Sinosporangium album gen. nov., sp. nov., a new member of the suborder *Streptosporangineae*

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A Gram-positive, aerobic, non-motile actinobacterium, designated strain 6014^T, was isolated from a soil sample collected from Qinghai province, north-west China, and subjected to a polyphasic taxonomic study. The isolate formed elementary branching hyphae and abundant aerial mycelia with globose sporangia on ISP 4 and R2A media. Whole-cell hydrolysates of strain 6014^T contained arabinose, galactose and ribose as diagnostic sugars and *meso*-diaminopimelic acid as the diagnostic diamino acid. The polar lipids consisted of phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, *N*-acetylglucosamine-containing phospholipids, two unknown phospholipids and an unknown glycolipid. The menaquinone system contained MK-9(H_2) and MK-9(H_4). The major fatty acids were $C_{14:0}$, i-$C_{15:0}$, $C_{16:0}$ and 10-methyl-$C_{16:1}$. The genomic DNA G+C content of the isolate was 69.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 6014^T fell within the radius of the suborder *Streptosporangineae*, in which the strain formed a distinct lineage next to genera of the family *Streptosporangiaceae*. Based on data from this polyphasic study, strain 6014^T can be readily distinguished from previously described organisms and represents a member of a novel species within a new genus in the suborder *Streptosporangineae*. The name *Sinosporangium album* gen. nov., sp. nov. is proposed with 6014^T (=DSM 45181^T =KCTC 19655^T) as the type strain.

The suborder *Streptosporangineae* was proposed by Stackebrandt *et al.* (1997) based on phylogenetic and signature nucleotide analysis. At the time of writing, there were only three families in this suborder, namely, *Streptosporangiaceae*, *Nocardiopsaceae* and *Thermomonosporaceae*, with *Streptosporangiaceae* as the type family. In this paper, characterization of strain 6014^T is reported, with proposals for *Sinosporangium* gen. nov. and *Sinosporangium album* sp. nov. in the suborder *Streptosporangineae* (Stackebrandt *et al.*, 1997).

Strain 6014^T was isolated on Czapek agar (Waksman, 1961) incubated at 28 °C for 3 weeks. The purified strain was maintained on ISP 4 agar and R2A slants at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained by cultivation in shake flasks using tryptic soy broth (Difco) at 28 °C for 10 days. Cultural characteristics of the isolate were determined after growth for 7–28 days at 28 °C on R2A, ISP 2, ISP 3, ISP 4 and ISP 5 (Shirling & Gottlieb, 1966), Czapek agar, nutrient agar (Difco) and potato agar (Waksman, 1961) media. The coverslip technique (Zhou *et al.*, 1998) was employed to observe characteristics of the hyphae, sporangia and spores. The sporangia and spore chain morphologies were recorded by examining gold-coated dehydrated specimens of 14 day cultures from R2A and ISP 4 agar by scanning electron microscopy (Quanta; FEI).

Growth was tested at 0, 4, 10, 15, 20, 28–37 (at intervals of 1 °C), 40, 45 and 55 °C on R2A. Other physiological and biochemical tests were performed at 28 °C. The pH range was examined at pH 4.0–11.0 (at intervals of 0.5 pH units).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 6014^T is EU438912.

A supplementary figure and a supplementary table are available with the online version of this paper.

Tolerance to sodium chloride [0, 1, 3 and 5–10 % (w/v), at intervals of 0.5 %] was examined using ISP 4 as basal medium. Carbon source utilization was tested as described by Shirling & Gottlieb (1966) and also using Biolog GEN III (MicroPlate) according to the manufacturer's instructions. Qualitative enzyme tests and acid production from carbohydrates were determined by using the API ZYM and API 50CH systems (bioMérieux) according to the manufacturer's instructions. Other physiological tests and antimicrobial activities of the strain were examined according to previously described procedures (Yuan *et al.*, 2008).

