-L., Xu, L.-H. & Jiang, C.-L. (2003). *Nocardiopsis xinjiangensis* sp. nov., a halophilic actinomycete isolated from a saline soil sample in China. *Int J Syst Evol Microbiol* **53**, 317–321.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.

Meyer, J. (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.

Mikami, Y., Miyashita, K. & Arai, T. (1982). Diaminopimelic acid profiles of alkalophilic and alkaline-resistant strains of actinomycetes. *J Gen Microbiol* **128**, 1709–1712.

Mikami, Y., Miyashita, K. & Arai, T. (1986). Alkaliphilic actinomycetes. *Actinomycetes* **19**, 176–191.

Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.

Miyashita, K., Mikami, Y. & Arai, T. (1984). Alkalophilic actinomycete, *Nocardiopsis dassonvillei* subsp. *prasina* subsp. nov., isolated from soil. *Int J Syst Bacteriol* **34**, 405–409.

Orsini, M. & Romano-Spica, V. (2001). A microwave-based method for nucleic acid isolation from environmental samples. *Lett Appl Microbiol* **33**, 17–20.

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.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sato, M., Beppu, T. & Arima, K. (1983). Studies on antibiotics produced at high alkaline pH. *Agric Biol Chem* **47**, 2019–2027.

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.

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 identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Waksman, S. A. (1961). *The Actinomycetes, vol. II. Classification, Identification and Descriptions of Genera and Species.* Baltimore: Williams & Wilkins.

Waksman, S. A. (1967). *The Actinomycetes. A Summary of Current Knowledge.* New York: Ronald Press.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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., 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.