Nocardia caishijiensis sp. nov., a novel soil actinomycete

Jianli Zhang,[1]† Zhiheng Liu[1] and Michael Goodfellow[2]

Correspondence
Zhiheng Liu
zhliu@sun.im.ac.cn

[1]State Key Laboratory of Microbial Resource, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

[2]Department of Agricultural and Environmental Science, University of Newcastle, Newcastle-upon-Tyne NE1 7RU, UK

A soil isolate, strain F829^T, which had provisionally been assigned to the genus *Nocardia*, was subjected to a polyphasic taxonomic study. An almost complete 16S rDNA sequence was determined for this strain; the sequence was aligned with available sequences for nocardiae and phylogenetic trees were inferred using three tree-making algorithms. The organism showed a combination of phenotypic properties typical of nocardiae and formed a distinct phyletic line within the evolutionary radiation of species of the genus *Nocardia*, being most closely related to *Nocardia asteroides* ATCC 19247^T. Strain F829^T was readily distinguished from representatives of species of *Nocardia* with validly published names on the basis of phenotypic data, notably from the type strain of *N. asteroides*. It is proposed that the organism be recognized as a novel species of *Nocardia*, *Nocardia caishijiensis* sp. nov. The type strain is F829^T (=AS 4.1728^T=JCM 11508^T).

The application of chemotaxonomic, numerical phenetic and molecular systematic methods has led to an improved description of the genus *Nocardia* (Goodfellow *et al.*, 1999). The genus belongs to the mycolic-acid-containing group of actinomycetes, i.e. the suborder *Corynebacterineae* Stackebrandt *et al.* 1997, which encompasses the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania*, *Tsukamurella* and *Williamsia*, and the genus *Turicella*, which lacks mycolic acids (Goodfellow *et al.*, 1998, 1999). Members of these taxa form a distinct phyletic line in the 16S rDNA tree and can be distinguished from one another using a combination of biochemical, chemical and morphological features (Goodfellow *et al.*, 1999). The revised genus *Nocardia* encompasses 27 species with validly published names at the time of writing; the taxonomic integrity of most of the latter is underpinned by a plethora of genotypic and phenotypic data (Goodfellow *et al.*, 1999; Maldonado *et al.*, 2000; Gürtler *et al.*, 2001; Hamid *et al.*, 2001; Wang *et al.*, 2001; Yassin *et al.*, 2001).

Much of the emphasis in nocardial systematics has focussed on the causal agents of actinomycetoma and nocardiosis (Goodfellow, 1992, 1998; McNeil & Brown, 1994), though it is evident that nocardiae are common in natural habitats, notably soil (Orchard *et al.*, 1977; Orchard, 1979, 1981; Maldonado *et al.*, 2000). It is also becoming increasingly clear that nocardial species diversity is underestimated in both clinical and non-clinical settings (Gürtler *et al.*, 2001; Hamid *et al.*, 2001; Wang *et al.*, 2001; Yassin *et al.*, 2001; Albuquerque de Barros *et al.*, 2003). It is important to unravel the species richness of nocardiae, especially to determine the roles that members of particular species play in the flow of nutrients and energy in natural habitats.

The aim of the present study was to determine the taxonomic position of a *Nocardia*-like strain, isolated from soil, using a polyphasic approach. The resultant data show that strain F829^T should be recognized as a novel species of *Nocardia*. The name *Nocardia caishijiensis* sp. nov. is proposed for this organism.

Strain F829^T was isolated on a Bennett's agar plate [1 % (w/v) D-glucose, 0·1 % (w/v) yeast extract, 0·1 % (w/v) beef extract, 0·2 % (w/v) casein enzymic hydrolysate (Sigma), 1·5 % (w/v) agar] that had been incubated at 28 °C for 7 days following inoculation with a suspension of a soil sample collected from Caishiji in Anhui Province, China. The isolate and the marker cultures used in the DNA–DNA relatedness and phenotypic characterization studies were maintained on modified Sauton's agar slants (Mordarska *et al.*, 1972) at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C.

The colonial properties of isolate F829^T were recorded from modified Sauton's and Bennett's agar plates that had been incubated for up to 7 days at 28 °C. The micromorphological properties of the isolate were recorded using samples taken from the modified Sauton's agar plate by light and

†Present address: College of Life Science and Technology, Beijing Institute of Technology, Beijing 100081, People's Republic of China.

