Plantactinospora endophytica sp. nov., an actinomycete isolated from *Camptotheca acuminata* Decne., reclassification of *Actinaurispora siamensis* as *Plantactinospora siamensis* comb. nov. and emended descriptions of the genus *Plantactinospora* and *Plantactinospora mayteni*

Wen-Yong Zhu,^{1†} Li-Xing Zhao,^{1†} Guo-Zhen Zhao,¹ Xue-Wei Duan,¹ Sheng Qin,² Jie Li,³ Li-Hua Xu¹ and Wen-Jun Li^{1,4}

Correspondence

Wen-Jun Li
wjli@ynu.edu.cn or
liact@hotmail.com

¹Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

²The Key Laboratory of Biotechnology for Medicinal Plant of Jiangsu Province, Xuzhou Normal University, Xuzhou, Jiangsu 221116, PR China

³Key Laboratory of Marine Bio-resources Sustainable Utilization CAS, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, PR China

⁴Key Laboratory of Biogeography and Bioresource in Arid Land, Chinese Academy of Science, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürümqi 830011, PR China

A novel endophytic actinomycete, designated strain YIM 68255^T, was isolated from healthy leaves of *Camptotheca acuminata* Decne. collected in Yunnan province, south-west China and characterized by using a polyphasic approach. The strain formed well-developed substrate mycelium, but no aerial mycelium. It grew at 10–45 °C, at pH 5–10 (optimum pH 7) and in the presence of 0–3 % (w/v) NaCl. The DNA G+C content was 73.0 mol%. Phylogenetic analyses showed that strain YIM 68255^T belonged to the genus *Plantactinospora*. However, it exhibited some differences from *Plantactinospora mayteni* YIM 61359^T and the level of DNA–DNA relatedness was 42.7 ± 1.3 %. Based on comparative analysis of physiological and chemotaxonomic data, it is proposed that strain YIM 68255^T represents a novel species of the genus *Plantactinospora*, *Plantactinospora endophytica* sp. nov., with strain YIM 68255^T (=DSM 45387^T=CCTCC AA 209047^T) as the type strain. In addition, it is also proposed that *Actinaurispora siamensis* Thawai *et al.* 2010 be transferred to the genus *Plantactinospora* as *Plantactinospora siamensis* comb. nov. [type strain CM2-8^T (=JCM 15677^T=BCC 34762^T)] based on chemotaxonomic characteristics and phylogenetic analysis. Emended descriptions of the genus *Plantactinospora* and *Plantactinospora mayteni* are also provided.

The genus *Plantactinospora* was described by Qin *et al.* (2009) with *Plantactinospora mayteni* as the type species.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 68255^T is GQ494033.

Four supplementary figures are available with the online version of this paper.

Shortly after the description of the genus *Plantactinospora*, the genus *Actinaurispora* was proposed with *Actinaurispora siamensis* as the type species (Thawai *et al.*, 2010). At present, each of the two genera comprises only a single species. In this study, phylogenetic trees based on 16S rRNA gene sequences of the type strains of the genera in the family *Micromonosporaceae* showed that these two genera belonged to one cluster. The main chemotaxonomic

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (1410 bp, by omitting unaligned regions) showing the relationships between strain YIM 68255^T and related members of the family Micromonosporaceae. Bootstrap values (>50 %) based on 1000 replicates are shown at the branch nodes. Asterisks indicate that the corresponding branches were also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods. *Glycomyces rutgersensis* IMSNU 22074^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

differences between these two genera are reported to be the whole-cell sugars (Ara, Gal and Xyl in *Plantactinospora* and Gal, Man and Xyl in *Actinaurispora*), major menaquinones [MK-10(H_{6,8,4}) in *Plantactinospora* and MK-9(H₆), MK-10(H₆) in *Actinaurispora*] and the fatty acid type (2d in *Plantactinospora* and 3b in *Actinaurispora*) (Qin *et al.*, 2009; Thawai *et al.*, 2010). During the course of investigating endophytic actinomycetes from medicinal plants in Yunnan province, south-west China, strain YIM 68255^T was isolated from the leaves of healthy *Camptotheca acuminata* Decne., a Chinese traditional medicine plant. On the basis of 16S rRNA gene sequence analysis, this isolate fell phylogenetically within the family Micromonosporaceae and clustered with *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T. In order to determine the phylogenetic and taxonomic relationships between strains YIM 68255^T, *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T, their morphological, physiological and chemotaxonomic characteristics were determined.

