Nocardia xishanensis sp. nov., a novel actinomycete isolated from soil

Jianli Zhang,[1] Zhiheng Liu[2] and Michael Goodfellow[3]

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

[1]School of Life Science and Technology, Beijing Institute of Technology, Beijing 100081, People's Republic of China

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

[3]School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

The taxonomic position of a soil isolate, strain 276^T, was established using a polyphasic approach. The organism showed a range of chemical and morphological properties consistent with its classification in the genus *Nocardia*. An almost complete 16S rRNA gene sequence determined for the strain was aligned with corresponding sequences of representatives of the genus *Nocardia* and related taxa using three tree-making algorithms. The organism formed a distinct phyletic line within the evolutionary radiation occupied by the genus *Nocardia* and was most closely related to the type strain of *Nocardia abscessus*. However, the two strains shared a low DNA–DNA relatedness value and were readily distinguished using a combination of phenotypic properties. The combined genotypic and phenotypic data show that strain 276^T should be assigned to the genus *Nocardia* as a novel species. The name proposed for this new taxon is *Nocardia xishanensis* sp. nov. The type strain is 276^T (=CGMCC 4.1860^T=JCM 12160^T).

The genus *Nocardia* has had a long and troubled taxonomic history but is now well defined due to the application of chemotaxonomic, molecular genetic and numerical phenetic methods (Goodfellow *et al.*, 1999). At the time of writing the genus contains 37 species with validly published names, most of which have been described within the last 5 years. Members of many of the novel species have been implicated as agents of human disease (Hamid *et al.*, 2001, Yassin *et al.*, 2000, 2001a, b, 2003; Kageyama *et al.*, 2004a, b) though it is evident that nocardial diversity is grossly underestimated in environmental samples, notably soil (Orchard & Goodfellow, 1980; Wang *et al.*, 1999; Maldonado *et al.*, 2000). Nocardiae form part of the soil microflora and play a role in the turnover of organic matter (Orchard, 1981); there is also evidence that some strains produce secondary metabolites of potential industrial value (Isik *et al.*, 1999; Kinoshita *et al.*, 2001). The present investigation was designed to clarify the taxonomic position of a soil isolate, strain 276^T, using a polyphasic approach.

Strain 276^T was isolated on modified Sauton's agar plate (Mordarska *et al.*, 1972) that had been incubated at 28 °C for 8 days following inoculation with a suspension of a soil sample collected from Xishan Mountain, Beijing, China. The isolate was maintained on modified Sauton's agar slants at 4 °C and as suspensions of mycelial fragments in glycerol (20 %, v/v) at −20 °C. Biomass for chemotaxonomic and molecular genetic studies was prepared as described previously (Zhang *et al.*, 2002).

The colonial and micromorphological properties of the isolate were observed using standard procedures following inoculation on modified Sauton's agar plates for 7 days at 28 °C. The remaining phenotypic tests were determined using procedures described by Zhang *et al.* (2003).

Standard procedures were used for the extraction and analysis of the diagnostic isomers of diaminopimelic acid (A_2pm; Hasegawa *et al.*, 1983), mycolic acids (Minnikin *et al.*, 1975), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Lechevalier & Lechevalier, 1980), using appropriate controls. Isoprenoid quinones were extracted and purified after Collins *et al.* (1987); purified menaquinones were determined by reverse-phase HPLC (Wu *et al.*, 1989). The fatty acids were extracted, purified, methylated and quantified by GC using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product from strain 276^T were carried out after Rainey *et al.* (1996) and the purified PCR product was sequenced directly using the method of Lu *et al.* (2001). The resultant 16S rRNA gene sequence was aligned manually with corresponding sequences of representatives of the genera classified in the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 276^T is AY333115.

suborder *Corynebacterineae*, including all of the type strains of *Nocardia* species, retrieved from the DDBJ/EMBL/GenBank databases using the CLUSTAL X 1.8 program (Thompson *et al.*, 1997). Phylogenetic trees were generated using the least-squares, maximum-likelihood and neighbour-joining algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated after Kimura (1980). The topologies of the resultant unrooted trees were evaluated by bootstrap analyses of the neighbour-joining dataset, based on 1000 resamplings, using the SEQBOOT and CONSENSE options from the PHYLIP package.

