Actinomadura catellatispora sp. nov. and *Actinomadura glauciflava* sp. nov., from a sewage ditch and soil in southern China

Zhitang Lu,^{1†} Liming Wang,¹ Yamei Zhang,¹ Yanlin Shi,¹ Zhiheng Liu,¹ Erika T. Quintana² and Michael Goodfellow²

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

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

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

Two soil isolates, strains 80-60^T and 3.24^T, were shown to have chemical and morphological properties consistent with their classification in the genus *Actinomadura*. The almost complete 16S rDNA sequences generated for the two organisms were aligned with available sequences of representatives of the genus *Actinomadura* and related taxa. It was apparent from the resultant phylogenetic trees that each of the strains formed a distinct phyletic line within the evolutionary radiation occupied by the genus *Actinomadura*. The two organisms could also be distinguished from one another and from representatives of all the validly described species of *Actinomadura* using a set of phenotypic properties. It is proposed that strains 3.24^T (= AS 4.1522^T = IFO 16341^T) and 80-60^T (= AS 4.1202^T = IFO 14668^T = JCM 16161^T) be classified in the genus *Actinomadura* as *Actinomadura catellatispora* sp. nov. and *Actinomadura glauciflava* sp. nov., respectively.

INTRODUCTION

Phylogenetic analyses using sequences of 16S rDNA, 23S rDNA and 16S–23S rRNA gene spacers have helped to clarify relationships between members of the family *Thermomonosporaceae* (Stackebrandt *et al.*, 1997; Zhang *et al.*, 1998, 2001). This taxon currently includes the genera *Actinocorallia* Iinuma *et al.* 1994, *Actinomadura* Lechevalier and Lechevalier 1968, *Spirillospora* Couch 1963 and *Thermomonospora* Henssen 1957, the type genus. Members of these taxa share a range of chemotaxonomic and molecular systematic properties that distinguish them from other actinomycetes (Zhang *et al.*, 1998, 2001; Miyadoh & Miyara, 2001), including near relatives classified in the families *Nocardiopsaceae* (Rainey *et al.* 1996) emend. Zhang *et al.* 2001 and *Streptosporangiaceae* (Goodfellow *et al.* 1990) emend. Stackebrandt *et al.* 1997.

Organisms previously classified as *Nocardia dassonvillei*, *Nocardia madurae* and *Nocardia pelletieri* were the founder

members of the genus *Actinomadura* (Lechevalier & Lechevalier, 1968), though *Actinomadura dassonvillei* was subsequently reclassified as *Nocardiopsis dassonvillei* (Meyer, 1976). Following its introduction, the genus *Actinomadura* became a dumping ground for an assortment of aerobic, Gram-positive, non-acid-fast, non-motile sporoactinomycetes that had cell walls rich in *meso*-diaminopimelic acid (*meso*-A₂pm), but lacking diagnostic sugars (wall chemotype III *sensu* Lechevalier & Lechevalier, 1970). The application of chemotaxonomic, molecular systematic and numerical phenetic procedures clearly showed that the genus was in need of taxonomic revision (Athalye *et al.*, 1985; Fischer *et al.*, 1983; Poscher *et al.*, 1985; Kroppenstedt *et al.*, 1990), a task that was initiated by the extensive studies of Zhang *et al.* (1998, 2001).

The revised genus *Actinomadura* accommodates aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes that typically form non-fragmenting, extensively branched substrate mycelia and aerial hyphae that differentiate into short to long, straight, hooked or spiral chains of spores with either folded, irregular, smooth, spiny or warty spores. Members of the genus *Actinomadura* contain *meso*-A₂pm, the sugars galactose, glucose, madurose, mannose and ribose, major proportions of hexahydrogenated menaquinones with nine isoprene units, complex fatty acids, including hexadecanoic, 14-methylpentadecanoic and

[†]Present address: College of Life Science, Hebei University, Baoding 071002, People's Republic of China.

Abbreviation: *meso*-A₂pm, *meso*-diaminopimelic acid.