The GenBank accession number for the 16S rDNA sequence of strain F829^T (=AS 4.1728^T) is AF459443.

scanning electron microscopy; in the latter case gold-coated dehydrated preparations from a 7 day culture were examined using a Hitachi S-570 scanning electron microscope. Gram (Hucker's modification; Society for American Bacteriologists, 1957) and Ziehl–Neelsen (Gordon, 1967) preparations were also observed by light microscopy.

The test strain was examined for a range of phenotypic properties using standard procedures (Goodfellow, 1971; Williams *et al.*, 1983). In addition, acid production from carbohydrates was carried out using media and methods described by Gordon *et al.* (1974) and the utilization of sole carbon and sole carbon/nitrogen sources was investigated after Gordon & Mihm (1957) and Tsukamura (1966). Resistance to lysozyme was determined by the method of Gordon *et al.* (1974). Tolerance of pH, temperature and sodium chloride regimes were determined on modified Sauton's agar plates incubated for up to 14 days. Resistance to antibiotics was examined using chloramphenicol (30 μg), erythromycin (15 μg), gentamicin sulfate (10 μg), midecamycin (15 μg), minocycline hydrochloride (30 μg), penicillin G (10 U), rifampicin (5 μg), streptomycin sulfate (10 μg), tobramycin sulfate (10 μg) and vancomycin (30 μg) disks (Goodfellow & Orchard, 1974) with glucose-yeast extract agar (Gordon & Mihm, 1962) as the basal medium; the results were recorded following incubation at 28 °C for up to 14 days.

Biomass for most of the chemotaxonomic studies was prepared following growth of the isolate and marker strains in shake flasks of modified Sauton's broth for 5 days at 28 °C; after checking for purity, the biomass was harvested by centrifugation, washed twice in distilled water and freeze-dried. Established TLC procedures were used to determine the diagnostic isomers of diaminopimelic acid (Lechevalier & Lechevalier, 1980), whole-organism sugars (Lechevalier & Lechevalier, 1980) and polar lipids (Minnikin *et al.*, 1984). The acid methanolysis procedure was used to detect mycolic acids (Minnikin *et al.*, 1975). The predominant isoprenoid quinones were extracted and purified by the method of Collins *et al.* (1977, 1987); purified menaquinones were determined by reversed-phase HPLC (Wu *et al.*, 1989). Biomass for the quantitative fatty acid analysis was prepared by scraping growth from TSB agar plates [trypticase soy broth (BBL), 3 % (w/v); Bacto agar (Difco), 1·5 % (w/v)] that had been incubated for 4 days at 28 °C. The fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Chromosomal DNA was extracted from biomass of strain F829^T grown in modified Sauton's broth for 3 days at 28 °C and purified following the methods of Saito & Miura (1963) and Whipple *et al.* (1987). The G+C content of the DNA was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as control. DNA–DNA relatedness values between strain F829^T and *Nocardia asteroides* ATCC 19247^T were determined spectrophotometrically from renaturation rates using established procedures (De Ley *et al.*, 1970; Huß *et al.*, 1983) and software (Jahnke, 1992).

Genomic DNA extraction, PCR amplification of 16S rDNA and purification of the PCR product from strain F829^T were carried out using procedures described by Rainey *et al.* (1996). The purified PCR product was sequenced directly using a *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) and universal primers as described previously (Lu *et al.*, 2001). Sequence gel electrophoresis was carried out and nucleotide sequences were obtained automatically using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer.

The 16S rDNA sequence of strain F829^T was aligned manually with corresponding nucleotide sequences of representatives of the suborder *Corynebacterineae*, including the type strains of *Nocardia* species, retrieved from the DDBJ/EMBL/GenBank databases using the program CLUSTAL X 1.8 (Thompson *et al.*, 1997). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were generated according to the method of Kimura (1980). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP suite of programs.

When the almost complete 16S rDNA sequence (1426 nt) obtained for strain F829^T was compared with corresponding sequences from representatives of genera in the suborder *Corynebacterineae*, it was found to contain signature nucleotides that are characteristic for members of the family *Nocardiaceae* (Stackebrandt *et al.*, 1997) and the genus *Nocardia* (Chun & Goodfellow, 1995). The high 16S rDNA gene sequence similarities found between the tested strain and representatives of the genus *Nocardia* (94·8–97·6 %) also support its assignment to this taxon.