Healthy leaves of *C. acuminata* Decne. were washed in running water to remove soil particles and sterilized by an established procedure (Li *et al.*, 2008). After being surface-sterilized, the samples were sliced into pieces, followed by plating on TWYE agar (containing 0.25 g yeast extract, 0.5 g K₂HPO₄ and 18 g agar per litre of tap water, pH 7.2) containing nalidixic acid (25 mg l⁻¹), nystatin (50 mg l⁻¹) and cycloheximide (50 mg l⁻¹) to repress growth of Gram-negative bacteria and fungi. The plates were incubated at 28 °C for 4–6 weeks until the outgrowth of endophytic actinomycetes was discerned. Colonies originating from leaf segments were selected and pure cultures were obtained by repeated streaking on medium YIM 38 [10 g malt extract, 4 g yeast extract, 4 g glucose, vitamin mixture (containing 0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxine-HCl, inositol, calcium pantothenate and *p*-aminobenzoic acid and 0.25 mg biotin), 20 g agar; pH 7.2] (Jiang *et al.*, 2007). Purified strain YIM 68255^T was maintained on tryptic soy agar slants at 4 °C and as 20 % (v/v) glycerol suspensions at -80 °C.

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Li *et al.* (2007). Multiple alignments with sequences of the most closely related actinobacteria were carried out using the program CLUSTAL_X 1.8 (Thompson *et al.*, 1997). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the software packages MEGA version 5.0 (Tamura *et al.*, 2011). The stability of relationships was assessed by performing bootstrap analyses with 1000 resamplings

(Felsenstein, 1985). The values for sequence similarity among the closest strains were determined using the EzTaxon server (Chun *et al.*, 2007). Levels of DNA–DNA relatedness were determined according to the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). The G+C content of the genomic DNA was determined by using the HPLC method (Mesbah *et al.*, 1989).

An almost complete 16S rRNA gene sequence (1515 bp) of strain YIM 68255^T was used for phylogenetic analysis with sequences of members of the family Micromonosporaceae. The highest sequence similarities (≥97.0 %) were between strain YIM 68255^T and members of the genera *Micromonospora*, *Actinoplanes*, *Asanoa*, *Dactylosporangium*, *Couchioplanes*, *Salinispora*, *Spirilliplanes*, *Jishengella*, *Actinaurispora*, *Polymorphospora* and *Plantactinospora* (97.0–99.1 %). Despite these high 16S rRNA gene sequence similarities, strain YIM 68255^T clustered with *P. mayteni* YIM 61359^T (99.1 % similarity) and *A. siamensis* CM2-8^T (97.8 % similarity) after phylogenetic analysis of the 16S rRNA gene sequences (Fig. 1; Figs S1 and S2, available in IJSEM Online). *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T exhibited 97.8 % 16S rRNA gene sequence similarity to each other. The phylogenetic results clearly demonstrated that strain YIM 68255^T was more closely related to *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T than other members of the family Micromonosporaceae. The levels of DNA–DNA relatedness between strain YIM 68255^T and *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T were 42.7 ± 1.3 % and 24.7 ± 0.9 %, respectively. *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T shared a DNA relatedness value of 31.3 ± 1.2 %. These values were well below the 70 % cut-off point for recognition of genomic species (Wayne *et al.*, 1987), thus suggesting that strain YIM 68255^T, *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T represent different species. The DNA G+C content of strain YIM 68255^T was 73.0 mol%.