The GenBank accession numbers for the 16S rDNA sequences of *A. catellatispora* 3.24^T, *A. glauciflava* 80-60^T, *A. latina* DSM 43382^T and *A. nitritigenes* DSM 44137^T are respectively AF154127, AF153881, AY035998 and AY035999.

10-methyloctadecanoic acids as predominant components and diphosphatidylglycerol and phosphatidylinositol as major phospholipids (Kroppenstedt *et al.*, 1990).

The genus presently contains 28 validly described species, though there is evidence that *Actinomadura spadix* may merit generic status (Athalye *et al.*, 1985; Ochi *et al.*, 1991; Zhang *et al.*, 2001). Further comparative studies are also needed to establish clear relationships between *Actinomadura echinospora*, *Actinomadura umbrina* and *Thermomonospora curvata* (the type species of the genus), as these organisms are well separated from other *Actinomadura* species on the basis of 16S and 23S rDNA sequence data (Zhang *et al.*, 2001). Phylogenetic data also indicate that *Spirillospora albida*, the type species of the genus, has a very close evolutionary relationship with some *Actinomadura* species.

The aim of the present study was to determine the taxonomic status of two soil isolates, strains 3.24^T and 80-60^T, which were considered to have phenotypic properties typical of actinomadurae. Isolate 80-60^T was invalidly described as '*Streptomyces glaucoflavus*' by Zhang *et al.* (1984), but was subsequently shown to have chemical and morphological properties typical of *Actinomadura* strains (Itoh *et al.*, 1987). The two organisms were examined for a range of genotypic and phenotypic properties and were found to form new centres of taxonomic variation within the genus *Actinomadura*; the names *Actinomadura catellatispora* sp. nov. and *Actinomadura glauciflava* sp. nov. are respectively proposed for strains 3.24^T and 80-60^T.

METHODS

Isolation, maintenance and cultivation. Strain 3.24^T was isolated on plates of glucose-asparagine agar (glucose, 10 g; L-asparagine, 0.5 g; K₂HPO₄, 0.5 g; agar, 15 g; distilled water, 1 l; pH 7.2–7.4) seeded with a soil suspension and incubated at 28 °C for 7 days. The soil suspension was prepared from a mud sample taken from a sewage ditch in Zhanjiang City, Canton Province, southern China. Similarly, strain 80-60^T was isolated, under the same conditions, using a soil suspension prepared from a soil sample collected at the Institute of Tropical Plants, Xishuang Bana, Yunnan Province, southern China, as described by Zhang *et al.* (1984). The isolates were maintained as glycerol suspensions (20%, v/v), as were the type strains of *Actinomadura citrea*, *Actinomadura coerulea*, *Actinomadura latina*, *Actinomadura livida*, *Actinomadura luteofluorescens*, *Actinomadura nitritigenes* and *Actinomadura verrucosospora*. Biomass for chemotaxonomic and molecular systematic studies was prepared by growing all of the organisms in shake flasks of modified Sauton's broth (Mordarska *et al.*, 1972) at 28 °C for 7 days. Cells for chemotaxonomic analyses (isolates 3.24^T and 80-60^T) were washed twice in distilled water and freeze-dried; those for molecular systematic studies (all strains) were washed in NaCl/EDTA buffer (0.1 M EDTA, pH 8.0; 0.1 M NaCl) and stored at –20 °C until required.

Cultural and morphological studies. The undisturbed arrangement of the aerial hyphae and spore chain morphology of the isolates was observed from cultures grown on glucose-yeast extract-malt extract agar (ISP 2 medium; Shirling & Gottlieb, 1966) at 28 °C for up to 4 weeks, using the cover-slip technique of Kawato & Shinobu (1959). Growth on the cover-slip was fixed and examined

following the procedure described by Zhou *et al.* (1998). Spore ornamentation was observed by examining gold-coated, dehydrated preparations, using a Hitachi 5-570 SEM.

Biochemical and physiological properties and chemotaxonomy. The isolates were examined for a broad range of phenotypic properties as described by Athalye *et al.* (1985).

The diagnostic isomers of A₂pm, the predominant menaquinones, sugars, polar lipids and the DNA base composition of the isolates were determined as described by Lu *et al.* (2001).

