NOTE

***Pseudonocardia spinosispora* sp. nov., isolated from Korean soil**Soon Dong Lee,^{1,2†} Eun Suk Kim,^{1,3} Sa-Ouk Kang¹ and Yung Chil Hah¹

¹ School of Biological Sciences and Research Centre for Molecular Microbiology, Seoul National University, Seoul 151-742, Republic of Korea

² Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, USA

³ T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40503, USA

Author for correspondence: Soon Dong Lee. Tel: +82 64 754 3282. Fax: +82 64 725 4902. e-mail: sdlee@cheju.ac.kr

A new strain of the genus *Pseudonocardia*, which was isolated from a Korean soil sample, is described. The organism shows distinct cultural characteristics in that it forms well-developed aerial mycelium that fragments into rod-shaped, spiny spores, and grows only in standing culture but not in shake culture. Substrate mycelium is sparse or absent in most media used. Phylogenetic analyses based on 16S rDNA sequences reveal that this isolate forms a distinct clade within the radiation encompassing the genera *Pseudonocardia* and *Actinobispora* of the family *Pseudonocardiaceae*. The affiliation to the genus *Pseudonocardia* is also supported by the cell chemistry, which is represented by the presence of meso-diaminopimelic acid, arabinose and galactose in the cell wall, tetrahydrogenated menaquinone with eight isoprene units as a predominant menaquinone, and phosphatidylcholine as a diagnostic phospholipid. The levels of 16S rDNA sequence similarity found between the isolate and the type strain of validly described species in the genus *Pseudonocardia* ranged from 94.8 to 96.3%. The organism is also readily differentiated from all of the validated species of the genus *Pseudonocardia* by means of a battery of phenotypic tests. The name proposed for the new species is *Pseudonocardia spinosispora* sp. nov. The type strain is LM 141^T (= IMSNU 50581^T = KCTC 9991^T = NRRL B-24156^T).

Keywords: *Pseudonocardia spinosispora* sp. nov., actinomycete, chemotaxonomy, phylogenetic analysis

The genus *Pseudonocardia* was proposed by Henssen (1957) for mycolate-less, nocardioform actinomycetes that contained meso-diaminopimelic acid, arabinose and galactose in the peptidoglycan (a type IV cell wall; Lechevalier & Lechevalier, 1970) and currently contains 11 validly described species: *Pseudonocardia alni*, *Pseudonocardia asaccharolytica*, *Pseudonocardia autotrophica*, *Pseudonocardia compacta*, *Pseudonocardia hydrocarbonoxydans*, *Pseudonocardia halophobica*, *Pseudonocardia petroleophila*, *Pseudonocardia saturnea*, *Pseudonocardia spinosa*, *Pseudonocardia sulfidoxydans* and *Pseudonocardia thermophila* (Henssen & Schäfer, 1971; Reichert *et al.*, 1998). Recently, a new member of the genus *Pseudonocardia*, *Pseudonocardia*

kongjuensis, was proposed (Lee *et al.*, 2001). ‘*Pseudonocardia nitrificans*’, which was described as a new species of this genus by Warwick *et al.* (1994), was shown to be a strain of *P. alni* on the basis of 16S rDNA sequence and physiological properties (Lee *et al.*, 2001).

The genus *Pseudonocardia* is morphologically characterized by the formation of well-developed vegetative and aerial mycelium, from which spore chains are produced by acropetal budding or fragmentation. It also has meso-diaminopimelic acid, arabinose and galactose in the cell wall (a type IV cell wall composition; Lechevalier & Lechevalier, 1970), tetrahydrogenated menaquinone with eight isoprene units [MK-8(H₄)] as a major menaquinone, a DNA G+C content of 68–79 mol%, no mycolic acids, and a phospholipid type PII (phosphatidylethanolamine or its derivatives as a diagnostic phospholipid) or PIII (phosphatidylcholine) pattern, according to the species (Lechevalier *et al.*, 1981). Phylogenetically, the genus

[†] Present address: Department of Science Education, Cheju National University, Jeju 690-756, Republic of Korea.

The EMBL accession number for the 16S rDNA sequence of strain LM 141^T (= IMSNU 50581^T = KCTC 9991^T = NRRL B-24156^T) is AJ249206.

