Jiangella alba sp. nov., an endophytic actinomycete isolated from the stem of *Maytenus austroyunnanensis*

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A novel actinomycete strain, YIM 61503^T, isolated from the stem of *Maytenus austroyunnanensis*, was characterized by using a polyphasic approach. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain YIM 61503^T belonged to the genus *Jiangella* and exhibited 16S rRNA gene sequence similarities of 98.8 and 98.6 % to *Jiangella alkaliphila* D8-87^T and *Jiangella gansuensis* YIM 002^T. The chemotaxonomic properties of strain YIM 61503^T were consistent with those of the genus *Jiangella*: the cell-wall peptidoglycan type was based on LL-diaminopimelic acid and MK-9(H₄) was the predominant menaquinone. The major fatty acids were anteiso-C₁₅:0, iso-C₁₆:0, iso-C₁₄:0, C₁₇:1ω8c and anteiso-C₁₇:0. The DNA G + C content was 71.9 mol%. Strain YIM 61503^T was phenotypically distinct from recognized *Jiangella* species and was shown to belong to a separate genomic species based on DNA–DNA hybridization results. Thus, strain YIM 61503^T is considered to represent a novel species of the genus *Jiangella*, for which the name *Jiangella alba* sp. nov. is proposed. The type strain is YIM 61503^T (=DSM 45237^T =CCTCC AA 208023^T).

The genus *Jiangella* was proposed by Song *et al.* (2005) to accommodate an aerobic, Gram-positive actinomycete with LL-2,6-diaminopimelic acid (LL-DAP) in the cell-wall peptidoglycan and MK-9(H₄) as the predominant menaquinone. At present, this genus comprises only two species, *Jiangella gansuensis* (Song *et al.*, 2005) and *Jiangella alkaliphila* (Lee, 2008), which were both isolated from soil samples. In this paper, we describe the polyphasic characterization of strain YIM 61503^T isolated from an endophytic environment.

Strain YIM 61503^T was isolated from the healthy stem of a traditional Chinese medicinal plant, *Maytenus austroyunnanensis*, collected from a tropical rainforest in Xishuangbanna, Yunnan Province, south-west China. Stem samples were air-dried at room temperature after being thoroughly washed under tap water and surface sterilized according to the five-step sterilization procedure (Qin *et al.*, 2008). Samples were then pulverized in a

ceramic mortar and processed with a calcium carbonate enrichment method described by Otoguro *et al.* (2001). The samples were serially diluted in sterile distilled water and spread-plated on glycerol-asparagine agar (ISP 5) (Shirling & Gottlieb, 1966). Strain YIM 61503^T was isolated after incubation at 28 °C for 21 days and was subsequently maintained as mycelial fragments in a 20 % (v/v) glycerol suspension at –80 °C.

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). The almost-complete 16S rRNA gene sequence of strain YIM 61503^T (1519 bases) was aligned using the CLUSTAL X program (Thompson *et al.*, 1997) with the 16S rRNA gene sequences of closely related strains retrieved from the GenBank database. Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods with the software package MEGA 3.1 (Kumar *et al.*, 2004); distances between sequences were calculated using Kimura's two-parameter model (Kimura, 1980). A bootstrap analysis was performed to assess the confidence limits of the branching (Felsenstein, 1985).

DNA–DNA hybridization was performed using the fluorometric microwell method (Ezaki *et al.*, 1989) with the

Abbreviation: DAP, 2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 61503^T is FJ157186.

A supplementary table showing the cellular fatty acid profiles of type strains of the genus *Jiangella* is available with the online version of this paper.

modifications described by He *et al.* (2005). The G+C content of the genomic DNA was determined using the HPLC method as described by Mesbah *et al.* (1989).