Gram staining was carried out by using the standard Gram stain and cell motility was established by the development of turbidity throughout a tube containing semi-solid medium (Leifson, 1960). Cultural characteristics of strain YIM 68255^T were recorded on ISP (International *Streptomyces* Project) media (Shirling & Gottlieb, 1966), Czapek's agar, potato-glucose agar (PDA) and nutrient agar prepared as described by Dong & Cai (2001). The colony colours were determined using the ISCC–NBS colour charts (Kelly, 1964). Microscopic observations of mycelia and spores of strain YIM 68255^T grown on ISP 2 medium for 3–7 weeks at 28 °C were made by light microscopy (BH 2; Olympus) and scanning electron microscopy (Quanta 200; FEI). Growth was tested at 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C on ISP 2 medium by

incubating the cultures for 35 days. The ability of the strain to grow at different pH values (pH 4, 5, 6, 7, 8, 9, 10 and 11, using the buffer system described by Xu *et al.*, 2005) and NaCl concentrations (0, 1, 2, 3, 4, 5, 7, 10, 12, 15 and 20%, w/v) was examined at 28 °C for 35 days. Catalase, oxidase and gelatinase activities, starch hydrolysis, nitrate reduction and urease were assessed as described by Smibert & Krieg (1994). Media and procedures used for the determination of other physiological characteristics, carbon source utilization and acid production from carbohydrates were those described by Gordon *et al.* (1974).

Strain YIM 68255^T was an aerobic, non-motile, Gram-positive actinomycete that formed well-developed substrate mycelium, but no aerial mycelium. Smooth-surfaced spores were borne singly on the substrate hyphae (Fig. 2). Strain YIM 68255^T grew well on ISP 2, ISP 3, PDA, nutrient agar and Czapek's agar, with moderate growth on ISP 4 medium and poor growth on ISP 5 medium. No soluble pigments were produced on any of the test media. The colour of colonies was orange-red before 6 weeks, and then brown on ISP 2, ISP 3, ISP 4, ISP 5 and PDA media, but orange-yellow before 5 weeks and then black on Czapek's agar and orange-yellow on nutrient agar. Strain YIM 68255^T could grow at 10–45 °C, at pH 5–10 (optimum pH 7) and in the presence of 0–3% (w/v) NaCl, but not in 4% NaCl. Detailed physiological and biochemical characteristics of strain YIM 68255^T are given in the species description and Table 1.

The freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in tryptic soy broth on a rotary shaker at 28 °C for 21 days. Amino acid and sugar analyses of whole-cell hydrolysates were performed according to the procedures described by Hasegawa *et al.* (1983); Lechevalier & Lechevalier (1970) and Tang *et al.* (2009). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were isolated according to Collins *et al.* (1977) and

separated by HPLC (Tamaoka *et al.*, 1983). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions. Fatty acid methyl esters were analysed by using the Microbial Identification software package (Sherlock Version 6.1; MIDI database TSBA6).

The peptidoglycan of isolate YIM 68255^T contained *meso*-diaminopimelic acid, whereas the whole-cell sugars detected were arabinose, galactose, glucose, mannose, xylose and rhamnose. The predominant menaquinones were MK-10(H₆) (59.6%), MK-10(H₈) (16.1%), MK-9(H₆) (10.9%) and MK-10(H₄) (6.9%), with MK-9(H₈) (3.3%), MK-9(H₄) (1.8%) and MK-10(H₂) (1.3%) as minor components. Phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol were detected (Fig. S3), corresponding to phospholipid type PII of Lechevalier *et al.* (1977). The major fatty acids were anteiso-C_{17:0} (26.20%), iso-C_{16:0} (22.75%), iso-C_{15:0} (12.71%), iso-C_{17:0} (8.99%) and anteiso-C_{15:0} (8.07%). The chemotaxonomic properties (whole-cell sugars, predominant menaquinones and fatty acid type) of the three strains, YIM 68255^T, *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T, were very similar to each other in this study (Table 2). Arabinose, galactose, glucose, mannose, xylose and rhamnose were detected as whole-cell sugars (Fig. S4). The major fatty acids were iso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and anteiso-C_{15:0}. The menaquinone components of these three strains were not significantly different, except that MK-9(H₆) was detected in *A. siamensis* CM2-8^T and strain YIM 68255^T as a predominant menaquinone (Table 2).

