Kineosporia mesophila sp. nov., isolated from surface-sterilized stems of *Tripterygium wilfordii*

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An endophytic actinomycete strain, designated YIM 65293^T, was isolated from a surface-sterilized stem sample of *Tripterygium wilfordii* collected from Yunnan province, south-west China, and its taxonomic position was investigated. The chemical and morphological properties of the organism were consistent with those of the genus *Kineosporia*. Phylogenetic analysis indicated that the levels of 16S rRNA gene sequence similarity between strain YIM 65293^T and other type strains of recognized members of the genus *Kineosporia* were 97.0–98.2%. However, the DNA–DNA hybridization values, in combination with differences in phenotypic characteristics, revealed that the strain differed from recognized species of the genus *Kineosporia*. Therefore, strain YIM 65293^T represents a novel species of the genus *Kineosporia*, for which the name *Kineosporia mesophila* sp. nov. is proposed. The type strain is YIM 65293^T (=CCTCC AA 208061^T=DSM 45271^T).

The genus *Kineosporia* was first described by Pagani & Parenti (1978) and then amended by Itoh *et al.* (1989) on the basis of morphology and chemotaxonomy. Subsequently, Kudo *et al.* (1998) amended the compositions of the diaminopimelic acid isomers and whole-cell sugars of this genus. At the time of writing, there are six recognized species belonging to the genus *Kineosporia*, including the recently described organism *Kineosporia babensis* (Sakiyama *et al.*, 2009). During an investigation into the diversity of endophytic actinomycetes from pharmaceutical plants, strain YIM 65293^T was isolated from surface-sterilized stems of *Tripterygium wilfordii*, which is a member of the Celastraceae family of plants and known as *lei gong teng* in Chinese.

Samples of *T. wilfordii* were collected from Yunnan province, south-west China, and were surface-sterilized according to the method of Coombs & Franco (2003). Strain YIM 65293^T was isolated by using the procedure described by Li *et al.* (2009) and grown on tap water-yeast extract agar (Coombs & Franco, 2003). The purified strain

was routinely cultivated on yeast extract-malt extract agar (ISP 2) at 28 °C and stored as an aqueous glycerol suspension (20%, v/v) at –70 °C.

The cultural characteristics of strain YIM 65293^T were determined after 3 weeks incubation at 28 °C, according to the methods of the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966). Czapek's agar, potato agar and nutrient agar were prepared as described by Waksman (1961). Colour determination was performed by comparison with chips from the *Inter-Society Color Council – National Bureau of Standards* colour charts (standard samples, no. 2106) (Kelly, 1964). After incubation on ISP 2 at 28 °C for 14 days, morphological properties were examined using a light microscope (BH-2; Olympus) and a scanning electron microscope (JSM-5600LV; JEOL).

For this study, the phenotypic characteristics of *K. mikuniensis* NBRC 16234^T, *K. succinea* I-273^T, *K. aurantiaca* JCM 3230^T, *K. rhamnosa* I-132^T and *K. rhizophila* I-449^T as well as those of strain YIM 65293^T were determined. Carbon source utilization was determined according to the methods of Shirling & Gottlieb (1966) and Locci (1989). Acid production from carbohydrates was assessed as described by Gordon *et al.* (1974). Nitrogen source utilization was assessed according to Williams *et al.* (1989). Gelatin hydrolysis was determined by incubating strain YIM 65293^T at 28 °C for 3 weeks on peptone-gelatin medium, comprising (l⁻¹ distilled water) 5 g peptone and

Abbreviation: TBSA, tuberculostearic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 65293^T is FJ214362.

Scanning electron micrographs of cells of strain YIM 65293^T and a table showing the fatty acid contents of *Kineosporia* strains are available with the online version of this paper.

120 g gelatin. Hydrolysis of urea was determined on peptone-glucose agar, comprising (1^{-1} distilled water) 1 g peptone, 1 g glucose, 5 g NaCl and 2 g KH_2PO_4 , which was supplemented with 2 % (w/v) urea and 0.001 % (w/v) phenol red. Milk coagulation and peptonization were determined by using 20 % (w/v) skimmed milk as the medium and incubation for 3 weeks at 28 °C. Decomposition of other compounds was examined on the basal medium of Gordon *et al.* (1974). Growth at different temperatures (4, 10, 15, 20, 28–37 °C at intervals of 1 °C, 45, 55 and 65 °C), pH values (pH 4.0–10.0, at intervals of 1.0 pH units) and NaCl concentrations (1, 3, 5, 7, 10, 12, 15 and 20 %; w/v) were determined on ISP 2 as the basal medium.