Pseudonocardia forms a distinct lineage within the radiation of the family *Pseudonocardiaceae* (Warwick *et al.*, 1994; Reichert *et al.*, 1998). However, our recent study shows that members of the genera *Pseudonocardia* and *Actinobispora* (Jiang *et al.*, 1991) of the family *Pseudonocardiaceae* are intermixed on the basis the 16S rDNA sequences (Lee *et al.*, 2000a).

During the taxonomic study of soil actinomycete isolates, strain LM 141^T was recovered from a soil sample from a gold mine cave near Kongju City, Korea. The present investigation was designed to establish the taxonomic characterization and classification of this organism. Phenotypic and genotypic data show that this strain should be recognized as a new species of the genus *Pseudonocardia*, for which the name *Pseudonocardia spinospora* sp. nov. is proposed.

Strain LM 141^T was isolated by the dilution plating of a soil suspension on tap-water agar (Lee, 1996). The soil sample was collected from a gold mine cave located in Kongju City, Korea. The isolate was maintained on yeast/malt-extract agar (medium 2 of the International *Streptomyces* Project) (Shirling & Gottlieb, 1966) at 4 °C and glycerol suspension (20%, v/v) at -20 °C. The isolate was lyophilized for long-term storage and maintained at the Institute of Microbiology, Seoul National University (IMSNU), Korea as IMSNU 50581^T. The following *Pseudonocardia* species were used for the comparison: *P. alni* IFO 14491^T (= IMSNU 20049^T), *P. asaccharolytica* DSM 44247^T (= IMSNU 22142^T), *P. autotrophica* IFO 12743^T (= IMSNU 20050^T), *P. compacta* IFO 14343^T (= IMSNU 20111^T), *P. hydrocarbonoxydans* DSM 43281^T (= IMSNU 22140^T), *P. halophobica* DSM 43089^T (= IMSNU 21327^T), *P. petroleophila* DSM 43193^T (= IMSNU 22072^T), *P. saturnea* IFO 14499^T (= IMSNU 20052^T), *P. sulfidoxydans* DSM 44248^T (= IMSNU 22143^T) and *P. thermophila* IFO 12133^T (= IMSNU 20112^T). *P. spinosa* JCM 3136^T was purchased from the Japan Collection of Microorganisms (JCM). Biomass for chemical analysis was obtained by growing isolate LM 141^T in a standing culture of trypticase soy broth (BBL) for 14 days at 30 °C. *P. spinosa* was grown in yeast/starch agar (yeast extract, 0.2%; soluble starch, 1%; agar, 1.5%; pH 7.3) for 21 days at 30 °C. The other *Pseudonocardia* reference strains were cultivated in shake flasks of trypticase soy broth (BBL) for 3 days at 30 °C. After harvesting, the cells were washed twice with distilled water and stored at room temperature by freeze-drying before use.

The cell morphology of isolate LM 141^T was observed by light and scanning electron microscopy of 14-day cultures grown on inorganic salts/starch agar (International *Streptomyces* Project medium 4; Difco). The ornamentation of the spore surface was determined by using a Stereoscan model 260 scanning electron microscope. Physiological characteristics were investigated as described previously (Lee *et al.*, 2000b).

Analysis of the isomer of diaminopimelic acid (A₂pm), the acyl type of muramic acid, whole-cell sugars, mycolic acids, fatty acids and phospholipids in strain LM 141^T was performed as described previously (Lee *et al.*, 1999). All the reference strains, except *P. spinosa*, were also subjected to phospholipid analysis. For genomic characterization, strain LM 141^T was grown in a standing culture of YD (0.1% yeast extract, 0.1% glucose) broth for 14 days at 30 °C. The cells were harvested by filtration, washed twice with distilled water, then stored at 4 °C before DNA extraction. After the cells had been disintegrated several times with small beads, the genomic DNA was extracted and purified by the previously described method (Lee *et al.*, 2000a). The G+C content of the genomic DNA was determined by HPLC, as described previously (Mesbah *et al.*, 1989).