Strain 6014^T grew well on R2A, ISP 4, ISP 5, Czapek solution agar, nutrient agar and potato agar media at 28–32 °C and pH 7.0–7.5. White to buff branching vegetative hyphae developed well and abundant white aerial hyphae were produced on the above media, whereas the strain grew very slowly with few aerial hyphae on ISP 2 and ISP 3 media. Diffusible pigments were not observed on any test media. Globose sporangia formed singly from the aerial hyphae (Fig. 1a). The sporangia were a mean size of 2.8–3.0 × 3.4–4.2 μm (Fig. 1b) and contained coiled spore chains (Fig. 1a). The non-motile, smooth-surfaced, cylindrical spores were about 0.5–0.6 × 0.6–1.2 μm. Gelatin, starch, urea and aesculin were not hydrolysed. Milk was not coagulated or peptonized and H_2S was not produced. The isolate could use most of the carbon sources listed in Biolog GEN III as sole carbon sources (Supplementary Table S1, available in IJSEM Online) for energy and growth except α-lactose, D-galactose and *myo*-inositol. Detailed physiological and biochemical characteristics of strain 6014^T are given in the species description.

The whole-cell sugar pattern and diagnostic diaminopimelic acid isomers were determined by TLC (Lechevalier & Lechevalier, 1965, 1980). Polar lipids were extracted and examined by two-dimensional TLC and identified using procedures described by Minnikin *et al.* (1984). Menaquinones were isolated using the method of Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). Analysis of the whole-cell fatty acid pattern followed described methods using the MIDI system (Microbial ID) (Kroppenstedt, 1985; Meier *et al.*, 1993). Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). The DNA G+C content was determined by reverse-phase HPLC of nucleosides according to Mesbah *et al.* (1989). 16S rRNA gene multiple alignments with sequences of most closely related taxa and calculations of sequence similarity levels were carried out using CLUSTAL X (Thompson *et al.*, 1997). A phylogenetic tree and distance matrix were reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983) using MEGA version 4.0 (Tamura *et al.*, 2007). A maximum-likelihood (Felsenstein, 1981) tree (not shown) was generated using the treeing algorithm contained in the PHYLIP package (Felsenstein, 1993). Topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

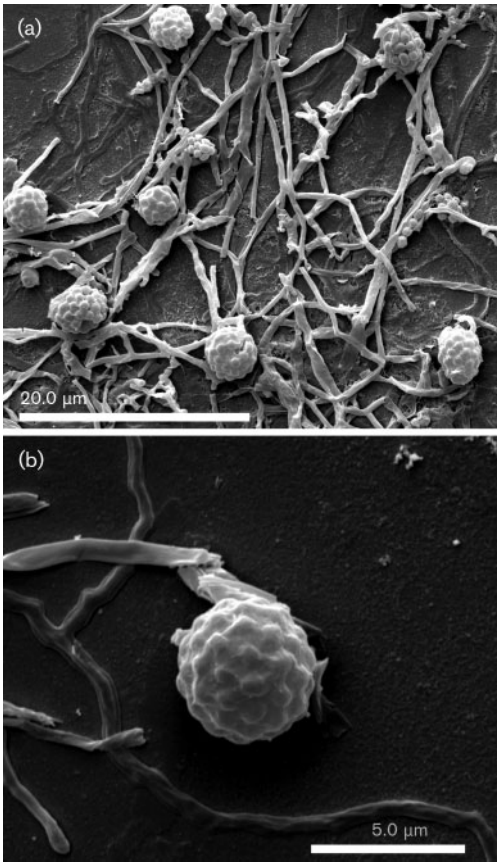

Fig. 1. Scanning electron micrographs of strain 6014^T after growth for 14 days at 28 °C on ISP 4 agar showing spore chains (a; bar, 20.0 μm) and a sporangium (b; bar, 5.0 μm).

Whole-cell hydrolysates of strain 6014^T contained ribose, galactose and arabinose. The diagnostic diamino acid was *meso*-diaminopimelic acid. The polar lipids comprised phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, *N*-acetylglucosamine-containing phospholipids, two unknown phospholipids and an unknown glycolipid (Supplementary Fig. S1). The menaquinone system contained MK-9(H_2) (53.1 %) and MK-9(H_4) (46.9 %). The major fatty acids detected were saturated, iso- and 10-methyl branched fatty acids. The detailed cellular fatty acid profile is as follows: $C_{12:0}$ (1.0 %), $C_{13:0}$ (0.6 %), $C_{14:0}$ (10.4 %), $C_{15:0}$ (2.1 %), $C_{16:0}$ (20.9 %), $C_{17:0}$ (0.6 %), $C_{18:0}$ (1.0 %), iso-$C_{14:0}$ (1.7 %), iso-$C_{15:0}$ (10.2 %), iso-$C_{16:0}$ (8.3 %), iso-$C_{17:0}$ (2.0 %), anteiso-$C_{17:0}$ (0.7 %), 10-methyl-$C_{16:0}$ (10.1 %), 10-methyl-$C_{17:0}$ (4.7 %), 10-methyl-$C_{18:0}$ (7.4 %), $C_{16:1}\omega7c$ (8.1 %), $C_{17:1}\omega8c$ (2.2 %), $C_{18:1}\omega9c$ (5.9 %). The genomic DNA G+C content was 69.4 mol%.

