Plantactinospora mayteni gen. nov., sp. nov., a member of the family *Micromonosporaceae*

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A novel Gram-positive, aerobic, spore-forming, endophytic actinomycete, designated strain YIM 61359^T, was isolated from the roots of *Maytenus austroyunnanensis* plants collected from tropical rainforest in Xishuangbanna, Yunnan Province, south-west China. The strain formed single or cluster spores with smooth surfaces from substrate mycelia. The strain contained meso-diaminopimelic acid in the cell wall and arabinose, xylose, galactose and glucose in whole-cell hydrolysates. The acyl type of the cell-wall polysaccharides was glycolyl. MK-10(H₆), MK-10(H₈) and MK-10(H₄) were the predominant menaquinones. The polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and several unknown phospholipids. The major fatty acids were iso-C_{15:0}, anteiso-C_{15:0}, C_{17:0}, anteiso-C_{17:0} and iso-C_{16:0}. The DNA G + C content of strain YIM 61359^T was 69.7 mol%. These chemotaxonomic data indicated that the strain belongs to the family *Micromonosporaceae*. Phylogenetic analysis based on 16S rRNA gene sequences also suggested that strain YIM 61359^T fell within the family *Micromonosporaceae*. On the basis of morphological and chemotaxonomic data, phylogenetic analysis and characteristic patterns of 16S rRNA gene signature nucleotides, strain YIM 61359^T is considered to represent a novel species of a new genus within the family *Micromonosporaceae*, for which the name *Plantactinospora mayteni* gen. nov., sp. nov. is proposed. The type strain of *Plantactinospora mayteni* is YIM 61359^T (=CCTCC AA 208022^T=DSM 45238^T).

The family *Micromonosporaceae* was first described by Krasil'nikov (1938), and its description has been subsequently emended by Goodfellow *et al.* (1990), Koch *et al.* (1996) and Stackebrandt *et al.* (1997) on the basis of chemotaxonomic data and 16S rRNA gene sequence analysis. At the time of writing, the family *Micromonosporaceae* comprises 19 genera: *Micromonospora* (Ørskov, 1923), *Actinoplanes* (Couch, 1950), *Pilimelia* (Kane, 1966), *Dactylosporangium* (Thiemann *et al.*, 1967), *Catellatospora* (Asano & Kawamoto, 1986), *Catenuloplanes* (Yokota *et al.*, 1993), *Couchioplanes* (Tamura *et al.*, 1994), *Spirilliplanes* (Tamura *et al.*, 1997), *Verrucosipora* (Rheims *et al.*, 1998), *Virgisporengium* (Tamura *et al.*, 2001), *Asanoa* (Lee & Hah, 2002), *Longispora* (Matsumoto *et al.*, 2003),

Salinispora (Maldonado *et al.*, 2005), *Actinocatenispora* (Thawai *et al.*, 2006), *Polymorphosporengium* (Tamura *et al.*, 2006), *Luedemannella* (Ara & Kudo, 2007a), *Krasilnikovia* (Ara & Kudo, 2007b), *Planosporengium* (Wiese *et al.*, 2008) and *Pseudosporengium* (Ara *et al.*, 2008).

Endophytic actinomycetes have attracted increasing attention in recent years, but they remain relatively unexplored as potential sources of novel species and novel natural products for medical and commercial exploitation. Some species of the plant genus *Maytenus* are known to produce the anti-cancer compound maytansine (a 19-membered macrocyclic lactam) (Reider & Roland, 1984), and endophytic micro-organisms and related secondary metabolites have been investigated (Zhao *et al.*, 2007). During our investigations of the diversity and taxonomy of rare actinomycetes associated with tropical rainforest medicinal plants from Xishuangbanna, Yunnan Province, south-west China, strain YIM 61359^T was isolated from the roots of healthy *Maytenus austroyunnanensis* plants. On the basis of its 16S rRNA gene sequence, this isolate falls phylogeneti-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 61359^T is FJ214343.