Determination of morphological traits and colour of the aerial and substrate mycelium, as well as of soluble pigments, was done as described by Shirling & Gottlieb (1966). Cell morphological characteristics were observed by using light microscopy (BH2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 30 days' growth on ISP 2 medium. Determination of growth at different temperatures (0, 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C) and pH (pH 4.0–11.0 at intervals of 0.5 pH units) and tolerance of NaCl (0–15 %, w/v, at intervals of 0.5 %) were performed using ISP 2 as basal medium. Other physiological and biochemical characteristics of strain YIM 61503^T were examined by using standard procedures (Gordon *et al.*, 1974).

For most chemotaxonomic analyses, strain YIM 61503^T was grown in yeast extract-malt extract broth in flasks on a rotary shaker at 170 r.p.m. at 28 °C. Biomass for quantitative fatty acid analysis was prepared by using cells grown in nutrient broth at 30 °C for 3 days, with shaking at 170 r.p.m. Fatty acids were analysed according to the standard protocol of the Microbial Identification System (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Analyses of amino acids and sugars were carried out using the methods of Hasegawa *et al.* (1983). Menaquinones were extracted and purified as described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). Phospholipids were extracted and identified by two-dimensional TLC (Minnikin *et al.*, 1979; Collins & Jones, 1980). Mycolic acids were determined using the method of Minnikin *et al.* (1980).

The results of the phylogenetic analysis (Fig. 1) revealed that strain YIM 61503^T formed a separate line of descent in

the phylogenetic cluster of the genus *Jiangella*. 16S rRNA gene sequence similarities between strain YIM 61503^T and *J. alkaliphila* D8-87^T and *J. gansuensis* YIM 002^T were 98.8 and 98.6 %, respectively. Hybridization experiments revealed that the DNA–DNA relatedness between strain YIM 61503^T and *J. alkaliphila* DSM 45079^T (45.3 %) and *J. gansuensis* YIM 002^T (41 %) was well below the 70 % cut-off point generally recognized for genomic species (Stackebrandt & Goebel, 1994), which suggests that this strain should be considered as representing a novel species.

Strain YIM 61503^T exhibited good growth on ISP 2, ISP 4, potato dextrose agar, Czapek's agar and nutrient agar and moderate growth on ISP 3 and ISP 5 agar. White aerial mycelium and yellowish-white to orange-yellow substrate mycelium were formed on the media tested. Both aerial and substrate mycelia fragmented into short or elongated rods (Fig. 2). No diffusible pigments were observed. Strain YIM 61503^T grew in the presence of up to 10 % NaCl. The optimal temperature and pH for growth were 28 °C and pH 7.0–8.0. Strain YIM 61503^T differs substantially from *J. alkaliphila* DSM 45079^T and *J. gansuensis* YIM 002^T in terms of phenotypic characteristics (Table 1). Detailed phenotypic characteristics are presented in the species description.

Most of the chemotaxonomic data confirmed that strain YIM 61503^T belongs to the genus *Jiangella*. The cell-wall peptidoglycan contained LL-DAP as the diagnostic diamino acid. The whole-cell sugars comprised glucose, ribose and galactose. The predominant menaquinone was MK-9(H₄) (92.8 %), with MK-9(H₂) (5.2 %) and MK-9(H₆) (2.0 %) as minor components. The phospholipid profile comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and some unknown phosphoglycolipids and phospholipids, which was somewhat different from that of *J. alkaliphila* DSM

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain YIM 61503^T and some related taxa. Percentages at nodes are levels of bootstrap support based on 1000 resamplings. Asterisks indicate nodes recovered in a phylogenetic tree constructed with the maximum-parsimony method. The sequence of *Streptomyces sparsogenes* NRRL 2940^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Scanning electron micrograph of growth of strain YIM 61503^T cultivated on ISP 2 medium for 30 days at 28 °C. Bar, 2 μm.