BLAST search results using the 16S rRNA gene sequence of strain 6014^T showed that the novel isolate exhibited highest similarities with members of the suborder *Streptosporangineae*, such as *Streptosporangium violaceochromogenes* DSM 43849^T (96.1 %). However, in the phylogenetic tree

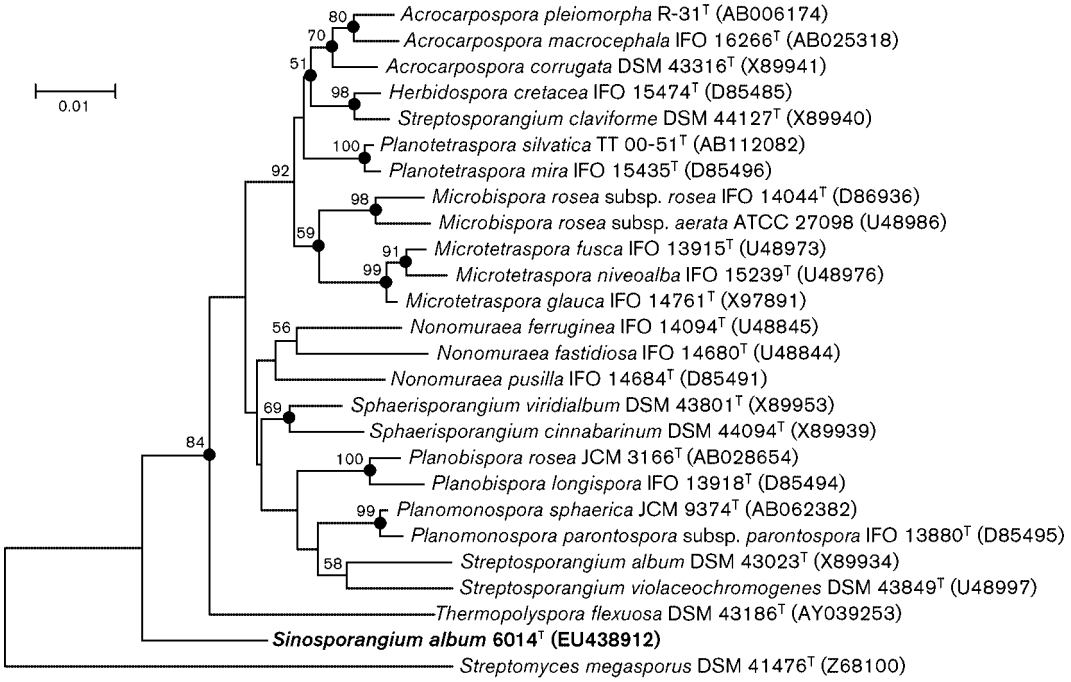

Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain 6014^T among its phylogenetically nearest neighbours. Bootstrap percentages (based on 1000 replications) >50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood method. Bar, 0.01 substitutions per nucleotide position.

Table 1. Signature nucleotide patterns of the genus *Sinosporangium* and related genera

Genera: 1, *Sinosporangium* gen. nov.; 2, *Sphaerosporangium*; 3, *Streptosporangium*; 4, *Planotetraspora*; 5, *Planomonospora*; 6, *Planobispora*; 7, *Nonomuraea*; 8, *Microtetraspora*; 9, *Microbispora*; 10, *Herbidospora*; 11, *Acrocarpospora*.