A table comparing 16S rRNA gene signature nucleotide positions of strain YIM 61359^T and related genera is available with the online version of this paper.

cally within the family *Micromonosporaceae* adjacent to the genera *Salinispora* and *Micromonospora*. Data from the present polyphasic taxonomic study indicate that this strain represents a novel species of a new genus within the family *Micromonosporaceae*.

Healthy root samples of *Maytenus austroyunnanensis*, a traditional Chinese medicinal plant, were used as the source for isolation of endophytic actinomycetes. Samples were air-dried for 48 h and then washed via an ultrasonic step to remove surface soil. Subsequently, samples were subjected to a modified five-step surface sterilization procedure according to Qin *et al.* (2008b): an 8-min wash in 5% NaOCl, followed by a 10-min wash in 2.5% Na₂S₂O₃, a 5-min wash in 75% ethanol, a wash in distilled water and a final rinse in 10% NaHCO₃ for 10 min. After air-drying at room temperature, surface-sterilized stems were subjected to air-drying at 80 °C for 30 min, then aseptically crumbled into small fragments and processed with a calcium carbonate enrichment method as described by Otoguro *et al.* (2001) for 3 weeks. The enriched samples were serially diluted in sterile distilled water, spread-plated onto modified cellulose-proline agar [per litre tap water: 2.5 g cellulose, 2.0 g sodium pyruvate, 0.25 g KNO₃, 1.0 g proline, 0.2 g MgSO₄·7H₂O, 0.2 g K₂HPO₄, 0.5 g CaCl₂, 10 mg FeSO₄·7H₂O and 15 g agar (pH 7.2–7.4)] and incubated at 28 °C for 2 weeks. The isolate was routinely cultured on International *Streptomyces* Project medium 2 (ISP 2) (Shirling & Gottlieb, 1966) and maintained as a glycerol suspension (20%, w/v) at –80 °C.

Cultural characteristics of strain YIM 61359^T were tested on ISP 2, ISP 3, ISP 4, ISP 5, nutrient agar, potato-glucose agar (PDA) and Czapek's agar plates, commonly used for characterization of *Streptomyces* species (Shirling & Gottlieb, 1966; Waksman, 1967) at 28 °C for 2–6 weeks. Colony colour was determined based on ISCC–NBS colour charts (Kelly, 1964). Observations of mycelia and spores of strain YIM 61359^T grown on ISP 2 for 21–45 days were made by light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL). Growth was tested at 0, 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C on ISP 2. The ability of the strain to grow at different pH and NaCl concentrations was examined according to Qin *et al.* (2008a). Media and procedures used for the determination of physiological characteristics, carbon source utilization and acid production from carbohydrates were those described by Gordon *et al.* (1974).

Biomass for most of the chemotaxonomic studies was obtained after cultivation at 28 °C for 7–14 days in shaken cultures with yeast extract-malt extract broth (ISP 2) or trypticase soy broth (for fatty acids). Amino acid and sugar analysis of whole-cell hydrolysates was performed according to the procedures described by Hasegawa *et al.* (1983). The *N*-acyl group of muramic acid was determined by the method of Uchida & Aida (1984). Cellular menaquinones were extracted and purified as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997).

Polar lipids were extracted, examined via two-dimensional TLC and identified by using established procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Analysis of whole-cell fatty acids followed the instructions of the MIDI System (Microbial ID) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) by using exponential-phase cultures. Determination of the DNA G+C content was performed according to Mesbah *et al.* (1989).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described by Li *et al.* (2007). 16S rRNA gene sequence similarities among closely related sequences obtained from the GenBank/EMBL/DDBJ databases were calculated manually after pairwise alignments by using the CLUSTAL_X program, v. 1.8 (Thompson *et al.*, 1997). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) by using the MEGA 3.1 software package (Kumar *et al.*, 2004). For construction of the maximum-likelihood tree, the online version of PhyML (Guindon *et al.*, 2005) was used (<http://www.atgc-montpellier.fr/phyml/>). The stability of relationships was assessed by performing bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings.