On the basis of the phenotypic, genotypic and phylogenetic data presented here, strain YIM 68255^T differed from its closest relatives, *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T. It could grow at 10–45 °C and pH 5–10 and was positive for catalase production and Tween 80 hydrolysis. D-Galactose, inositol, lactose and mannitol were not used as sole carbon sources. L-Hydroxyproline was used as a sole nitrogen source. Furthermore, the levels of DNA–DNA relatedness of strain YIM 68255^T with *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T were 42.7 ± 1.3% and 24.7 ± 0.9%, respectively. Based on the data presented, it is proposed that strain YIM 68255^T should be recognized as a novel species of *Plantactinospora*, for which the name *Plantactinospora endophytica* sp. nov. is proposed. Furthermore, the results of phylogenetic analysis showed that the relationship between these three strains, YIM 68255^T, *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T, was much closer than with other members of the family *Micromonosporaceae* and their chemotaxonomic characteristics were very similar to each other. All three strains had the same peptidoglycan diagnostic amino acid (*meso*-diaminopimelic acid), whole-cell sugars (arabinose, galactose, glucose, mannose, xylose and rhamnose), polar lipid type (PII) and major fatty acids (iso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and anteiso-C_{15:0}), and the predominant menaquinones were not significantly different. Based on our

Fig. 2. Scanning electron micrograph of strain YIM 68255^T grown on ISP 2 medium for 7 weeks at 28 °C. Bar, 5 µm.

Table 1. Differential phenotypic characteristics of strain YIM 68255^T and related strains

Strains: 1, YIM 68255^T; 2, *P. mayteni* YIM 61359^T; 3, *A. siamensis* CM2-8^T. +, Positive; w, weakly positive; –, negative. Data are from this study, tested under the same conditions, unless indicated otherwise.

Characteristic	1	2	3
Aerial mycelium	None	White, sparse	None
Spore shape	Smooth, single	Smooth, single/cluster*	Rough, single†
Temperature for growth (°C)			
Range	10–45	4–45	10–40
Optimum	20–37	15–37	20–37
pH for growth	5–10	6–8	5–8
NaCl concentration for growth (% w/v)	0–3	0–3	0–2
Catalase	w	–	–
Oxidase	–	+	–
Gelatinase	–	–	w
Hydrolysis of:			
Tween 20	+	–	+
Tween 40	+	+	–
Tween 80	+	–	–
Utilization as sole carbon source:			
D-Arabinose	–	+	–
Dulcitol	–	+	–
D-Galactose	–	+	+
Inositol	–	+	+
Lactose	–	+	+
Mannitol	–	+	+
D-Mannose	–	+	–
Xylitol	–	+	–
Raffinose	–	+	–
D-Ribose	–	+	–
Sorbitol	–	+	–
Trehalose	–	+	–
Utilization as sole nitrogen source:			
L-Arginine	+	+	–
L-Lysine	+	–	+
Hypoxanthine	–	+	–
L-Hydroxyproline	+	–	–
L-Ornithine	+	+	–
L-Serine	+	–	+
L-Valine	+	+	–
Xanthine	–	+	–
DNA G + C content (mol%)	73.0	69.7*	72.6†

*Data from Qin *et al.* (2009).

†Data from Thawai *et al.* (2010).

data, the two genera *Actinaurispora* and *Plantactinospora* should be combined, and *A. siamensis* should be reclassified as *Plantactinospora siamensis* comb. nov. The descriptions of the genus *Plantactinospora* and *P. mayteni* are emended.

Emended description of the genus *Plantactinospora* Qin *et al.* 2009

The genus is as described by Qin *et al.* (2009), with the following emendations. Smooth/rough-surfaced spores are borne singly or in clusters. Arabinose, galactose, glucose, mannose, xylose and rhamnose are detected as whole-cell

sugars. The phospholipid type is PII. The predominant menaquinone is MK-9(H₆) or MK-10(H₆). The major fatty acids are iso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and anteiso-C_{15:0}. The DNA G + C content is 69.0–73.0 mol%.

Emended description of *Plantactinospora* *mayteni* Qin *et al.* 2009

The emended description of this species is based on that given by Qin *et al.* (2009), with the following supplements and changes. Utilizes inositol and xylitol as sole carbon sources, but not glycerol. L-Asparagine, L-alanine, glycine,

Table 2. Chemotaxonomic characteristics that differentiate strain YIM 68255^T from its closest relatives

Strains: 1, YIM 68255^T; 2, *P. mayteni* YIM 61359^T; 3, *A. siamensis* CM2-8^T. All data are taken from this study and all three strains have the same diagnostic amino acid in the peptidoglycan (*meso*-diaminopimelic acid), whole-cell sugars (arabinose, galactose, glucose, mannose, xylose, rhamnose) and polar lipid type (PII). —, Not detected.