Chromosomal DNA, extracted from biomass of strain 276^T following growth of the organism in modified Sauton's broth for 3 days at 28 °C, was purified using established procedures (Saito & Miura, 1963; 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. The extent of DNA–DNA relatedness between strain 276^T and *Nocardia abscessus* DSM 44432^T and *Nocardia asteroides* ATCC 19247^T was determined from the DNA–DNA liquid reassociation rate (De Ley *et al.*, 1970; Huß *et al.*, 1983) using a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermal controller and standard software (Jahnke, 1992); the results were expressed as the mean of two determinations.

Comparison of the almost complete 16S rRNA gene sequence (1411 nt) of isolate 276^T with those of representatives of the genera classified in the suborder *Corynebacterineae* show that it contains all of the signature nucleotides expected for members of this taxon (Stackebrandt *et al.*, 1997) and, more specifically, those characteristic of members of the family *Nocardiaceae* (Stackebrandt *et al.*, 1997) and the genus *Nocardia* (Chun & Goodfellow, 1995). The high 16S rRNA gene sequence similarities found between the tested organism and representatives of the species of the genus *Nocardia* with validly published names, namely 95·1–98·2 %, provide further evidence of its assignment to this taxon.

Strain 276^T has phenotypic properties typical of members of the genus *Nocardia* (Goodfellow *et al.*, 1999; Wang *et al.*, 2004). The organism is an aerobic, Gram-positive, slightly acid–alcohol-fast actinomycete which forms an extensively branched substrate mycelium that fragments into irregular, non-motile, coccoid and rod-shaped elements and which supports sparse to abundant white aerial hyphae on modified Sauton's agar. Whole-organism hydrolysates were rich in *meso*-A_2pm, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970) and contained major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid type II *sensu* Lechevalier *et al.*, 1977). The isolate also contained predominant amounts of hexahydrogenated menaquinone with eight isoprene units in which the two terminal isoprene moieties were cyclized; this menaquinone is characteristic of 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 a lower spot that corresponded to mycolic acids and a higher one corresponding to non-hydroxylated fatty acids. The mycolic acids of the tested strain co-migrated (R_f value around 0·47) with those from marker strains of *Nocardia*. The non-hydroxylated fatty acid profile consisted of straight-chain saturated, unsaturated and 10-methyl-branched components.

It is apparent from Fig. 1 that strain 276^T forms a distinct phyletic line within the 16S rRNA *Nocardia* gene tree. The organism is most closely related to the type strains of *N. abscessus* and *N. asteroides*. It shares 98·2 % 16S rRNA gene sequence similarity with the type strain of *N. abscessus* and 97·9 % with that of *N. asteroides*, values that correspond respectively to 25 and 29 nt differences at 1411 positions. Much higher 16S rRNA gene sequence similarities have been recorded between representatives of several *Nocardia* species with validly published names that have DNA–DNA relatedness values well below the 70 % cut-off point recommended for the delineation of genomic species by Wayne *et al.* (1987). The type strains of *Nocardia cerradoensis* and *Nocardia veterana*, for instance, share a 16S rRNA gene sequence similarity of 99 % and have a DNA–DNA relatedness value of 58 % (Albuquerque de Barros *et al.*, 2003). In the present study, strain 276^T showed a mean DNA–DNA relatedness value of 39 % with *N. abscessus* DSM 44432^T and 35 % with *N. asteroides* ATCC 19247^T. A range of phenotypic properties separated strain 276^T from the type strains of phylogenetically close species (Table 1).

It is evident from the combination of genotypic and phenotypic data that strain 276^T can be distinguished from all species of *Nocardia* with validly published names; hence, it is proposed that it be classified in the genus *Nocardia* as *Nocardia xishanensis* sp. nov.

Description of *Nocardia xishanensis* sp. nov.