Strain YIM 61359^T was an aerobic, Gram-positive, non-acid-fast actinomycete that formed extensively branched substrate mycelia and sparse aerial mycelia. Non-motile spores were borne singly or in clusters from substrate mycelia. The spore surface was smooth (Fig. 1). Strain YIM 61359^T grew well on ISP 2, ISP 3 and Czapek's agar, moderately well on ISP 4 and PDA, but poorly on ISP 5 and nutrient agar. No soluble pigments were produced on any of the media tested. Colonies on ISP 2 were orange-yellow and lacked aerial hyphae initially; after 4 weeks growth colonies were black, raised and folded. The colour of colonies on other media was orange-yellow (nutrient agar/Czapek's agar), yellowish (ISP 5), pale orange-yellow to black (ISP 3/PDA) and black (ISP 4). Strain YIM 61359^T grew well at 15–37 °C and pH 6.0–8.0 but could not grow in the presence of 4% NaCl. Other physiological and biochemical characteristics are presented in the species description below.

The novel isolate contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell wall, and the whole-cell sugars detected were arabinose, xylose, galactose and glucose. The acyl type of the cell-wall polysaccharides was glycolyl. The predominant menaquinones were MK-10(H₆) (53%), MK-10(H₈) (23%) and MK-10(H₄) (12%), with MK-10(H₂) (6%) and MK-9(H₆) (6%) as minor components. Mycolic acids were not detected. The polar lipids detected were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol, corresponding to phospholipid type PII of Lechevalier *et al.* (1977). The fatty acid profile of strain YIM 61359^T comprised iso-C_{15:0} (20.3% of the total), anteiso-C_{15:0} (13.8%), C_{17:0} (12.5%), anteiso-C_{17:0} (12.1%), iso-C_{16:0} (10.2%), C_{16:0} (6.3%),

Fig. 1. Scanning electron micrographs of cells of strain YIM 61359^T growing on ISP 2 after incubation for 5 weeks at 28 °C. Bars, 10 µm (a), 2 µm (b) and 5 µm (c).

C_{18:1}9c (6.1 %), iso-C_{17:0} (3.6 %), C_{18:0} (3.2 %), iso-C_{16:1} G (2.5 %), iso-C_{15:1} G (1.8 %), C_{17:1}9c (1.6 %), anteiso-C_{17:1} A (1.5 %), iso-C_{14:0} (1.0 %), C_{19:0} (1.0 %), C_{15:0} (0.9 %), C_{16:1}9c (0.7 %), iso-C_{18:0} (0.6 %), C_{14:0} (0.3 %) and C_{16:1} B (0.3 %), which corresponds to fatty acid type 2d of Kroppenstedt (1985). The G + C content of the DNA of strain YIM 61359^T was 69.7 mol%.

The almost-complete 16S rRNA gene sequence (1427 bp) of strain YIM 61359^T was used for phylogenetic analysis together with those of members of the family *Micromonosporaceae*. 16S rRNA gene sequence comparisons revealed that strain YIM 61359^T was most closely related to the type strains of members of the genera *Micromonospora* (96.6–98.1 % similarity) and *Salinispora* (97.7 %) and *Polymorphospora rubra* (97.6 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 61359^T belonged to a separate cluster within the family *Micromonosporaceae* and formed a

distinct monophyletic clade that was different from each of the 19 recognized genera in this family. It did not cluster within the genus *Micromonospora*, but was related to the genus *Salinispora* (Fig. 2). The topology of the tree generated with the maximum-likelihood algorithm was similar (data not shown). The 16S rRNA gene signature nucleotide positions of strain YIM 61359^T were compared with those of its closest phylogenetic relatives (see Supplementary Table S1 in IJSEM Online).