Characteristic	1	2	3
Predominant menaquinones (>5 %)	MK-10(H ₆) (59.6 %), MK-10(H ₈) (16.1 %), MK-9(H ₆) (10.9 %), MK-10(H ₄) (6.9 %)	MK-10(H ₆) (49.4 %), MK-10(H ₈) (26.6 %), MK-10(H ₄) (11.2 %), MK-9(H ₆) (7.5 %)	MK-9(H ₆) (39.6 %), MK-10(H ₆) (17.2 %), MK-9(H ₈) (8.5 %), MK-10(H ₈) (6.6 %)
Fatty acid (%)			
C _{16:0}	0.48	0.39	0.74
C _{17:0}	3.19	4.28	8.79
C _{18:0}	2.53	0.54	0.88
C _{19:0}	1.06	0.40	0.73
C _{16:1} 2-OH	2.20	1.98	—
C _{17:1} ω8c	0.98	6.53	2.42
C _{18:1} ω9c	1.26	1.08	0.41
iso-C _{14:0}	1.61	1.00	1.16
iso-C _{15:0}	12.71	18.18	23.37
iso-C _{16:0}	22.75	17.77	16.04
iso-C _{17:0}	8.99	4.65	11.87
iso-C _{18:0}	3.22	0.95	0.4
iso-C _{19:0}	0.38	—	—
iso-C _{15:1} G	0.05	0.58	—
iso-C _{16:1} G	1.19	1.08	—
anteiso-C _{15:0}	8.07	17.45	11.15
anteiso-C _{17:0}	26.2	20.37	17.38
anteiso-C _{19:0}	0.79	—	—
anteiso-C _{17:1} A	0.66	1.08	—
10-methyl C _{17:0}	0.58	0.68	3.93
10-methyl C _{18:0}	0.21	—	0.28
Summed feature 6*	—	0.45	—
Summed feature 9*	0.40	0.27	0.45

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 6 consisted of C_{19:1}ω11c and/or C_{19:1}ω9c. Summed feature 9 consisted of iso-C_{17:1}ω9c and/or 10-methyl-C_{16:0}.

hypoxanthine, L-ornithine, L-valine and xanthine are used as sole nitrogen sources, but not L-hydroxyproline, L-lysine or L-serine. Arabinose, galactose, glucose, mannose, xylose and rhamnose are detected as whole-cell sugars. Major fatty acids are anteiso-C_{17:0}, anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0}, C_{17:1}ω8c and iso-C_{17:0}.

Description of *Plantactinospora siamensis* comb. nov.

Plantactinospora siamensis (si.am.en'sis. N.L. fem. adj. *siamensis* of or pertaining to Siam, the old name for Thailand, the country of origin of the soil from which the type strain was isolated).

Basonym: *Actinaurispora siamensis* Thawai *et al.* 2010.

This description is based on that given by Thawai *et al.* (2010), with the following additions and changes. Inositol and sucrose can be utilized as sole carbon sources, but not dulcitol, D-mannose, sorbitol, trehalose or xylitol. Utilizes

L-alanine, L-asparagine, glycine, L-lysine and L-serine as sole nitrogen sources, but not L-arginine, L-hydroxyproline, hypoxanthine, L-ornithine, L-valine or xanthine. Arabinose, galactose, glucose, mannose, xylose and rhamnose are detected as whole-cell sugars.

The type strain is CM2-8^T (=JCM 15677^T=BCC 34762^T), isolated from temperate peat swamp forest soil in Chiang Mai Province, Thailand.

Description of *Plantactinospora endophytica* sp. nov.

Plantactinospora endophytica (en.do.phy'ti.ca. Gr. pref. *endo* within; Gr. n. *phuton* plant; L. fem. suff. *-ica* adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica* within plant, endophytic).