Nocardia xishanensis (xi.shan.en′sis. N.L. fem. adj. *xishanensis* referring to Xishan Mountain, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, catalase-positive, slightly acid–alcohol-fast, non-motile actinomycete that forms an extensively branched substrate mycelium which fragments *in situ* into coccoid and rod-shaped elements. A yellow to orange substrate mycelium carries sparse to abundant, white aerial hyphae on modified Sauton's agar. Diffusible pigments are not formed. Colony elevation is convex to irregular and colony margins are filamentous. Nitrate is reduced and aesculin, arbutin and urea are hydrolysed. Tweens 20, 60 and 80 are degraded, but not adenine, casein, elastin, guanine, hypoxanthine, starch, tyrosine or xanthine. Growth occurs between 22 and 38 °C and from pH 5·5 to 10. Chemotaxonomic properties are typical of

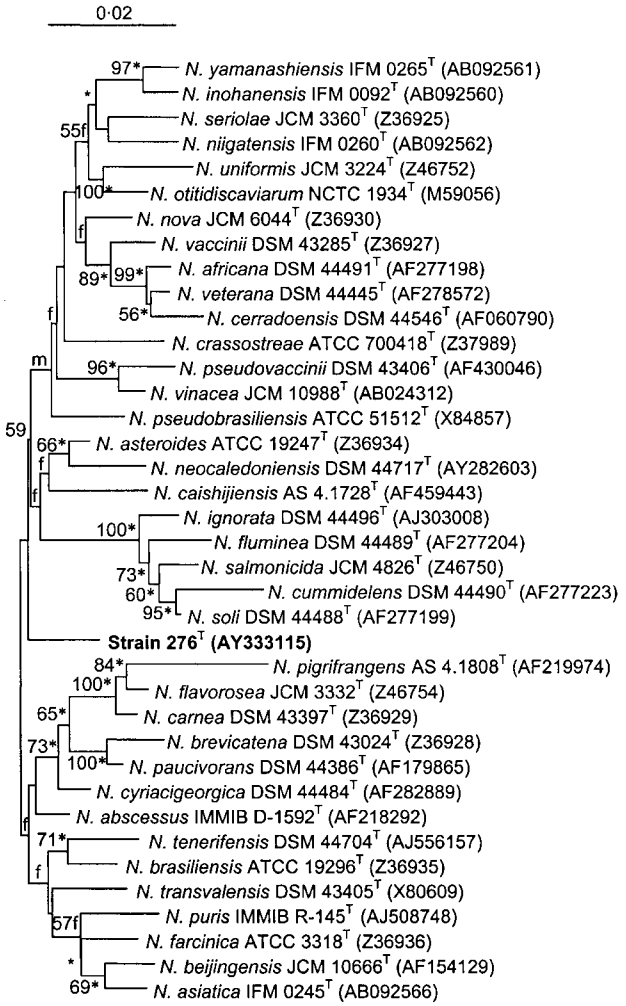

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain 276^T and representatives of species of *Nocardia* with validly published names. Asterisks indicate branches of the tree that were also recovered using the least-squares and maximum-likelihood tree-making algorithms. The symbols f and m denote branches of the tree that were also recovered using the least-squares and maximum-likelihood methods, respectively. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. The scale bar indicates 0·02 substitutions per nucleotide position.

Table 1. Phenotypic properties that distinguish strain 276^T from representatives of phylogenetically close *Nocardia* species

Strains: 1, strain 276^T; 2, *N. abscessus* DSM 44432^T; 3, *N. asteroides* ATCC 19247^T; 4, *Nocardia beijingensis* JCM 10666^T; 5, *Nocardia cyriacigeorgica* DSM 44484^T; 6, *Nocardia otitidiscaviarum* NCTC 1934^T; 7, *Nocardia paucivorans* DSM 44386^T; 8, *Nocardia pseudobrasiliensis* ATCC 51512^T. Data were taken from this and previous studies (Yassin *et al.*, 2000, 2001b; Zhang *et al.*, 2003). +, Positive; −, negative; ND, not determined. All strains are positive for urea hydrolysis and grow on 0·1 % (w/v) sodium acetate.