Strain YIM 61359^T formed single or cluster spores from substrate mycelia, similar to members of the genus *Salinispora*, but was clearly different from members of the genera *Micromonospora* and *Polymorphospora*, which formed only single spores or short spore chains from substrate mycelia (Ørskov, 1923; Tamura *et al.*, 2006). Members of the genus *Salinispora* have MK-9(H₄) as the major menaquinone and fatty acid profile type 3a. Members of the genus *Micromonospora* have MK-10(H_{4,6}) and MK-9(H_{4,6}) as major menaquinones and fatty acid profile type 3b. The genus *Polymorphospora* has MK-10(H_{6,4}) and MK-9(H_{6,4}) as major menaquinones and fatty acid profile type 2a. Strain YIM 61359^T can be distinguished from the genera *Salinispora*, *Micromonospora* and *Polymorphospora* based on the major menaquinones and the fatty acid profile (Table 1). In addition, the 16S rRNA gene signature nucleotides clearly distinguished the new isolate from members of these three genera.

Strain YIM 61359^T was not affiliated with any other recognized genus of the family *Micromonosporaceae* based on the distinctness of its 16S rRNA gene sequence and phylogenetic position. On the basis of morphological, chemotaxonomic and physiological data and the signature nucleotide pattern of the 16S rRNA gene, strain YIM 61359^T is readily distinguishable from the genera *Salinispora*, *Micromonospora* and *Polymorphospora*. Therefore, we suggest that strain YIM 61359^T represents a novel species of a new genus within the family *Micromonosporaceae*, for which the name *Plantactinospora mayteni* gen. nov., sp. nov. is proposed.

Description of *Plantactinospora* gen. nov.

Plantactinospora (plan.tac.ti.no.spo'ra. L. n. *planta* a plant; Gr. n. *actis actinos* a ray; Gr. n. *spora* a seed, and in biology a spore; N.L. fem. n. *Plantactinospora* pertaining to a spore-forming actinomycete isolated from plant tissues).

Aerobic, Gram-positive and non-acid-fast actinomycetes. Cells form extensively branched substrate mycelia (0.16–0.23 µm in diameter), which carry smooth-surfaced spores (0.63–1.10 µm in diameter) borne singly or in clusters. Spores are non-motile. White aerial mycelia are sparse. The cell wall contains *meso*-diaminopimelic acid as the diagnostic diamino acid. Arabinose, xylose, galactose and glucose are detected as whole-cell sugars. The acyl type of the cell-wall polysaccharides is glycolyl. Polar lipids detected are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol and several unknown

Fig. 2. Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences showing the relationship between strain YIM 61359^T and the type strains of species of related genera in the family Micromonosporaceae. Numbers at branch nodes are bootstrap percentages (based on 1000 replications; only values >50% are given). Bar, 0.01 substitutions per nucleotide position.

phospholipids, corresponding to phospholipid type PII. Mycolic acids are absent. The predominant menaquinones are MK-10(H₆), MK-10(H₈) and MK-10(H₄); MK-10(H₂)

and MK-9(H₆) are present as minor components. The major cellular fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, C_{17:0}, anteiso-C_{17:0} and iso-C_{16:0} (fatty acid type 2d). The

Table 1. Differential characteristics between strain YIM 61359^T and recognized genera in the family *Micromonosporaceae*