The 16S rDNA sequence of strain LM 141^T was determined as described previously (Lee *et al.*, 2000b) and initially compared with those of all the representatives of the families *Pseudonocardiaceae* and *Actinosynnemataceae* of the class *Actinobacteria* by using the CLUSTAL X program (Thompson *et al.*, 1997). The aligned sequences were further optimized by manual comparison with the *Escherichia coli* sequence. The final phylogenetic tree was generated on the basis of those of members of the genera *Pseudonocardia* and *Actinobispora* with *Amycolatopsis albidoflavus* (AJ252832). The evolutionary distances were calculated by the method of Jukes & Cantor (1969) and the phylogenetic tree was constructed by the neighbour-joining method of Saitou & Nei (1987) contained in the PHYLIP package of Felsenstein (1993). The sequences were also subjected to parsimony analysis using the PAUP program for Macintosh computers (Swofford, 1998). The topologies of the resulting trees were evaluated by bootstrap analysis of the data with 1000 resamplings. Evolutionary trees were displayed using version 1.5 of the TREEVIEW program of Page (1996).

Chemotaxonomic and molecular systematic data indicate that strain LM 141^T belongs to the genus *Pseudonocardia*. An almost complete 16S rDNA sequence determined for isolate LM 141^T (1512 nucleotides) was initially compared with all of the representatives of the families *Pseudonocardiaceae* and *Actinosynnemataceae*. Strain LM 141^T fell within the radiation encompassing members of the family *Pseudonocardiaceae*. An abbreviated phylogenetic tree (Fig. 1) showed that strain LM 141^T formed a distinct lineage within the radius of the genera *Pseudonocardia* and *Actinobispora* of the family *Pseudonocardiaceae*. Phylogenetic analysis using the parsimony method generated similar tree topologies. The 16S rDNA sequence of isolate LM 141^T also showed the sets of 16S rDNA signature nucleotides previously proposed for the family *Pseudonocardiaceae* (Stackebrandt *et al.*, 1997) and the genera *Pseudonocardia*-*Actinobispora* (Lee *et al.*, 2000b). The exceptions were 139-224 (U-A) nucleotide pairs and the 1336 (C) nucleotide.

Fig. 1. Abbreviated phylogenetic tree showing the relationship between strain LM 141^T and members of the genera *Pseudonocardia* and *Actinobispora* of the family *Pseudonocardiaceae*. The tree was based on a comparison of 1441 unambiguous nucleotides. The bootstrap values indicated at the branching points are expressed as percentages out of 1000 replications. Asterisks indicates branching nodes that were also recovered in the parsimony analysis. The scale bar indicates one nucleotide substitution per 100 nucleotides.

This evolutionary relationship of isolate LM141^T is well reflected in the cell chemistry, most of which is in agreement with that of the genus *Pseudonocardia*, in that it has a type IV cell wall composition (containing *meso*-diaminopimelic acid, arabinose and galactose; Lechevalier & Lechevalier, 1970), an *N*-acetylated type of muramic acid and MK-8(H₄) as the predominant menaquinone. The phospholipid profiles of isolate LM 141^T showed that it contains phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylmethylethanolamine, hydroxy-phosphatidylethanolamine and an unknown phospholipid (the type PIII pattern of Lechevalier *et al.*, 1981). The combination of the chemical markers supports the view that strain LM 141^T belongs to the genus *Pseudonocardia* and can be differentiated from members of all other genera having a type IV cell wall (Embley, 1992; Jiang *et al.*, 1991; Tamura *et al.*, 1995; Kim & Goodfellow, 1999).

Sequencing of the 16S rDNA has provided a rapid and precise way of determining the taxonomic position of the environmental isolate (Lee *et al.*, 1999). Phylogenetic analysis (Fig. 1) indicates that strain LM 141^T occupies a distinct place within the radiation encompassing the genera *Actinobispora* and *Pseudonocardia*

of the family *Pseudonocardiaceae*. Our previous study (Lee *et al.*, 2000a) suggested the unification of the genera *Actinobispora* and *Pseudonocardia* on the basis of the combination of 16S rDNA-based phylogenetic clustering and the presence of 16S rDNA signature nucleotides consistent for both genera, as well as the coherence of most chemical markers. The nucleotide sequence of strain LM 141^T shows substantial differences from the corresponding sequences of members of both genera. Pairwise comparison of 1441 unambiguous nucleotide positions reveals that isolate LM 141^T has levels of 16S rDNA sequence similarity ranging from 94.8 to 96.3 % with the type species of the genus *Pseudonocardia*, and ranging from 94.9 to 96.9 % with the type species of the genus *Actinobispora*, showing that the isolate may constitute a new phylogenetic nucleus within the genus *Pseudonocardia*. The nearest phylogenetic neighbour was *P. asaccharolytica* (the level of sequence similarity being 95.5 %). However, this relationship was supported by only a low bootstrap value (39 %).