DNA extraction and 16S rDNA sequencing. Standard procedures were used to extract genomic DNA from all of the test organisms (Pitcher *et al.*, 1989; Kim *et al.*, 1998).

PCR amplification of 16S rDNA samples prepared from the isolates and from the type strains of *A. latina* and *A. nitritigenes* was carried out as described previously (Kim *et al.*, 1996). The PCR products were purified according to the Wizard PCR purification system (Promega) and then sequenced using a 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 373A) and software provided by the manufacturer.

Analysis of sequence data. The almost complete 16S rDNA sequences of the organisms were aligned manually with corresponding sequences of representatives of the family *Thermomonosporaceae* retrieved from the DDBJ/EMBL/GenBank databases using the program PHYDIT (J. Chun, unpublished data). Evolutionary trees were inferred using the least-squares, maximum-likelihood, maximum-parsimony and neighbour-joining treeing algorithms from the PHYLIP suite of programs (Felsenstein, 1993) and evolutionary distance matrices were generated for the least-squares and neighbour-joining methods as described by Jukes & Cantor (1969). The stability of the groupings was evaluated by bootstrap analyses (1000 replicates) of the neighbour-joining dataset using the programs SEQBOOT and CONSENSE (Felsenstein, 1993).

DNA–DNA relatedness studies. Levels of DNA–DNA relatedness between isolate 80-60^T and the type strains of *A. citrea*, *A. coerulea*, *A. luteofluorescens*, *Actinomadura macra* and *A. verrucosospora* were determined, in duplicate, following the procedure described by De Ley *et al.* (1970).

RESULTS AND DISCUSSION

Almost complete 16S rDNA sequences were generated for isolates 3.24^T and 80-60^T and for the type strains of *A. latina* and *A. nitritigenes* (>1445 nt). Comparison of these sequences with those of representatives of the family *Thermomonosporaceae sensu* Zhang *et al.* 2001 showed that all of the strains fall within the range of the evolutionary radiation occupied by the genus *Actinomadura* (data not shown). The isolates also exhibited chemical markers typical of members of this taxon, i.e. they contained: *meso*-A₂pm as the wall diamino acid; the sugars galactose, glucose, madurose, mannose and ribose; hexahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (~80%), minor amounts (~10–12%) of tetra- and octahydrogenated components with nine isoprene units, diphosphatidylglycerol and phosphatidylinositol as major polar lipids; and complex mixtures of fatty acids,

notably major proportions of hexadecanoic (~40 % of total fatty acids), 14-methylpentadecanoic (~20 %) and 10-methyloctadecanoic (~20 %) acids. These chemical properties distinguish the isolates from members of the family *Thermomonosporaceae*, apart from those classified in the genus *Actinomadura* (Kroppenstedt *et al.*, 1990; Miyadoh & Miyara, 2001). In addition, strains 3.24^T and 80-60^T have G+C-rich DNA (70.8 and 72.0 mol%, respectively), but lack mycolic acids.

The phenotypic properties of the isolates are also consistent with their classification in the genus *Actinomadura*. The organisms are aerobic, non-motile, Gram-positive, non-acid/alcohol-fast actinomycetes that form extensively branched, non-fragmenting substrate mycelia. Isolate 80-60^T produces abundant bluish-green aerial hyphae on ISP 2 medium (Shirling & Gottlieb, 1966) that differentiate into short, curved or hooked or spiral (one turn) spore chains with a warty ornamentation (Fig. 1a). In contrast, strain 3.24^T formed short, straight chains of smooth-surfaced spores on the aerial mycelium (Fig. 1b).

The positions of the tested strains in the 16S rDNA *Actinomadura* tree are shown in Fig. 2. Strain 3.24^T is most closely related to the type strain of *A. livida*; the two

Fig. 1. SEM of strain 80-60^T (a), showing hooked and spiral chains of warty ornamented spores, and strain 3.24^T (b), showing short chains of smooth ornamented spores, after 4 weeks growth at 28°C on ISP 2 medium. Bars, 1 µm.