Property	1	2	3	4	5	6	7	8
Biochemical tests:								
Aesculin hydrolysis	+	−	+	+	+	+	−	+
Arbutin hydrolysis	+	+	+	−	ND	+	+	−
Nitrate reduction	+	+	+	+	+	+	+	−
Decomposition of (%, w/v):								
Adenine (0·4)	−	−	−	−	−	−	−	+
Casein (1·0)	−	−	−	−	−	−	−	+
Elastin (0·3)	−	−	−	−	−	−	−	+
Hypoxanthine (0·4)	−	−	−	−	−	+	−	+
Tyrosine (0·5)	−	−	−	−	−	−	−	+
Xanthine (0·4)	−	−	−	+	−	+	−	−
Growth on sole carbon sources (%, w/v):								
(+)-D-Cellobiose (1·0)	+	−	−	+	−	−	−	ND
(+)-D-Galactose (1·0)	+	−	−	+	−	−	−	+
(+)-D-Maltose (1·0)	+	+	−	+	−	−	−	ND
(+)-D-Mannitol (1·0)	−	−	+	+	−	+	−	+
α-L-Rhamnose (1·0)	+	+	−	+	−	−	−	−
(+)-D-Sorbitol (1·0)	+	−	−	+	−	−	−	+
Sodium citrate (0·1)	−	+	+	+	−	−	−	+
Growth at 45 °C	−	−	−	−	−	+	−	−

Nocardia. Acid is formed from (+)-D-glucose and glycerol but not from arbutin, (+)-D-cellobiose, (+)-D-fructose, (+)-D-galactose, *meso*-inositol, inulin, (+)-D-maltose, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (+)-D-raffinose, α-L-rhamnose, (−)-D-ribose, starch, (+)-D-sucrose, (+)-D-trehalose, (+)-D-turanose or (+)-D-xylose. (+)-D-Cellobiose, (+)-D-fructose, (+)-D-fucose, (+)-D-galactose, (+)-D-glucose, glycerol, *meso*-inositol (weak), (+)-D-maltose, (+)-D-mannose (weak), (+)-D-melibiose, α-L-rhamnose, (−)-D-ribose, (+)-D-salicin, (+)-D-sorbitol (weak), starch (weak), (+)-D-sucrose, (+)-D-trehalose, acetate, adipic acid, fumarate, lactate, malate, paraffin, pyruvate and succinate are used as sole carbon and energy sources, but not adonitol, (+)-L-arabinose, (+)-D-arabitol, arbutin, dulcitol, *meso*-erythritol, ethanol, glycogen, inulin, (+)-D-lactose, (+)-D-mannitol, (+)-D-melezitose, methyl α-D-glucoside, (+)-D-raffinose, xylitol, (+)-D-xylose, benzoate, citrate, formate, hippurate, malonate, sebacic acid, oxalate or tartrate. L-Alanine, L-proline and L-valine are used as sole carbon and nitrogen sources but not acetamide, L-asparagine, L-aspartate, gelatin, D-glucosamine, L-leucine, L-phenylalanine or L-serine. Growth occurs in the presence of sodium chloride at 3 %, w/v but not at 5 %, w/v. Resistance is shown to lysozyme, gentamicin sulphate (10 μg ml^{-1}) and penicillin G (10 U), but not to chloramphenicol (30 μg ml^{-1}), erythromycin (15 μg ml^{-1}), midecamycin (15 μg ml^{-1}), minocycline hydrochloride (30 μg ml^{-1}), rifampicin (5 μg ml^{-1}), streptomycin sulphate (10 μg ml^{-1}), tobramycin sulphate (10 μg ml^{-1}) or vancomycin hydrochloride (30 μg ml^{-1}). The fatty acid profile is composed of $C_{16:0}$ (33·1 %), $C_{18:0}$ (7·6 %),

cis-9-$C_{18:1}$ (32·7 %) and 10-methyl-$C_{18:0}$ (11·3 %). The G+C content of the DNA is 68·8 mol%.