Members of the genus *Pseudonocardia* are divided into two groups according to their phospholipid patterns. One group is characterized by the presence of phosphatidylcholine (a type PIII pattern) and contains the isolate and eight other species (*P. alni*, *P. autotrophica*,

Fig. 2. Scanning electron micrograph of strain LM 141^T grown on inorganic salts/starch agar (medium 4 of the International *Streptomyces* Project) for 14 days at 28 °C. Bar, 2 μm.

P. compacta, *P. hydrocarbonoxydans*, *P. kongjuensis*, *P. petroleophila*, *P. saturnea* and *P. thermophila*), whereas the other group possesses nitrogenous phospholipids (a type PII pattern) in the polar lipid profiles and is composed of *P. asaccharolytica*, *P. halophobica* and *P. sulfidoxydans* (Embley, 1992; Reichert *et al.*, 1998; Lee *et al.*, 2001; this study). Strain LM 141^T is distinguished from the nearest phylogenetic neighbour, *P. asaccharolytica*, in that the isolate contains phosphatidylcholine as a diagnostic phospholipid.

In addition to molecular systematic data, isolate LM 141^T can be readily differentiated from members of the validly described *Pseudonocardia* species by using a combination of morphological (Fig. 2), cultural and physiological characteristics (Table 1). Strain LM 141^T is morphologically similar to the type strain of *P. spinosa*, in that both organisms have non-motile, spiny spores, whereas all the other type strains of the genus *Pseudonocardia* are morphologically characterized by the presence of non-motile, smooth spores. *P. spinosa*, which was originally proposed by Henssen & Schäfer (1971), was listed in the approved lists of bacterial names (Skerman *et al.*, 1980), but it was thereafter reported that the culture of *P. spinosa* is no longer viable and was therefore unavailable for further study (Embley, 1992). However, the type strain of *P. spinosa* is still maintained only in one of the public culture collections, i.e. the JCM. The aerial mycelium of strain LM 141^T was well developed, and fragmented into rod-shaped elements (approx. 0.54×2.25 μm). The spore surfaces were covered with numerous spines (Fig. 2). The vegetative hyphae were sparse or absent on most agar media used. As the culture aged, the reverse of the colony was a deep brownish colour. The mat-like mass structures were produced on the surface of liquid culture broth. These morphological and cultural characteristics of *P. spinosa* had been previously described in detail (Henssen & Schäfer, 1971). Strain LM 141^T differed morphologically from *P.*

Table 1. Characteristics that differentiate isolate LM 141^T from reference strains of the genus *Pseudonocardia*

1, Isolate LM 141^T; 2, *P. asaccharolytica* IMSNU 22142^T; 3, *P. compacta* IMSNU 20111^T; 4, *P. saturnea* IMSNU 20052^T; 5, *P. thermophila* IMSNU 20112^T. All results for *P. spinosa* JCM 3136^T are negative.

Characteristic	1	2	3	4	5
Acid produced from:					
L-Arabinose	+	—	—	+	+
D-Cellobiose	—	—	+	+	+
D-Fructose	+	—	+	+	+
D-Galactose	—	—	—	—	+
D-Glucose	+	—	+	+	+
Maltose	—	—	—	+	+
D-Mannose	+	—	—	+	—
D-Melezitose	—	—	—	+	+
Methyl α-D-glucoside	—	—	—	+	—
L-Rhamnose	+	—	—	—	+
Salicin	—	—	—	—	+
Sucrose	—	—	—	+	—
D-Trehalose	—	—	—	+	+
D-Xylose	+	—	—	+	+
Adonitol	+	—	—	—	+
meso-Erythritol	+	—	—	—	+
meso-Inositol	+	—	—	+	+
D-Mannitol	—	—	+	+	+
1,2-Propanediol	—	+	—	+	+
D-Sorbitol	—	—	—	—	+
D-Xylitol	+	—	—	—	+
Enzyme activity:					
H ₂ S production	+	—	—	+	+
Urease	+	—	—	+	+
Decomposition of tyrosine:	—	—	—	+	—
Hydrolysis of:					
Casein	—	—	—	—	+
Gelatin	—	—	—	+	—
Starch	—	—	—	—	+
Growth at 37 °C	—	—	—	+	+
Growth on 3 % NaCl	—	—	—	+	+