Aerobic, non-motile, Gram-positive actinomycete that forms well-developed substrate mycelium, but no aerial mycelium. Smooth-surfaced spores are borne singly on the

substrate hyphae. Grows well on ISP 2, ISP 3, PDA, nutrient agar and Czapek's agar, with moderate growth on ISP 4 medium and poor growth on ISP 5 medium. No soluble pigments are produced on the media tested. The colour of colonies is orange-red before 6 weeks, and then brown on ISP 2, ISP 3, ISP 4, ISP 5 and PDA media, orange-yellow before 5 weeks and then black on Czapek's agar, and orange-yellow on nutrient agar. Grows at 10–45 °C, at pH 5–10 (optimum pH 7) and in the presence of 0–3 % (w/v) NaCl, but not in 4 % NaCl. Negative for production of oxidase, gelatinase, urease and H₂S. Negative for milk coagulation and peptonization, starch hydrolysis and nitrate reduction. Weakly positive for production of catalase. Tweens 20, 40, 80 and cellulose are hydrolysed. Utilizes cellobiose, D-fructose, glucose, maltose, L-rhamnose, sucrose and D-xylose as sole carbon sources. D-Arabinose, dulcitol, glycerol, D-galactose, inositol, lactose, D-mannose, mannitol, raffinose, D-ribose, sorbitol, trehalose and xylitol are not utilized. L-Alanine, L-arginine, L-asparagine, glycine, L-hydroxyproline, L-lysine, L-ornithine, L-serine and L-valine are used as sole nitrogen sources, but not hypoxanthine or xanthine. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and several unknown lipids. The cell wall contains *meso*-diaminopimelic acid and the whole-cell sugars detected are arabinose, galactose, glucose, mannose, xylose and rhamnose. The predominant menaquinones are MK-10(H₆), MK-10(H₈), MK-9(H₆) and MK-10(H₄). Major fatty acids are anteiso-C_{17:0}, iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{17:0}.

The type strain, YIM 68255^T (=DSM 45387^T=CCTCC AA 209047^T), was isolated from leaves of *Camptotheca acuminata* Decne. collected in Yunnan Province, south-west China. The DNA G+C content of the type strain is 73.0 mol%.

Acknowledgements

The authors are grateful to Professor Chitti Thawai from King Mongkut's Institute of Technology Ladkrabang, Thailand, for kindly providing the reference type strain *A. siamensis* CM2-8^T and Dr Paul Meyers from University of Cape Town, South Africa for his helpful comments on our manuscript. This research was supported by the National Basic Research Program of China (2010CB833801), the National Natural Science Foundation of China (no. U0932601) and the Basic Research Foundation of Yunnan University (no. 2010ZD001). W.-J. L. was also supported by 'Hundred Talents Program' of the Chinese Academy of Sciences.

References

Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans 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.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Dong, X. Z. & Cai, M. Y. (2001). *Manual of Systematics and Identification of General Bacteria*. Beijing: Science Press (in Chinese).

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.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

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.

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

HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

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

Jiang, Y., Tang, S. K., Wiese, J., Xu, L. H., Imhoff, J. F. & Jiang, C. L. (2007). *Streptomyces hainanensis* sp. nov., a novel member of the genus *Streptomyces*. *Int J Syst Evol Microbiol* **57**, 2694–2698.

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.

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., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Leifson, E. (1960). *Atlas of Bacterial Flagellation*. London: Academic Press.

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.

Li, J., Zhao, G. Z., Chen, H. H., Wang, H. B., Qin, S., Zhu, W. Y., Xu, L. H., Jiang, C. L. & Li, W. J. (2008). Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. *Lett Appl Microbiol* **47**, 574–580.

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.

Qin, S., Li, J., Zhang, Y. Q., Zhu, W. Y., Zhao, G. Z., Xu, L. H. & Li, W. J. (2009). *Plantactinospora mayteni* gen. nov., sp. nov., a member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* **59**, 2527–2533.

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.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, D.C.: American Society for Microbiology.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983).** Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011).** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.
- Tang, S. K., Wang, Y., Chen, Y., Lou, K., Cao, L. L., Xu, L. H. & Li, W. J. (2009).** *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int J Syst Evol Microbiol* **59**, 2025–2031.
- Thawai, C., Tanasupawat, S., Suwanborirux, K. & Kudo, T. (2010).** *Actinaurispora siamensis* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* **60**, 1660–1666.
- 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.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & 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.
- Xu, P., Li, W. J., Tang, S. K., Zhang, Y. Q., Chen, G. Z., Chen, H. H., Xu, L. H. & Jiang, C. L. (2005).** *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family ‘*Oxalobacteraceae*’ isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.