Fig. 2. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rDNA sequences of strains 3.24^T and 80-60^T and representatives of validly described species of *Actinomadura*. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) treeing algorithms; the symbols F and P respectively indicate branches recovered using the least-squares and maximum-parsimony methods. Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0.02 substitutions per nucleotide position. *A.*, *Actinomadura*; *S.*, *Spirillospora*; *T.*, *Thermomonospora*.

organisms share 96.8 % 16S rDNA similarity, which corresponds to 45 nt differences at 1450 locations. Much higher 16S rDNA similarities have been found between representatives of validly described *Actinomadura* species. The type strains of *A. citrea* and *A. coerulea*, for instance, share 99.4 % similarity, which corresponds to 8 nt differences at 1450 sites. Representatives of these taxa have a DNA–DNA relatedness value of 48 % (Poscher *et al.*, 1985), a value well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for the delineation of genomic species.

It is also apparent from Fig. 2 that strain 80-60^T belongs to the *A. luteofluorescens* subclade. The taxonomic integrity of this group is supported by a bootstrap value of 61 % and by

results obtained with all four treeing algorithms. Strain 80-60^T is most closely related to the type strain of *A. citrea*. The two organisms share 98.4% 16S rDNA similarity, which corresponds to 23 nt differences at 1450 sites. DNA–DNA relatedness studies are now commonly used to resolve the finer taxonomic relationships between representatives of such closely related species. In the present investigation, it is evident that isolate 80-60^T belongs to a distinct genomic species, as it shares mean DNA–DNA relatedness values of 62% with *A. luteofluorescens* JCM 4491^T, 53% with *A. citrea* JCM 3295^T and *A. verrucosospota* JCM 3147^T and 38% with *A. coerulea* JCM 3320^T. These strains can also be distinguished from one another, and from isolate 3.24^T, using a range of phenotypic properties (Table 1).

It is evident from the genotypic and phenotypic data that isolates 3.24^T and 80-60^T form the core of two novel species within the genus *Actinomadura*. It is therefore proposed that these organisms be given the names *Actinomadura catellatispora* sp. nov. and *Actinomadura glauciflava* sp. nov., respectively. It is also clear from the phylogenetic data that *A. latina* DSM 43382^T and *A. nitritigenes* DSM 44137^T form distinct phyletic lines, thereby underpinning the taxonomic status of these species, which were circumscribed mainly on the basis of phenotypic data (Trujillo & Goodfellow, 1997; Lipski & Altendorf, 1995).

Description of *Actinomadura catellatispora* sp. nov.

Actinomadura catellatispora (ca.tel.la.ti.spo'ra. L. n. *catella* small chain; Gr. n. *spora* a seed; N.L. fem. n. *catellatispora* organism forming small chains of spores).

Aerobic, Gram-positive, non-acid/alcohol-fast, non-motile actinomycete that forms an extensively branched, non-fragmenting, light-yellow substrate mycelium on glucose-yeast extract-malt extract agar (ISP 2 medium). Short chains of spores (0.85 µm diameter) are formed on the aerial mycelium. The spore surface is smooth and the aerial spore mass is yellow. Diffusible pigments are not formed. Aesculin and gelatin are degraded but not casein, hypoxanthine, starch, tyrosine or xanthine. Nitrate is reduced. The organism is chemo-organotrophic, has an oxidative type of metabolism and grows at temperatures between 18 and 35 °C. Isolated from a mud sample taken from a sewage ditch in Zhanjiang City, Canton Province, southern China. The type strain is isolate 3.24^T (= AS 4.1522^T = IFO 16341^T), which has a DNA G + C content of 70.8 mol%. The species description is based upon a single strain and hence serves as the description of the type strain.

Description of *Actinomadura glauciflava* sp. nov.

Actinomadura glauciflava (glau'ci fla.va. L. fem. adj. *glaucia* bluish-green; L. adj. *flaveus* yellow; N.L. adj. *glauciflava* bluish-green yellow).