spinosa in that the cells of the isolate did not produce a zig-zag-shaped morphology and a yellow pigment, and it showed growth only in standing culture but not in shake culture; *P. spinosa* did not grow in liquid culture, only on agar plates.

The physiological profile differentiating isolate LM 141^T from the reference strains of the genus *Pseudonocardia* is shown in Table 1. With respect to the reference strains, *P. spinosa* had been previously characterized for a few physiological characteristics, such as the absence of growth at 37 °C and the ability to hydrolyse casein and starch (Henssen & Schäfer, 1971). In our study, the type strain of *P. spinosa* showed a negative response for all of the physiological characteristics tested when the 14-day cultures obtained from yeast/

starch agar plate were used as inoculum sources (Table 1). The other reference strain of the genus *Pseudonocardia*, *P. assacharolytica*, also produced negative results for most of the characteristics. The exception was acid production from 1,2-propanediol, which had not been described previously (Reichert *et al.*, 1998). The physiological properties of both species are very similar. However, isolate LM 141^T is readily distinguished from its nearest phylogenetic neighbour, *P. assacharolytica*, and the spiny spore-producer *P. spinosa* in terms of acid-production profiles from carbohydrates and enzyme activities such as H₂S production and urease.

The genotypic and phenotypic data show that isolate LM 141^T forms a new nucleus of taxonomic variation within the genus *Pseudonocardia*. It is proposed that the isolate be recognized as a new species of the genus *Pseudonocardia*, namely *Pseudonocardia spinosispora* sp. nov.

Description of *Pseudonocardia spinosispora* sp. nov.

Pseudonocardia spinosispora (spi.no.si.spo'ra. L. adj. *spinus* thorny; Gr. fem. n. *spora* seed; N.L. fem. n. *spora* spore; N.L. fem. n. *spinosispora* thorny spore).

Aerobic, Gram-positive, non-acid/alcohol-fast. Catalase-positive. Urease-positive. Nitrate is reduced to nitrite. Growth occurs between 4 and 30 °C. Growth does not occur at or above 37 °C. H₂S production is observed. Forms a white aerial mycelium that fragments into spiny, rod-shaped spores. Substrate mycelium is sparse or absent on most media used. Growth occurs only in standing culture but not in shake culture. Produces acid from L-arabinose, D-fructose, D-glucose, D-mannose, L-rhamnose, D-xylose, adonitol, *meso*-erythritol, glycerol, *meso*-inositol and D-xylitol. Does not decompose adenine, hypoxanthine, DL-tyrosine or xanthine. Does not produce acid from D-cellobiose, D-galactose, D-lactose, maltose, D-melbitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, D-raffinose, salicin, L-sorbose, sucrose, D-trehalose, 2,3-butanediol, dulcitol, D-mannitol, 1,2-propanediol or D-sorbitol. Does not hydrolyse casein, gelatin or starch. Growth does not occur on 3 % NaCl. Type III phospholipid pattern (phosphatidylcholine is a diagnostic phospholipid). The fatty acid profiles contain i-C_{16:0} (33.8%), i-C_{15:0} (13.8%), i-C_{17:0} (10.0%), C_{17:1} (9.5%), i-C_{16:1} (7.4%), i-C_{17:1} (7.2%) and C_{16:1} (6.0%) acids. A small amount of 10-methyl branched fatty acids (10Me-C_{16:0}, 2.7%, and 10Me-C_{17:0}, 1.3%) is also detected, but tuberculostearic acid or hydroxy fatty acids are not present. The G+C content of the DNA is 70.4 mol%. Isolated from soil from a gold mine cave near Kongju City, Korea. The type strain is strain LM 141^T (= IMSNU 50581^T = KCTC 9991^T = NRRL B-24156^T).