The type strain, 276^T (=CGMCC 4.1860^T=JCM 12160^T), was isolated from a soil sample collected from Xishan Mountain, Beijing, China. The species description is based on a single strain and hence serves as the description of the species.

Acknowledgements

This work was supported through the Royal Society–Chinese Academy of Sciences Exchange Scheme (grant no. Q814) and by the University Basic Fund of Beijing Institute of Technology of China (grant no. BIT-UBF-200306C08). The authors are grateful to Dr T. Kudo (JCM) and Professor R. M. Kroppenstedt (DSMZ) for providing some of the type strains of *Nocardia* species. We also thank Dr Yajun Song for carrying out the quantitative fatty acid analysis.

References

Albuquerque de Barros, E. V. S., Manfio, G. P., Ribeiro Maitan, V., Mendes Bataus, L. A., Kim, S. B., Maldonado, L. & 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., 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 measurements of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.

Goodfellow, M., Isik, K. & Yates, E. (1999). Actinomycete systematics: an unfinished synthesis. *Nova Acta Leopold* **NF 80** (**312**), 47–82.

Hamid, M. E., Maldonado, L., Sharaf Eldin, G. 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.

Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.

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.

Isik, K., Chun, J., Hah, Y. C. & Goodfellow, M. (1999). *Nocardia uniformis* nom. rev. *Int J Syst Bacteriol* **49**, 1227–1230.

Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from a Gilford System 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

Kageyama, A., Poonwan, N., Yazawa, K., Mikami, Y. & Nishimura, K. (2004a). *Nocardia asiatica* sp. nov., isolated from patients with nocardiosis in Japan and clinical specimens from Thailand. *Int J Syst Evol Microbiol* **54**, 125–130.

Kageyama, A., Yazawa, K., Nishimura, K. & Mikami, Y. (2004b). *Nocardia inohanaensis* sp. nov., *Nocardia yamanashiensis* sp. nov. and *Nocardia niigatensis* sp. nov. isolated from clinical specimens. *Int J Syst Evol Microbiol* **54**, 563–569.

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.

Kinoshita, N., Homma, Y., Igarashi, M., Ikeno, S., Hori, M. & Hamada, M. (2001). *Nocardia vinacea* sp. nov. *Actinomycetologica* **15**, 1–5.

Lechevalier, H. A. & Lechevalier, M. P. (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 base composition of deoxyribonucleic acid from its denaturation temperature. *J Mol Biol* **5**, 109–118.

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, 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.

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

Orchard, V. A. & Goodfellow, M. (1980). Numerical classification of some named strains of *Nocardia asteroides* and related isolates from soil. *J Gen Microbiol* **118**, 295–312.

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 *Nocardiopsiaceae* fam. nov. *Int J Syst Bacteriol* **46**, 28–96.

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

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids.* Technical Note 101. Newark, DE: Microbial ID.

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.

Wang, Y., Zhang, Z., Ruan, J., Wang, Y. & Ali, S. (1999). Investigations of actinomycete diversity in the tropical rainforests of Singapore. *J Ind Microbiol Biotechnol* **23**, 178–187.

Wang, L., Zhang, Y., Huang, Y., Maldonado, L. A., Liu, Z. & Goodfellow, M. (2004). *Nocardia pigrifrangens* sp. nov., a novel actinomycete isolated from a contaminated plate. *Int J Syst Evol Microbiol* **54**, 1683–1686.

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., Le Febvre, 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.

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., Mendroch, U., Brzezinka, H. & Schaal, K. P. (2000). *Nocardia abscessus* sp. nov. *Int J Syst Evol Microbiol* **50**, 1487–1493.

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

Yassin, A. F., Rainey, F. A. & Steiner, U. (2001b). *Nocardia ignorata* sp. nov. *Int J Syst Evol Microbiol* **51**, 2127–2131.

Yassin, A. F., Sträubler, B., Schumann, P. & Schaal, K. P. (2003). *Nocardia puris* sp. nov. *Int J Syst Evol Microbiol* **53**, 1595–1599.

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.