The description is based on data taken from this and earlier studies (Zhang *et al.*, 1984; Itoh *et al.*, 1987). Aerobic, Gram-positive, non-acid/alcohol-fast, non-motile actinomycete that forms an extensively branched, non-fragmenting, substrate mycelium that carries aerial hyphae which differentiate into curved or hooked or spiral chains of spores (1 µm diameter) on glucose-yeast extract-malt extract agar (ISP 2 medium). The spore surface is warty,

Table 1. Phenotypic characters that distinguish strains 3.24^T and 80-60^T from the type strains of closely related *Actinomadura* species

Strains: 1, 3.24^T; 2, 80-60^T; 3, *A. citrea* JCM 3295^T; 4, *A. coerulea* JCM 3320^T; 5, *A. livida* JCM 3387^T; 6, *A. luteofluorescens* JCM 4491^T; 7, *A. verrucosospota* JCM 3147^T. +, Positive; –, negative, absent. Data were taken from the present study and from Holt *et al.* (1994), Itoh *et al.* (1987), Meyer (1979) and Zhang *et al.* (1984). ISP 2 medium is glucose-yeast extract-malt extract agar. All strains degrade gelatin.

Characteristic	1	2	3	4	5	6	7
Morphology:							
Spore chain arrangement	Straight	Hooks, spirals	Hooks	Hooks	Hooks, spirals	Hooks	Hooks, spirals
Spore surface ornamentation	Smooth	Warty	Irregular	Warty	Irregular	Warty	Warty
Growth on ISP 2 medium:							
Aerial mycelium	Yellow	Bluish-green	None	None	None	White-yellow	White
Substrate mycelium	Light yellow	Yellow-brown	Yellow-brown	Brown	Brown	Yellow	Yellow
Diffusible pigment	None	Yellow	None	None	None	Yellow	None
Degradation of:							
Aesculin	+	+	+	–	+	–	+
Casein	–	+	+	+	+	+	+
Hypoxanthine	–	+	+	+	–	+	+
Starch	–	+	+	–	–	+	+
Tyrosine	–	–	+	–	+	+	+
Xanthine	–	–	–	–	–	+	–
Nitrate reduction	+	+	+	+	+	+	–

the aerial spore mass is bluish-green. A yellow diffusible pigment is produced. Aesculin, casein, gelatin, starch and hypoxanthine are degraded, but not tyrosine or xanthine. Nitrate is reduced. The organism is chemo-organotrophic, has an oxidative type of metabolism and grows at temperatures between 18 and 35 °C. Isolated from soil collected in Yunnan Province, southern China. The type strain is isolate 80-60^T (= AS 4.1202^T = IFO 14668^T = JCM 16161^T), which has a DNA G + C content of 72 mol%. The species description is based on a single strain and hence serves as the description of the type strain.

ACKNOWLEDGEMENTS

This work was supported through the Royal Society–Chinese Academy of Sciences Exchange Scheme and by the National Natural Science Foundation of China (grant no. 39670001). The authors are indebted to Dr T. Kudo (Japan Collection of Microorganisms, Saitama, Wako-shi, Japan) and to Dr K. Hatano (Institute for Fermentation, Osaka, Japan) for providing some of the type strains of *Actinomadura*. E. T. Q. was supported by a studentship from the Consejo Nacional de Ciencia y Tecnologia (CONACYT), Mexico City, Mexico.