Strain F829^T showed a range of phenotypic properties typical of members of the genus *Nocardia* (Goodfellow, 1998; Goodfellow *et al.*, 1999). The organism is an aerobic, Gram-positive, slightly acid–alcohol-fast actinomycete which forms an extensively branched substrate mycelium that fragments into non-motile, rod-shaped elements on modified Sauton's agar. Whole-organism hydrolysates of the organism were rich in *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970) and diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl inositol mannosides (phospholipid type II *sensu* Lechevalier *et al.*, 1977). The isolate also contained predominantly hexahydrogenated menaquinones with eight isoprene units, the end two being cyclized; this menaquinone is restricted to members of the genera *Nocardia* and *Skermania* (Chun *et al.*, 1997; Goodfellow *et al.*, 1999).

One-dimensional TLC of whole-organism acid methanolysates revealed the presence of two lipid spots, the lower one corresponded to mycolic acids, as identified by its relative front value (0·47), and the higher one to non-hydroxylated fatty acids. The fatty acid profile contained mainly straight chain saturated, unsaturated and 10-methyl-branched fatty acids. The predominant components, as a proportion of the total fatty acid composition, were: $C_{15:0}$, 1·2 %; $C_{16:0}$, 21·8 %; $C_{17:0}$, 5·9 %; $C_{18:0}$, 31·7 %; *cis*9-$C_{16:1}$, 10·7 %; *cis*9-$C_{17:1}$, 1·3 %; *cis*9-$C_{18:1}$, 16·1 %; *cis*11,14-$C_{20:2}$, 2·4 %; and 10-methyl-$C_{18:0}$, 7·8 %. In addition, the DNA of strain F829^T was rich in guanine and cytosine (G+C content 69·4 mol%).

The position of strain F829^T in the unrooted 16S rDNA tree based on three tree-making algorithms is shown in Fig. 1. Strain F829^T is most closely related to the type strain of *N. asteroides*. The two strains share 97·6 % 16S rDNA sequence similarity, which corresponds to 34 nt differences over 1400 positions. However, it is evident from the 16S rDNA tree that the isolate and the type strain of *N. asteroides* are in different subclades; these organisms also show a relatively low level of DNA–DNA relatedness, 32 %, a value well below the 70 % cut-off point recommended for assignment of bacterial strains to the same genomic species (Wayne *et al.*, 1987). Strain F829^T can be distinguished from the type strains of all validly described species of *Nocardia*, including *N. asteroides* ATCC 19247^T, using a combination of phenotypic properties (Table 1).

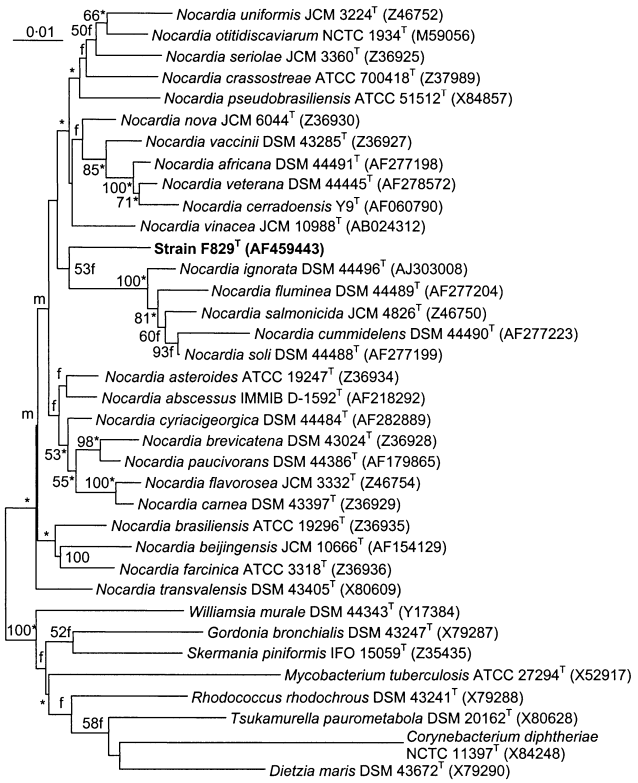

Fig. 1. Unrooted neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rDNA sequences showing the position of strain F829^T. Asterisks indicate branches of the tree that were also recovered using both the least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) treeing algorithms. Symbols 'f' and 'm' respectively denote branches that were also recovered using the least-squares or maximum-likelihood methods. Numbers at nodes indicate levels 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.