Position	1	2	3	4	5	6	7	8	9	10	11
263	G	G	A	G	G	G	G	G	A	G	G
264	U	U	C	U	U	U	U	U	U	U	U
442 : 492	G–C	G–C	G–C	G–U	G–C	G–C	G–C	G–U	G–U	G–U	G–U
594 : 645	C–G	C–G	C–G	U–G	C–G	C–G	C–G	C–G	C–G/U–G	C–G	C–G
595	G	G	A	G	G	G	G	G	G	G	G
600 : 638	C–G	U–G	G–C	U–G	G–C	G–C	U–G	U–G	U–G	U–G	U–G
602 : 636	C–G	C–G	G–U	C–G	A–U	A–U	C–G	C–G	C–G	C–G	C–G
603 : 635	C–G	C–G	U–A	C–G	U–A	U–A	C–C	C–C	C–C	C–C	C–C
625	C	G	C	G	C	G	G	C	A	A	G
626	C	U	C	C	C	C	C	U	C	U	C
627	G	G	A	G	A	G	G	A	G	G	G
657 : 749	G–C	G–C	G–C	G–U	C–C	G–C	G–C	G–U	G–U	G–U	G–U
658 : 748	C–G	U–A	U–A	C–U	U–A	U–A	U–A	C–U	C–U	C–U	C–U
659 : 746	A–U	A–U	A–U	U–A	A–U	A–U	A–U	U–A	U–A	U–A	U–G
668 : 738	A–U	C–G	A–U	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
669 : 737	G–C	A–U	G–C	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U
671 : 735	A–U	G–C	A–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
835 : 851	G–C	G–U	G–U	G–C	G–U	G–C	G–C	G–C	G–C	G–C	G–C
990 : 1215	U–G	C–G	C–G	G–C/G–U	G–U	G–C	G–U	G–C	G–C	G–U	G–C
1012 : 1017	G–U	G–C	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U
1116	G	U	U	U	U	U	U	U	U	U	U
1137	C	U	U	U	U	U	U	U	U	U	U
1263 : 1272	G–C	G–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	G–U
1436 : 1465	C–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C

Table 2. Morphological and chemotaxonomic characteristics of strain 6014^T and members of genera in the family *Streptosporangiaceae*

Taxa: 1, 6014^T; 2, *Streptosporangium*; 3, *Acrocarpospora*; 4, *Herbidospora*; 5, *Microbispora*; 6, *Microtetraspora*; 7, *Nonomuraea*; 8, *Planobispora*; 9, *Planomonospora*; 10, *Planotetraspora*; 11, *Sphaerisporangium*; 12, *Thermopolyspora*. Data were taken from this and previous studies (Greiner-Mai *et al.*, 1987; Goodfellow *et al.*, 1990; Kudo *et al.*, 1993; Tamura *et al.*, 2000; Stackebrandt *et al.*, 2001; Tamura & Sakane, 2004; Ara & Kudo, 2007; Goodfellow *et al.*, 2005; Cao *et al.*, 2009). For all taxa, the fatty acid type [saturated fatty acids, unsaturated fatty acids, iso-fatty acids, variable and methyl-branched fatty acids (Kroppenstedt, 1985)] was 3c. +, Present; −, absent.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Sporangium formation	Globose sporangia on aerial hyphae	Globose sporangia on aerial hyphae	Club or globose spore vesicles on aerial hyphae	Straight chains of smooth-surfaced spores on aerial hyphae	Smooth-surfaced spores in characteristic longitudinal pairs on aerial hyphae	Spore chains typically containing four smooth-surfaced spores on short aerial hyphae	Spore chains or pseudo-sporangia formed on aerial hyphae	Cylindrical to clavate spore vesicles containing longitudinal pairs of spores on aerial hyphae	Cylindrical to clavate spore vesicles containing single spores on aerial hyphae	Spore vesicles containing four spores on aerial hyphae	Globose sporangia on aerial hyphae	Hooked or irregular spiral chains of four to ten warty to spiny ornamented spores on aerial hyphae
Motile spores	−	−	−	−	−	−	−	+	+	+	−	−
Whole-cell sugar pattern*	A	B	B, C	B	B, C	B, C	B, C	B	B	A, D	B	C
Major menaquinone(s)	MK-9(H_2, H_4)	MK-9(H_2, H_4)	MK-9(H_2, H_4, H_6)	MK-10(H_4, H_6, H_8)	MK-9(H_2, H_4)	MK-9(H_2, H_4)	MK-9(H_2, H_4)	MK-9(H_2, H_4)	MK-9(H_2)	MK-9(H_4)	MK-9(H_4)	MK-9(H_2, H_4)
Phospholipid type†	PIV	PIV	PIV, PII	PIV	PIV	PIV	PIV	PIV	PIV	PIV	PIV	PIV
DNA G+C content (mol%)	69.4	69–71	68–69	69–71	71–73	69–71	64–69	70–71	72	71	67–72	77