REFERENCES

- Athalye, M., Goodfellow, M., Lacey, J. & White, R. P. (1985). Numerical classification of *Actinomadura* and *Nocardiopsis*. *Int J Syst Bacteriol* **35**, 86–98.
- Couch, J. N. (1963). Some new genera and species of *Actinoplanaceae*. *J Elisha Mitchell Sci Soc* **79**, 53–70.
- 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. (1993). PHYLIP (phylogenetic inference package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA, USA.
- Fischer, A., Kroppenstedt, R. M. & Stackebrandt, E. (1983). Molecular-genetic and chemotaxonomic studies on *Actinomadura* and *Nocardiopsis*. *J Gen Microbiol* **129**, 3433–3446.
- 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., Stanton, L. J., Simpson, K. E. & Minnikin, D. E. (1990). Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. *J Gen Microbiol* **136**, 19–36.
- Henssen, A. (1957). Beiträge zur Morphologie und Systematik der thermophilen Actinomyceten. *Arch Mikrobiol* **26**, 373–414.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (editors) (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.
- Iinuma, S., Yokota, A., Hasegawa, T. & Kanamaru, T. (1994). *Actinocorallia* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **44**, 230–234.
- Itoh, T., Kudo, T. & Seino, A. (1987). Chemotaxonomic studies on new genera of actinomycetes proposed in Chinese papers. *Actinomycetologica* **1**, 43–59.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kawato, M. & Shinobu, R. (1959). *Streptomyces herbaricolor* sp. nov., supplement: a single technique for microscopical observation. *Mem Osaka Univ Lib Arts Educ* **8**, 114.
- Kim, D., Chun, J., Sahin, N., Hah, Y. C. & Goodfellow, M. (1996). Analysis of thermophilic clades within the genus *Streptomyces* by 16S ribosomal DNA sequence comparisons. *Int J Syst Bacteriol* **46**, 581–587.
- Kim, S. B., Falconer, C., Williams, E. & Goodfellow, M. (1998). *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxy-dotrophic species from soil. *Int J Syst Bacteriol* **48**, 59–68.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Kroppenstedt, R. M., Stackebrandt, E. & Goodfellow, M. (1990). Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetraspora*. *Syst Appl Microbiol* **13**, 148–160.
- Lechevalier, H. A. & Lechevalier, M. P. (1968). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer.
- Lechevalier, M. P. & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lipski, A. & Altendorf, K. (1995). *Actinomadura nitritigenes* sp. nov., isolated from experimental biofilters. *Int J Syst Bacteriol* **45**, 717–723.
- 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.
- Meyer, J. (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.
- Meyer, J. (1979). New species of the genus *Actinomadura*. *Zentbl Allg Mikrobiol* **19**, 37–44.
- Miyadoh, S. & Miyara, T. (2001). Family *Thermomonosporaceae*. In *Identification Manual of Actinomycetes*, pp. 281–291. Edited by the Society for Actinomycetes, Japan. Tokyo: Business Center for Academic Societies.
- Mordarska, H., Mordarski, M. & Goodfellow, M. (1972). Chemotaxonomic characters and classification of some nocardioform bacteria. *J Gen Microbiol* **71**, 77–86.
- Ochi, K., Miyadoh, S. & Tamura, T. (1991). Polyacrylamide gel electrophoresis analysis of ribosomal protein AT-L30 as a novel approach to actinomycete taxonomy: application to the genera *Actinomadura* and *Microtetraspora*. *Int J Syst Bacteriol* **41**, 234–239.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Poscher, J., Kroppenstedt, R. M., Fischer, A. & Stackebrandt, E. (1985). DNA:DNA reassociation and chemotaxonomic studies on *Actinomadura*, *Microbispora*, *Microtetraspora*, *Micropolyspora* and *Nocardiopsis*. *Syst Appl Microbiol* **6**, 264–270.
- 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.
- 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.
- 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.

- Trujillo, M. E. & Goodfellow, M. (1997).** Polyphasic taxonomic study of clinically significant actinomadurae including the description of *Actinomadura latina* sp. nov. *Zentbl Bakteriol* **285**, 212–233.
- 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.
- Zhang, G., Xing, G. & Yan, X. (1984).** Studies of classification of *Streptomycetaceae*. III. A new genus – *Streptomycooides* in the *Streptomycetaceae*. *Acta Microbiol Sin* **24**, 189–194.
- Zhang, Z., Wang, Y. & Ruan, J. (1998).** Reclassification of *Thermomonospora* and *Microtetraspora*. *Int J Syst Bacteriol* **48**, 411–422.
- Zhang, Z., Kudo, T., Nakajima, Y. & Wang, Y. (2001).** Clarification of the relationship between the members of the family *Thermomonosporaceae* on the basis of 16S rDNA, 16S–23S rRNA internal transcribed spacer and 23S rDNA sequences and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 373–383.
- Zhou, Z.-H., Liu, Z.-H., Qian, Y.-D., Kim, S. B. & Goodfellow, M. (1998).** *Saccharopolyspora spinosporotrichia* sp. nov., a novel actinomycete from soil. *Int J Syst Bacteriol* **48**, 53–58.