Taxa: 1, strain YIM 61359^T; 2, *Salinispora*; 3, *Micromonospora*; 4, *Polymorphospora*; 5, *Pseudosporangium*; 6, *Couchioplanes*; 7, *Krasilnikovia*; 8, *Actinoplanes*; 9, *Luedemannella*; 10, *Longispora*; 11, *Actinocatenispora*; 12, *Asanoa*; 13, *Catellatospora*; 14, *Catenuloplanes*; 15, *Dactylosporangium*; 16, *Pilimelia*; 17, *Spirilliplanes*; 18, *Verrucosipora*; 19, *Virgisporangium*; 20, *Planosporangium*. Data for reference genera were taken from Ørskov (1923), Couch (1950), Kane (1966), Thiemann *et al.* (1967), Asano & Kawamoto (1986), Yokota *et al.* (1993), Rheims *et al.* (1998), Kudo *et al.* (1999), Tamura *et al.* (1994, 1997, 2001, 2006), Lee & Hah (2002), Matsumoto *et al.* (2003), Maldonado *et al.* (2005), Thawai *et al.* (2006), Ara & Kudo (2006, 2007a, b), Wiese *et al.* (2008) and Ara *et al.* (2008). +, Present; –, absent; +/–, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Sporangium	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+	+	–	–	+	+
Spore motility	–	–	–	–	–	+	–	+	–	–	–	–	–	+	+	+	+	–	–	+/–
Diamino acid(s)*	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> - and 3-OH-DAP	L-Lysine	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	L-Lysine	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP
Diagnostic sugars†	Ara, Gal, Xyl	Ara, Gal, Xyl	Ara, Xyl	Xyl	Gal, Glu, Rib, Man, Xyl, Ara	Ara, Gal, Xyl	Gal, Man, Xyl, Ara, Rib	Ara, Xyl	Xyl, Gal, Man Rha, Rib, Ara	Ara, Gal, Xyl	Ara, Gal, Xyl	Ara, Gal, Xyl	Xyl, Man, Gal, Ara, Rha, Rib	Xyl	Ara, Xyl	Ara, Xyl	Ara, Xyl	Man, Xyl	Ara, Gal, Xyl	Ara, Xyl
Fatty acid type	2d	3a	3b	2a	2d	2c	2d	2d	2d	2d	3b	2d	3b	2c	3b	2d	2d	2b	2d	3b
Major mena-quinone(s)	MK-10(H _{6,8,4})	MK-9(H ₄)	MK-10(H _{4,6}), MK-9(H _{4,6})	MK-10(H _{6,4}), MK-9(H _{6,4})	MK-9(H ₆)	MK-9(H ₄)	MK-9(H _{6,4,8})	MK-9(H ₄)	MK-9(H _{6,4,2,8})	MK-10(H _{4,6})	MK-9(H _{4,6})	MK-10(H _{6,8})	MK-9(H _{4,6}) or MK-10(H _{4,6})	MK-9(H ₈), MK-10(H ₈)	MK-9(H _{4,6,8})	MK-9(H _{4,2})	MK-10(H ₄)	MK-9(H ₄)	MK-10(H _{4,6,8})	MK-9(H ₄), MK-10(H ₄)
Phospholipid type	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII
DNA G+C content (mol%)	69.7	70–73	71–72	71	73	70–72	71	72–73	71	70	72	71–72	70–71	71–73	71–73	ND	69	70	71	71.4

**m*-DAP, *meso*-diaminopimelic acid.

†Ara, Arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

DNA G+C content is approximately 69–70 mol%. The type species is *Plantactinospora mayteni*.

Description of *Plantactinospora mayteni* sp. nov.

Plantactinospora mayteni (may.te'ni. N.L. n. *Maytenus* a botanical genus name; N.L. gen. n. *mayteni* of the plant genus *Maytenus*).

Has the following characteristics in addition to those described for the genus. Colonies develop well on ISP 2, ISP 3 and Czapek's agar, moderately well on ISP 4 and PDA, but poorly on ISP 5 and nutrient agar. Soluble pigments are not formed. Colony colour varies from yellowish, orange–yellow, pale orange–yellow to black. Colonies can become darkened, raised and folded during sporulation. Grows at 15–37 °C and pH 6–8.0; unable to grow in the presence of 4% NaCl. Optimal temperature and pH for growth are 28 °C and pH 7.0. Degrades adenine, hypoxanthine, starch and xanthine. Milk is not coagulated or peptonized. Nitrate and H₂S are reduced. Utilizes D-arginine, L-asparagine, L-phenylalanine and L-proline as nitrogen resources. Utilizes L-arabinose, cellobiose, dulcitol, erythritol, D-fructose, D-galactose, glucose, inositol, lactose, maltose, mannitol, mannose, raffinose, L-rhamnose, D-ribose, sorbitol, sucrose, trehalose and D-xylose as carbon resources. Acid is not produced from L-rhamnose, inositol or D-fructose.