*Whole-cell sugar patterns of actinomycetes containing *meso*-diaminopimelic acid: A, arabinose and galactose; B, madurose; C, no diagnostic sugar; D, arabinose and xylose (Lechevalier & Lechevalier, 1970).

†Diagnostic phospholipids: PII, phosphatidylethanolamine; PIV, glucosamine (with phosphatidylethanolamine and phosphatidylmethylethanolamine variable) (Lechevalier *et al.*, 1977).

(not shown) based on the 16S rRNA gene sequences of all genera in the suborder *Streptosporangineae*, strain 6014^T formed a distinct lineage among families in the suborder *Streptosporangineae*, next to the family *Streptosporangiaceae* (Fig. 2), which showed that strain 6014^T could not be placed into any known family. Analysis of 16S rRNA signature nucleotide patterns demonstrated that strain 6014^T contained the signature nucleotide pattern defined for the suborder *Streptosporangineae*, namely 127 : 234 (A–U), 657 : 749 (G–C) and 955 : 1225 (C–G) (Stackebrandt *et al.*, 1997); additionally, strain 6014^T possessed its own signature nucleotides, which distinguished it from its nearest neighbour, namely 1116 (G), 1137 (C) and 1436 : 1465 (C–U), instead of 1116 (U), 1137(U) and 1436 : 1465 (G–C) for the family *Streptosporangiaceae*. The specific diagnostic nucleotide signature patterns of the isolate and genera in the family *Streptosporangiaceae* are listed in Table 1.

Based on its phylogenetic position (Fig. 2) and chemotaxonomic data (Table 2), it is proposed that strain 6014^T represents a novel species in a new genus, *Sinosporangium album* gen. nov., sp. nov.

Description of *Sinosporangium* gen. nov.

Sinosporangium [Si.no.spo.ran′gi.um. M.L. n. *sina* China; N.L. n. *sporangium* from Gr. n. *spora* a seed and, in biology, a spore and Gr. n. *angeion* (Latin transliteration *angium*) vessel, sporangium; N.L. neut. n. *Sinosporangium* an organism isolated in China bearing sporangia].

Cells are Gram-positive and form branching hyphae. Globose sporangia are borne on aerial mycelia. Coiled spore chains are contained in the sporangia. Smooth-surfaced spores are non-motile. Grows at pH 6.5–8.5 and 10–37 °C. Catalase-positive, oxidase-negative. The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. Whole-cell hydrolysates contain arabinose, galactose and ribose. Phospholipids consist of phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, *N*-acetylglucosamine-containing phospholipids, two unknown phospholipids and an unknown glycolipid. The menaquinone system contains MK-9(H_2) (53.1 %) and MK-9(H_4) (46.9 %). Major cellular fatty acids are $C_{16:0}$, $C_{14:0}$, iso-$C_{15:0}$ and 10-methyl-$C_{16:0}$. The 16S rRNA contains a genus-specific diagnostic nucleotide signature pattern, namely 600 : 638 (C–G), 658 : 748 (C–G), 990 : 1215 (U–G), 1012 : 1017 (G–U) and 1263 : 1272 (G–C). The type species is *Sinosporangium album*.

Description of *Sinosporangium album* sp. nov.

Sinosporangium album (al′bum. L. neut. adj. *album* white).

In addition to properties given for the genus, forms white to buff branching substrate mycelia and white aerial mycelia. No diffusible pigment is produced on any of the media tested. Optimum growth occurs at pH 7.0–7.5 and 28–32 °C. Can tolerate up to 1 % (w/v) NaCl at 28 °C. Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase activities. Negative for gelatin liquefaction, milk coagulation and peptonization, hydrolysis of starch, urea and aesculin, nitrate reduction, H_2S production and hydrolysis of cellulose. Most of the substrates listed in Biolog GEN III can be utilized as sole carbon sources for energy and growth, except α-lactose, D-galactose and *myo*-inositol. Acid can be produced from D-adonitol, D-glucose, trehalose and potassium 5-ketogluconate.