Biomass for chemotaxonomic analyses was obtained by growing the novel strain in tryptic soy broth (TSB) in shake flasks (at 180 r.p.m.) for 4–7 days at 28 °C. The analysis of cellular fatty acid composition followed the instructions of the Microbial Identification System (Sasser, 1990). The fatty acid compositions of *K. mikuniensis* NBRC 16234^T, *K. succinea* I-273^T and *K. rhizophila* I-449^T were determined as well that of strain YIM 65293^T. The purified cell-wall hydrolysate was prepared as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysate were analysed by precolumn derivatization with *o*-phthalaldehyde by HPLC (Tang *et al.*, 2009). Whole-cell hydrolysate sugars were determined using TLC as described by Stanek & Roberts (1974). Phospholipids were extracted and identified according to published methods (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were extracted as described by Collins *et al.* (1977), and analysed by HPLC (Tamaoka *et al.*, 1983). The G + C content of the genomic DNA was determined by the HPLC method according to Mesbah *et al.* (1989).

The 16S rRNA gene sequence of strain YIM 65293^T was amplified by PCR and sequenced as described previously (Li *et al.*, 2007). The resulting 16S rRNA gene sequence was compared with those of the type strains of the genus *Kineosporia*, which were retrieved from the GenBank database, and aligned using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analysis was performed using MEGA version 4.0 (Tamura *et al.*, 2007). Evolutionary distances and the K_{nuc} value (Kimura, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replications.

Strain YIM 65293^T grew well on ISP 2, ISP 3, ISP 4, ISP 5 agars, potato agar and nutrient agar at 28 °C. However, poor growth was observed on Czapek's agar. Strain YIM 65293^T lacked aerial mycelium on any of the media tested. Colonies were moderate orange–yellow on ISP 2, ISP 4 and potato agars, orange–yellow on Czapek's agar, deep orange–yellow on ISP 5 agar and light orange on ISP 3 and nutrient agars. There was no diffusible pigment on any of the media tested. After incubation for nearly 1 week, the surfaces of the colonies became moist and glossy. Single

spores, spherical to oval or pyriform, were borne at the tips of substrate hyphae (see Supplementary Fig. S1, available in IJSEM Online). Detailed physiological results are given in Table 1 and in the species description.

HPLC analysis of amino acids obtained from submerged cultures in shake flasks revealed that strain YIM 65293^T contained Asp, Glu, Gly, Ala and Lys, but no diaminopimelic acid. Whole-cell hydrolysates contained glucose, galactose, mannose and ribose. The phospholipids comprised phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol and phosphatidylinositol mannosides. The menaquinones were represented by MK-9(H_4) (94.8 %) and MK-9(H_2) (5.2 %). Major fatty acids of the strain (>10 %) were $\text{C}_{16:0}$ (27.98 %), $\text{C}_{18:1}$ *cis*-9 (20.67 %) and tuberculostearic acid (TBSA, $\text{C}_{18:0}$ 10-methyl; 18.60 %). The detailed fatty acid profile is given in Supplementary Table S1 (in IJSEM Online). The G + C content of the genomic DNA from strain YIM 65293^T was 70.3 mol%.

The almost-complete 16S rRNA gene sequence (1425 bp) for strain YIM 65293^T was determined. The results of the phylogenetic analysis indicated that strain YIM 65293^T clustered with members of the genus *Kineosporia* (Fig. 1). The closest phylogenetic neighbour was *Kineosporia mikuniensis* NBRC 16234^T (98.2 % sequence similarity). Sequence similarities between strain YIM 65293^T and other members of the genus *Kineosporia* were 97.0–98.1 %. Genomic relatedness between strain YIM 65293^T and other members of the genus *Kineosporia* was examined according to the fluorometric micro-well method (Ezaki *et al.*, 1989; He *et al.*, 2005). The levels of DNA–DNA relatedness of strain YIM 65293^T to *K. mikuniensis* NBRC 16234^T, *K. succinea* I-273^T, *K. aurantiaca* JCM 3230^T, *K. rhamnosa* I-132^T and *K. rhizophila* I-449^T were 59.0, 48.9, 48.1, 34.3 and 34.5 %, respectively. All of the DNA–DNA hybridization values were less than 70 %, the recommended threshold value for the delineation of genomic species (Stackebrandt & Goebel, 1994), which supports the placement of strain YIM 65293^T in a novel species of the genus *Kineosporia*.

Differences between strain YIM 65293^T and other members of the genus *Kineosporia* were found for the reduction of nitrate, production of H_2S , tolerance of NaCl concentration and utilization of sole carbon and nitrogen sources (Table 1). A striking difference was also observed in the composition of the diaminopimelic acid isomers. It was reported by Kudo *et al.* (1998) that strains in the genus *Kineosporia* showed variety in the ratio of diaminopimelic acid isomers and could be classified into LL type, *meso* type and LL-plus-*meso* type, and it has also been reported that the proportions of the two isomers in shake-flask cultures can vary with culture condition (Eke *et al.*, 1989; Kudo *et al.*, 1998). Nevertheless, this study detected no diaminopimelic acid isomers in submerged cultures of strain YIM 65293^T in shake flasks. Strain YIM 65293^T also had remarkable differences in the fatty acid profile, such as the quantitative differences in the proportions of $\text{C}_{16:0}$, $\text{C}_{17:0}$, $\text{C}_{18:1}$ *cis*-9 and TBSA.