The type strain, YIM 61359^T (=CCTCC AA 208022^T=DSM 45238^T), was isolated from surface-sterilized roots of *Maytenus austroyunnanensis* collected from tropical rain-forest in Xishuangbanna, Yunnan Province, south-west China. The DNA G+C content of the type strain is 69.7 mol%.

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References

- Ara, I. & Kudo, T. (2006). Three novel species of the genus *Catellatospora*, *Catellatospora chokoriensis* sp. nov., *Catellatospora coxensis* sp. nov. and *Catellatospora bangladeshensis* sp. nov., and transfer of *Catellatospora citrea* subsp. *methionotrophica* Asano and Kawamoto 1988 to *Catellatospora methionotrophica* sp. nov., comb. nov. *Int J Syst Evol Microbiol* **56**, 393–400.
- Ara, I. & Kudo, T. (2007a). *Luedemannella* gen. nov., a new genus of the family *Micromonosporaceae* and description of *Luedemannella helvata* sp. nov. and *Luedemannella flava* sp. nov. *J Gen Appl Microbiol* **53**, 39–51.
- Ara, I. & Kudo, T. (2007b). *Krasilnikovia* gen. nov., a new member of the family *Micromonosporaceae* and description of *Krasilnikovia cinnamonea* sp. nov. *Actinomycetologica* **21**, 1–10.
- Ara, I., Matsumoto, A., Bakir, M. A., Kudo, T., Omura, S. & Takahashi, Y. (2008). *Pseudosporangium ferrugineum* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* **58**, 1644–1652.
- Asano, K. & Kawamoto, I. (1986). *Catellatospora*, a new genus of the *Actinomycetales*. *Int J Syst Bacteriol* **36**, 512–517.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycan based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Couch, J. N. (1950). *Actinoplanes*. A new genus of the *Actinomycetales*. *J Elisha Mitchell Sci Soc* **66**, 87–92.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Goodfellow, M. L., Stanton, J., Simpson, K. E. & Minnikin, D. E. (1990). Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. *J Gen Microbiol* **136**, 19–36.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terrigena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.
- Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. (2005). PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33**, W557–W559.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kane, W. D. (1966). A new genus of the *Actinoplanaceae*, *Pilimelia*, with a description of two species, *Pilimelia terevasa* and *Pilimelia anulata*. *J Elisha Mitchell Sci Soc* **82**, 220–230.
- Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- Koch, C., Kroppenstedt, R. M., Rainey, F. A. & Stackebrandt, E. (1996). 16S ribosomal DNA analysis of the genera *Micromonospora*, *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium*, and *Pilimelia* and emendation of the family *Micromonosporaceae*. *Int J Syst Bacteriol* **46**, 765–768.
- Krasil'nikov, N. A. (1938). *Ray Fungi and Related Organisms–Actinomycetales*. Moscow: Izdatel'stvo Akademii Nauk SSSR (in Russian).
- 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., Nakajima, Y. & Suzuki, K. (1999). *Catenuloplanes crispus* (Petroli et al. 1993) comb. nov.: incorporation of the genus *Planopolyspora* Petroli et al. 1993 into the genus *Catenuloplanes* Yokota et al. 1993 with an amended description of the genus *Catenuloplanes*. *Int J Syst Bacteriol* **49**, 1853–1860.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* 5, 249–260.
- Lee, S. D. & Hah, Y. C. (2002). Proposal to transfer *Catellatospora ferrugineum* and '*Catellatospora ishkariense*' to *Asanoa* gen. nov. as *Asanoa ferrugineum* comb. nov. and *Asanoa ishkariensis* sp. nov., with the emended description of the genus *Catellatospora*. *Int J Syst Evol Microbiol* 52, 967–972.
- 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.