The genotypic and phenotypic data show that strain F829^T merits recognition as a novel species in the genus *Nocardia*. It is, therefore, proposed that the organism be classified in this taxon as *Nocardia caishijiensis* sp. nov.

Description of *Nocardia caishijiensis* sp. nov.

Nocardia caishijiensis (cai.shi.ji.en′sis. N.L. adj. *caishijiensis* referring to Caishiji, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, slightly acid–alcohol-fast, non-motile actinomycete that forms an extensively branched substrate mycelium that fragments *in situ* into rod-shaped elements. An orange to brown substrate mycelium carries sparse to abundant, white to pinkish aerial hyphae on modified Sauton's agar. A brown substrate mycelium bears white to greyish aerial hyphae on Bennett's agar. Colony elevation is convex to irregular and colony margins are filamentous. Diffusible pigments are not formed. The organism is catalase-positive and reduces nitrate, but is oxidase-negative. Aesculin and urea are hydrolysed, but not arbutin. Tweens 20 and 80 are degraded, but not adenine, casein, elastin, guanine, hypoxanthine, starch, Tween 60, tyrosine or xanthine. Acid is formed from D-fructose, D-galactose, D-glucose, glycerol, D-mannose, D-ribose, D-trehalose and D-xylose, but not from arbutin, D-cellobiose, *myo*-inositol, inulin, D-maltose, D-melezitose, D-melibiose, D-raffinose, α-L-rhamnose, starch, D-sucrose or D-turanose. Arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, inulin, D-maltose, D-mannose, D-melezitose, D-melibiose, methyl α-D-glucoside, D-raffinose, α-L-rhamnose, D-ribose, starch (weak), D-sucrose, D-trehalose, D-turanose, D-xylose, acetate, fumarate (weak), lactic acid, propionate, pyruvate and succinate (weak) are utilized as sole carbon and energy sources, but not adonitol, L-arabinose, arabitol, dulcitol, *meso*-erythritol, ethanol, D-fucose, glycogen, *myo*-inositol, lactose, D-mannitol, paraffin, salicin, D-sorbitol, xylitol, adipic acid, benzoate, citrate, formate, hippurate, malate, malonate, sebacic acid, oxalate or tartrate. L-Alanine, L-aspartate (weak), D-glucosamine, L-proline and L-serine are used as sole carbon and nitrogen sources, but not acetamide, L-asparagine, gelatin, L-leucine, phenylalanine or L-valine. Grows between 17 and 37 °C, from pH 5·2 to 10·0 and in the presence of sodium chloride at 5 %, but not at 6 or 7 % (w/v). Resistant to lysozyme, gentamicin sulfate, penicillin G and streptomycin sulfate, but sensitive to

Table 1. Phenotypic characteristics that distinguish strain F829^T from the type strains of *Nocardia* species