The type strain is 6014^T (=DSM 45181^T =KCTC 19655^T), isolated from soil collected in Qinghai, China. The genomic DNA G+C content of the type strain is 69.4 mol%.

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References

Ara, I. & Kudo, T. (2007). *Sphaerosporangium* gen. nov., a new member of the family *Streptosporangiaceae*, with descriptions of three new species as *Sphaerosporangium melleum* sp. nov., *Sphaerosporangium rubeum* sp. nov. and *Sphaerosporangium cinnabarinum* sp. nov., and transfer of *Streptosporangium viridialbum* Nonomura and Ohara 1960 to *Sphaerosporangium viridialbum* comb. nov. *Actinomycetologica* **21**, 11–21.

Cao, Y.-R., Jiang, Y., Xu, L.-H. & Jiang, C.-L. (2009). *Sphaerisporangium flaviroseum* sp. nov. and *Sphaerisporangium album* sp. nov., isolated from forest soil in China. *Int J Syst Evol Microbiol* **59**, 1679–1684.

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

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 (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

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.

Goodfellow, M., Maldonado, L. A. & Quintana, E. T. (2005). Reclassification of *Nonomuraea flexuosa* (Meyer 1989) Zhang *et al.* 1998 as *Thermopolyspora flexuosa* gen. nov., comb. nov., nom. rev. *Int J Syst Evol Microbiol* **55**, 1979–1983.

Greiner-Mai, E., Kroppenstedt, R. M., Korn-Wendisch, F. & Kutzner, H. J. (1987). Morphological and biochemical characterization and emended descriptions of thermophilic actinomycetes. *Syst Appl Microbiol* **9**, 97–109.

Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terragena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.

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.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution.* Cambridge: Cambridge University Press.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.

Kudo, T., Itoh, T., Miyadoh, S., Shomura, T. & Seino, A. (1993). *Herbidospora* gen. nov., a new genus of the family *Streptosporangiaceae* Goodfellow *et al.* 1990. *Int J Syst Bacteriol* **43**, 319–328.

Lechevalier, H. & Lechevalier, M. P. (1965). Classification of aerobic actinomycetes based on their morphology and their chemical composition. *Ann Inst Pasteur (Paris)* **108**, 662–673 (in French).

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. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy* (SIM Special Publication no. 6), pp. 227–291. Edited by A. Dietz & D. W. Thayer. 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.

Li, W.-J., Xu, P., Schumann, P., Zhang, Y.-Q., Pukall, R., Xu, L.-H., Stackebrandt, E. & Jiang, C.-L. (2007). *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.

Meier, A., Kirschner, P., Schröder, K. H., Wolters, J., Kroppenstedt, R. M. & Böttger, E. C. (1993). *Mycobacterium intermedium* sp. nov. *Int J Syst Bacteriol* **43**, 204–209.

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.

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.

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.

Stackebrandt, E., Wink, J., Steiner, U. & Kroppenstedt, R. M. (2001). *Nonomuraea dietzii* sp. nov. *Int J Syst Evol Microbiol* **51**, 1437–1441.

Tamura, T. & Sakane, T. (2004). *Planotetraspora silvatica* sp. nov. and emended description of the genus *Planotetraspora*. *Int J Syst Evol Microbiol* **54**, 2053–2056.

Tamura, T., Suzuki, S. & Hatano, K. (2000). *Acrocarpospora* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Evol Microbiol* **50**, 1163–1171.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

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.

Waksman, S. A. (1961). *The Actinomycetes*, vol. 2. Baltimore: The Williams & Wilkins Co.

Yuan, L.-J., Zhang, Y.-Q., Guan, Y., Wei, Y.-Z., Li, Q.-P., Yu, L.-Y., Li, W.-J. & Zhang, Y.-Q. (2008). *Saccharopolyspora antimicrobica* sp. nov., an actinomycete from soil. *Int J Syst Evol Microbiol* **58**, 1180–1185.

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.