- Maldonado, L. A., Fenical, W., Jensen, P. R., Kauffman, C. A., Mincer, T. J., Ward, A. C., Bull, A. T. & Goodfellow, M. (2005). *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 55, 1759–1766.
- Matsumoto, A., Takahashi, Y., Shinose, M., Seino, A., Iwai, Y. & Omura, S. (2003). *Longispora albida* gen. nov., sp. nov., a novel genus of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 53, 1553–1559.
- 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., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* 47, 87–95.
- Ørskov, J. (1923). *Investigations into the Morphology of the Ray Fungi*. Copenhagen: Levin and Munksgaard.
- Otoguro, M., Hayakawa, M., Yamazaki, T. & Imura, Y. (2001). An integrated method for the enrichment and selective isolation of *Actinokineospora* spp. in soil and plant litter. *J Appl Microbiol* 91, 118–130.
- Qin, S., Su, Y. Y., Zhang, Y. Q., Wang, H. B., Jiang, C. L., Xu, L. H. & Li, W. J. (2008a). *Pseudonocardia ailaonensis* sp. nov., isolated from soil in China. *Int J Syst Evol Microbiol* 58, 2086–2089.
- Qin, S., Wang, H. B., Chen, H. H., Zhang, Y. Q., Jiang, C. L., Xu, L. H. & Li, W. J. (2008b). *Glycomyces endophyticus* sp. nov., an endophytic actinomycete isolated from the root of *Carex baccans* Nees. *Int J Syst Evol Microbiol* 58, 2525–2528.
- Reider, P. J. & Roland, D. M. (1984). Maytansinoids. In *The Alkaloids*, pp. 71–156. Edited by A. Brossi. New York: Academic Press.
- Rheims, H., Schumann, P., Rohde, M. & Stackebrandt, E. (1998). *Verrucosipora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst Bacteriol* 48, 1119–1127.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- 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.
- Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E. & Yokota, A. (1994). A new genus of the order *Actinomycetales*, *Couchioplanes* gen. nov., with descriptions of *Couchioplanes caeruleus* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int J Syst Bacteriol* 44, 193–203.
- Tamura, T., Hayakawa, M. & Hatano, K. (1997). A new genus of the order *Actinomycetales*, *Spirilliplanes* gen. nov., with description of *Spirilliplanes yamanashiensis* sp. nov. *Int J Syst Bacteriol* 47, 97–102.
- Tamura, T., Hayakawa, M. & Hatano, K. (2001). A new genus of the order *Actinomycetales*, *Virgisporangium* gen. nov., with descriptions of *Virgisporangium ochraceum* sp. nov. and *Virgisporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* 51, 1809–1816.
- Tamura, T., Hatano, K. & Suzuki, K. (2006). A new genus of the family *Micromonosporaceae*, *Polymorphospora* gen. nov., with description of *Polymorphospora rubra* sp. nov. *Int J Syst Evol Microbiol* 56, 1959–1964.
- Thawai, C., Tanasupawat, S., Itoh, T. & Kudo, T. (2006). *Actinocatenispora thailandica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 56, 1789–1794.
- Thiemann, J. E., Pagani, H. & Beretta, G. (1967). A new genus of the *Actinoplanaceae*: *Dactyloporangium* gen. nov. *Arch Mikrobiol* 58, 42–52.
- 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.
- Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of acyl type of bacterial cell walls. *J Gen Appl Microbiol* 30, 131–134.
- Waksman, S. A. (1967). *The Actinomycetes. A Summary of Current Knowledge*. New York: Ronald Press.
- Wiese, J., Jiang, Y., Tang, S. K., Thiel, V., Schmaljohann, R., Xu, L. H., Jiang, C. L. & Imhoff, J. F. (2008). A new member of the family *Micromonosporaceae*, *Planosporangium flavigriseum* gen. nov., sp. nov. *Int J Syst Evol Microbiol* 58, 1324–1331.
- Yokota, A., Tamura, T., Hasegawa, T. & Huang, L. H. (1993). *Catenuloplanes japonicus* gen. nov., sp. nov., nom. rev., a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* 43, 805–812.
- Zhao, P. J., Wang, H. X., Li, G. H., Li, H. D., Liu, J. & Shen, Y. M. (2007). Secondary metabolites from endophytic *Streptomyces* sp. Lz531. *Chem Biodivers* 4, 899–904.