Strains: 1, F829^T; 2, *N. abscessus* DSM 44432^T; 3, *N. africana* DSM 44491^T; 4, *N. asteroides* ATCC 19247^T; 5, *N. beijingensis* JCM 10666^T; 6, *N. brasiliensis* ATCC 19296^T; 7, *N. brevicatena* DSM 43024^T; 8, *N. carnea* DSM 43397^T; 9, *N. cerradoensis* Y9^T; 10, *N. crassostreae* ATCC 700418^T; 11, *N. cummidelens* DSM 44490^T; 12, *N. cyriacigeorgica* DSM 44484^T; 13, *N. farcinica* ATCC 3318^T; 14, *N. flavorosea* JCM 3332^T; 15, *N. fluminea* DSM 44489^T; 16, *N. ignorata* DSM 44496^T; 17, *N. nova* JCM 6044^T; 18, *N. otitidiscaviarum* NCTC 1934^T; 19, *N. paucivorans* DSM 44386^T; 20, *N. pseudobrasiliensis* ATCC 51512^T; 21, *N. salmonicida* JCM 4826^T; 22, *N. seriolae* JCM 3360^T; 23, *N. soli* DSM 44488^T; 24, *N. transvalensis* DSM 43405^T; 25, *N. uniformis* JCM 3224^T; 26, *N. vaccinii* DSM 43285^T; 27, *N. veterana* DSM 44445^T; 28, *N. vinacea* JCM 10988^T. Data were taken from this and previous studies (Maldonado *et al.*, 2000; Wang *et al.*, 2001; Yassin *et al.*, 2001; Albuquerque de Barros *et al.*, 2003). +, Positive; −, negative; d, doubtful; ND, not determined.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Aesculin hydrolysis	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	d	d
Arbutin hydrolysis	−	+	−	+	−	+	+	−	ND	ND	+	ND	+	+	+	ND	+	+	+	−	+	+	+	+	+	−	ND	ND
Nitrate reduction	+	+	+	+	+	+	−	+	+	−	+	ND	+	−	+	ND	+	+	+	−	+	+	+	+	+	+	−	+
Urea hydrolysis	+	+	−	+	+	+	−	−	+	−	+	+	+	−	−	ND	+	+	+	+	+	−	+	+	+	+	+	+
Decomposition of (%, w/v):																												
Adenine (0·4)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	−	−	−	+	−	−	−	−	−	−	−	−
Casein (1·0)	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Elastin (0·3)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	−
Hypoxanthine (0·4)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+	+	−	−	+
Tyrosine (0·5)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−	+	−	−	−
Xanthine (0·4)	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	+	−	−	−
Growth on sole carbon source (%, w/v):																												
D(+)-Mannitol (1·0)	−	−	−	+	+	+	−	+	−	−	−	−	−	+	−	+	+	+	−	+	+	−	−	+	−	+	−	+
α-L-Rhamnose (1·0)	+	+	−	−	+	−	+	−	+	+	−	−	+	−	+	−	−	−	−	−	−	−	−	+	+	−	+	−
D(+)-Sorbitol (1·0)	−	−	−	−	+	−	−	+	+	−	−	−	−	+	−	ND	+	−	−	+	+	−	−	+	−	−	−	+
Sodium acetate (0·1)	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	ND
Sodium citrate (0·1)	−	+	−	+	+	+	−	−	−	−	−	−	−	+	+	−	−	−	−	+	+	+	−	−	−	−	−	d
Growth at 45 °C	−	−	+	−	−	+	−	−	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	+	−

chloramphenicol, erythromycin, midecamycin, minocycline hydrochloride, rifampicin, tobramycin sulfate and vancomycin. The major cellular fatty acids are $C_{16:0}$ (21·8 %), $C_{18:0}$ (31·7 %), *cis*9-$C_{16:1}$ (10·7 %), *cis*9-$C_{18:1}$ (16·1 %) and 10-methyl-$C_{18:0}$ (7·8 %). The G+C content of the DNA is 69·4 mol%.

The type strain, F829^T (=AS 4.1728^T=JCM 11508^T), was isolated from a soil sample collected in Caishiji, Anhui Province, China.

Acknowledgements

This work was supported through the Royal Society–Chinese Academy of Sciences Exchange Scheme (grant no. Q814), by the National Natural Science Foundation of China (grant no. 39570002) and by the Key Laboratory for Microbial Resources of the Education Board of China. The authors are grateful to Dr T. Kudo (JCM) and Professor R. M. Kroppenstedt (DSMZ) for providing some of the type strains of *Nocardia*. We also thank Dr Long Hong for his excellent technical assistance.

References

Albuquerque de Barros, E. V. S., Manfio, G. P., Ribiero Maitan, V., Mendes Bataus, L. A., Kim, S. B., Maldonado, L. A. & Goodfellow, M. (2003). *Nocardia cerradoensis* sp. nov., a novel isolate from Cerrado soil in Brazil. *Int J Syst Evol Microbiol* **53**, 29–33.

Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.

Chun, J., Blackall, L. L., Kang, S.-O., Hah, Y. C. & Goodfellow, M. (1997). A proposal to reclassify *Nocardia pinensis* Blackall et al. as *Skermania piniformis* gen. nov., comb. nov. *Int J Syst Bacteriol* **47**, 127–131.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Collins, M. D., Howarth, O. W., Grund, E. & Kroppenstedt, R. M. (1987). Isolation and structural determination of new members of the vitamin K_2 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**, 143–153.

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 (phylogenetic inference package), version 3.6. Department of Genetics, University of Washington, Seattle, WA, USA.