Table 1. Phenotypic characteristics that differentiate strain YIM 65293^T from other species of the genus *Kineosporia*

Strains: 1, YIM 65293^T; 2, *K. mikuniensis* NBRC 16234^T; 3, *K. succinea* I-273^T; 4, *K. aurantiaca* JCM 3230^T; 5, *K. rhamnosa* I-132^T; 6, *K. rhizophila* I-449^T. Data were taken from this study. All strains were positive for hydrolysis of urea, starch and Tweens 20, 40 and 80 and for utilization of L-arabinose, cellobiose, D-fructose, D-galactose, glucose, maltose, D-mannitol, L-rhamnose, turanose, glycine and L-valine. All strains were negative for decomposition of adenine, cellulose, gelatin, hypoxanthine, L-tyrosine and xanthine and for the utilization of sodium acetate and milk coagulation and peptonization. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6
Isolation source*	Stems of <i>Tripterygium wilfordii</i>	Sphagnum	Fallen leaves	Soil	Leaves of <i>Typha latifolia</i>	Roots of <i>Cyperus microiria</i>
Nitrate reduction	–	–	–	–	–	+
H ₂ S production	–	+	–	–	–	–
Highest NaCl tolerance (% w/v)	3	1	5	3	3	5
Sole carbon source utilization						
D-Arabinose	+	–	+	+	–	+
Dulcitol	–	+	+	–	–	–
Inositol	–	+	+	+	+	–
Lactose	–	+	+	–	–	+
D-Mannose	+	+	–	+	–	–
Melezitose	+	+	+	+	–	+
Melibiose	+	+	+	+	–	+
Raffinose	+	–	–	–	+	+
D-Ribose	–	+	+	+	–	+
Sodium oxalate	+	–	–	+	+	+
D-Sorbitol	+	–	+	–	+	–
Sucrose	+	+	+	+	–	+
Trehalose	+	+	+	+	–	+
Trisodium citrate	+	–	–	+	+	–
D-Xylose	+	+	+	–	+	+
Sole nitrogen source utilization						
L-Alanine	+	+	+	+	–	+
L-Arginine	–	+	–	+	+	+
L-Asparagine	+	+	+	+	–	+
L-Hydroxyproline	+	+	–	+	+	+
L-Lysine	–	+	–	–	–	+
L-Phenylalanine	+	+	+	–	+	+
L-Serine	+	+	–	+	+	+
DNA G + C content (mol%)*	70.3	69.4	70.7	69.5	68.3	70.0

*Data from Pagani & Parenti (1978) and Kudo *et al.* (1998) except for strain YIM 65293^T.

On the basis of the phenotypic and phylogenetic studies, it is clear that strain YIM 65293^T represents a novel species of the genus *Kineosporia*, for which the name *Kineosporia mesophila* sp. nov. is proposed.

Description of *Kineosporia mesophila* sp. nov.

Kineosporia mesophila (me.so'phi.la. Gr. adj. *mesos* medium; Gr. adj. *philos* loving; N.L. fem. adj. *mesophila* medium-temperature-loving, mesophilic).

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain YIM 65293^T and other members of the genus *Kineosporia*. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Bar, 1 substitution per 100 nucleotide positions.

Substrate mycelia are light orange to deep orange–yellow. Does not produce any diffusible pigments on ISP 2, ISP 3, ISP 4, ISP 5 agars, potato agar, Czapek's agar or nutrient agar. Single spores, spherical to oval or pyriform, are borne at the tips of substrate hyphae. Growth occurs at 10–32 °C and pH 6.0–8.0. Tolerates 3 % (w/v) NaCl. Negative for production of H₂S, nitrate reduction and milk coagulation and peptonization. Urea, starch and Tweens 20, 40 and 80 are hydrolysed. Adenine, cellulose, gelatin, hypoxanthine, L-tyrosine and xanthine are not decomposed. Utilizes D-arabinose, D-fructose, glucose, D-xylose, maltose, D-mannitol, D-mannose, L-rhamnose, sodium oxalate, D-sorbitol and trisodium citrate as sole carbon sources, but not dulcitol, inositol, lactose, D-ribose or sodium acetate. Acid is produced from L-arabinose, cellobiose, D-galactose, melezitose, melibiose, raffinose, sucrose, trehalose and turanose. L-Alanine, L-asparagine, glycine, L-hydroxyproline, L-phenylalanine, L-serine and L-valine are used as sole nitrogen sources, but not L-arginine or L-lysine. The cell-wall peptidoglycan contains aspartic acid, glutamic acid, glycine, alanine and lysine. Glucose, galactose, mannose and ribose are present in whole-cell hydrolysates. Phospholipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol and phosphatidylinositol mannosides. The predominant menaquinone is MK-9(H₄). Major fatty acids (>10 %) are C_{16:0}, C_{18:1} *cis*-9 and TBSA.

The type strain, YIM 65293^T (=CCTCC AA 208061^T=DSM 45271^T), was isolated from a surface-sterilized stem sample of *Tripterygium wilfordii* from Yunnan province, south-west China. The DNA G+C content of the type strain is 70.3 mol%.

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