Acknowledgements

The Brain Korea 21 Project supported this work for the School of Biological Sciences, Seoul National University.

We thank Dr Takuji Kudo for his helpful advice on the cultivation of *P. spinosa*.

References

- Embley, T. M. (1992). The family *Pseudonocardiaceae*. In *The Prokaryotes*, pp. 996–1027. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3. Seattle: Department of Genetics, University of Washington.
- Henssen, A. (1957). Beiträge zur Morphologie und Systematik der thermophilen Actinomyceten. *Arch Mikrobiol* **26**, 373–414.
- Henssen, A. & Schäfer, D. (1971). Emended description of the genus *Pseudonocardia* Henssen and description of a new species *Pseudonocardia spinosa* Schäfer. *Int J Syst Bacteriol* **21**, 29–34.
- Jiang, C., Xu, L., Yang, Y.-R., Guo, G.-Y., Ma, J. & Liu, Y. (1991). *Actinobispora*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **41**, 526–528.
- 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.
- Kim, S. B. & Goodfellow, M. (1999). Reclassification of *Amycolatopsis rugosa* Lechevalier *et al.* 1986 as *Prauserella rugosa* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 507–512.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. *Zentbl Bakteriol Hyg Abt 1 Suppl* **11**, 111–116.
- Lee, S. D. (1996). Classification of novel actinomycetes from gold mine cave in Kongju, Korea. PhD thesis, Seoul National University.
- Lee, S. D., Goodfellow, M. & Hah, Y. C. (1999). A phylogenetic analysis of the genus *Catellatospora* based on 16S ribosomal DNA sequences, including transfer of *Catellatospora matsumotoense* to the genus *Micromonospora* as *Micromonospora matsumotoense* comb. nov. *FEMS Microbiol Lett* **178**, 349–354.
- Lee, S. D., Kim, E. S. & Hah, Y. C. (2000a). Phylogenetic analysis of the genera *Pseudonocardia* and *Actinobispora* based on the 16S rDNA sequence studies. *FEMS Microbiol Lett* **182**, 125–129.
- Lee, S. D., Kim, E. S., Roe, J.-H., Kim, J.-H., Kang, S.-O. & Hah, Y. C. (2000b). *Saccharothrix violacea* sp. nov., isolated from a gold mine cave, and *Saccharothrix albidocapillata* comb. nov. *Int J Syst Evol Microbiol* **50**, 1315–1323.
- Lee, S. D., Kim, E. S., Min, K.-L., Lee, W. Y., Kang, S.-O. & Hah, Y. C. (2001). *Pseudonocardia kongjuensis* sp. nov., isolated from a gold mine cave. *Int J Syst Evol Microbiol* **51**, 1505–1510.
- 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.
- Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *CABIOS* **12**, 357–358.
- Reichert, K., Lipski, A., Pradella, S., Stackebrandt, E. & Altendorf, K. (1998). *Pseudonocardia asaccharolytica* sp. nov. and *Pseudonocardia sulfidoxydans* sp. nov., two new dimethyl disulfide-degrading actinomycetes and emended description of the genus *Pseudonocardia*. *Int J Syst Bacteriol* **48**, 441–449.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.
- 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.

Swofford, D. (1998). PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0. Sunderland, MA: Sinauer.

Tamura, T., Hayakawa, M., Nonomura, H., Yokota, A. & Hatano, K. (1995). Four new species of the genus *Actinokineospora*: *Actinokineospora inagensis* sp. nov., *Actinokineospora globicatena* sp. nov., *Actinokineospora terrae* sp. nov., and *Actinokineospora diospyrosa* sp. nov. *Int J Syst Bacteriol* **45**, 371–378.

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* **24**, 4876–4882.

Warwick, S., Bowen, T., McVeigh, H. & Embley, T. M. (1994). A phylogenetic analysis of the family *Pseudonocardiaceae* and the genera *Actinokineospora* and *Saccharothrix* with 16S rRNA sequences and a proposal to combine the genera *Amycolata* and *Pseudonocardia* in an emended genus *Pseudonocardia*. *Int J Syst Bacteriol* **44**, 293–299.