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.

Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* **69**, 33–80.

Goodfellow, M. (1992). The family *Nocardiaceae*. In *The Prokaryotes*, 2nd edn, pp. 1188–1213. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.

Goodfellow, M. (1998). *Nocardia* and related genera. In *Topley and Wilson's Microbiology and Microbial Infections*, 9th edn, vol. 2, *Systematic Bacteriology*, pp. 463–489. Edited by A. Balows & B. I. Duerden. London: Arnold.

Goodfellow, M. & Orchard, V. A. (1974). Antibiotic sensitivity of some nocardioform bacteria and its value as a criterion for taxonomy. *J Gen Microbiol* **83**, 375–387.

Goodfellow, M., Alderson, G. & Chun, J. (1998). Rhodococcal systematics: problems and developments. *Antonie van Leeuwenhoek* **74**, 3–20.

Goodfellow, M., Isik, K. & Yates, E. (1999). Actinomycete systematics: an unfinished synthesis. *Nova Acta Leopold* **NF80**, (**312**) 47–82.

Gordon, R. E. (1967). The taxonomy of soil bacteria. In *The Ecology of Soil Bacteria*, pp. 293–321. Edited by T. R. G. Gray & D. Parkinson. Liverpool: Liverpool University Press.

Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J Bacteriol* **73**, 15–27.

Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.

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.

Gürtler, V., Smith, R., Mayall, B. C., Potter-Reinemann, G., Stackebrandt, E. & Kroppenstedt, R. M. (2001). *Nocardia veterana* sp. nov., isolated from human bronchial lavage. *Int J Syst Evol Microbiol* **51**, 933–936.

Hamid, M. E., Maldonado, L., Eldin, G. S. S., Mohamed, M. F., Saeed, N. S. & Goodfellow, M. (2001). *Nocardia africana* sp. nov., a new pathogen isolated from patients with pulmonary infections. *J Clin Microbiol* **39**, 625–630.

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.

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.

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*, Special Publication 6, pp. 227–291. 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.

Maldonado, L., Hookey, J. V., Ward, A. C. & Goodfellow, M. (2000). The *Nocardia salmonicida* clade, including descriptions of *Nocardia cummidelens* sp. nov., *Nocardia fluminea* sp. nov. and *Nocardia soli* sp. nov. *Antonie van Leeuwenhoek* **78**, 367–377.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **4**, 109–118.

McNeil, M. M. & Brown, J. M. (1994). The medically important aerobic actinomycetes: epidemiology and microbiology. *Clin Microbiol Rev* **7**, 357–417.

Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *J Gen Microbiol* **88**, 200–204.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & 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.

Orchard, V. A. (1979). Effect of sewage sludge additions on *Nocardia* in soil. *Soil Biol Biochem* **11**, 217–220.

Orchard, V. A. (1981). The ecology of *Nocardia* and related taxa. *Zentbl Bakteriol* Suppl. 11, 167–180.

Orchard, V. A., Goodfellow, M. & Williams, S. T. (1977). Selective isolation and occurrence of nocardiae in soil. *Soil Biol Biochem* **9**, 233–238.

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.

Saito, H. & Miura, K.-I. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.

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.

Society for American Bacteriologists (1957). *Manual of Microbiological Methods*. New York: McGraw-Hill.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.

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.

Tsukamura, M. (1966). Adansonian classification of mycobacteria. *J Gen Microbiol* **45**, 253–273.

Wang, L., Zhang, Y., Lu, Z., Shi, Y., Liu, Z., Maldonado, L. & Goodfellow, M. (2001). *Nocardia beijingensis* sp. nov., a novel isolate from soil. *Int J Syst Evol Microbiol* **51**, 1783–1788.

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.

Whipple, D. L., LeFebvre, R. B., Andrews, R. E., Jr & Thiermann, A. B. (1987). Isolation and analysis of restriction endonuclease digestive patterns of chromosomal DNA from *Mycobacterium paratuberculosis* and other *Mycobacterium* species. *J Clin Microbiol* **25**, 1511–1515.

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.

Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* (English translation of *Mikrobiologiya*) **16**, 176–178.

Yassin, A. F., Rainey, F. A. & Steiner, U. (2001). *Nocardia cyriacigeorgici* sp. nov. *Int J Syst Evol Microbiol* **51**, 1419–1423.