45079^T in that phosphatidylcholine was not detected. Mycolic acids were not present. The major fatty acids were anteiso-C_{15:0} (26.1 %), iso-C_{16:0} (20.6 %), iso-C_{14:0} (10.0 %), C_{17:1ω8c} (9.8 %) and anteiso-C_{17:0} (8.2 %). This fatty acid profile is similar to those of the two recognized *Jiangella* species, although there are differences in the proportions of some fatty acids. A detailed comparison of the fatty acid profiles is given in Supplementary Table S1 (available in IJSEM Online).

On the basis of the genotypic and phenotypic data, strain YIM 61503^T is clearly distinguished from its phylogenetically closest relatives. Therefore, strain YIM 61503^T represents a novel species of the genus *Jiangella*, for which we propose the name *Jiangella alba* sp. nov.

Description of *Jiangella alba* sp. nov.

Jiangella alba (al'ba. L. fem. adj. *alba* white, referring to the white aerial hyphae).

Aerobic, Gram-positive-staining, non-acid-alcohol-fast actinomycete. Grows at 15–45 °C (optimum 28 °C) and pH 6.5–9.0 (optimum pH 7.0). Grows in the presence of 10 % NaCl. Forms well-developed white aerial mycelium and yellowish-white to orange-yellow substrate mycelium that fragment into short or elongated rods. No diffusible pigment is produced on any of the media tested. Adenine, casein, hypoxanthine and xanthine are degraded, but chitin, starch, DL-tyrosine and urea are not. Positive for catalase production, gelatin liquefaction and nitrate reduction, but negative for milk coagulation and peptonization, cellulose hydrolysis and H₂S production. Positive for utilization of D- and L-arabinose, *meso*-erythritol, D-fructose, D-glucose, glycerol, inulin, maltose, D-mannitol, D-mannose, melezitose, raffinose, L-rhamnose, D-ribose, salicin, sucrose, trehalose and D-xylose as sole carbon and energy sources. Negative for utilization of acetate, adonitol, cellobiose, citrate, D-dulcitol, formate, D-galactose, *myo*-inositol, lactose, malate, methyl α-D-mannoside, D-sorbitol, L-sorbose and D-xylitol. The cell wall contains LL-DAP. The whole-cell sugars are glucose, ribose and galactose. The

Table 1. Differential characteristics between *Jiangella alba* sp. nov. YIM 61503^T and other type strains of the genus *Jiangella*

Strains: 1, YIM 61503^T; 2, *J. alkaliphila* D8-87^T (data from Lee, 2008); 3, *J. gansuensis* YIM 002^T (Song *et al.*, 2005). All strains utilized D- and L-arabinose, D-fructose, D-glucose, glycerol, inulin, maltose, D-mannitol, D-mannose, L-rhamnose, sucrose and D-xylose as sole carbon and energy sources, but not methyl α-D-mannoside, L-sorbose, D-dulcitol, formate or malate. All strains degraded casein and liquefied gelatin, but did not degrade chitin, starch or DL-tyrosine or produce H₂S.

Characteristic	1	2	3
Growth at/with:			
5 % NaCl	+	–	+
pH 10.0	–	+	+
45 °C	+	–	–
Utilization of:			
Acetate	–	–	+
Adonitol	–	+	+
Cellobiose	–	+	+
Citrate	–	–	+
<i>meso</i> -Erythritol	+	–	–
D-Galactose	–	–	+
<i>myo</i> -Inositol	–	–	+
Lactose	–	+	+
D-Mannitol	+	+	–
Melezitose	+	–	+
Raffinose	+	–	–
D-Ribose	+	–	–
Salicin	+	–	+
D-Sorbitol	–	–	+
Trehalose	+	–	+
D-Xylitol	–	–	+
Degradation of:			
Urea	–	–	+
Hypoxanthine	+	+	–
Xanthine	+	–	–
Reduction of nitrate	+	–	–
G + C content (mol%)	71.9	71.5	70

predominant menaquinone is MK-9(H₄). Major fatty acids (>5 %) are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{14:0}, C_{17:1ω8c}, anteiso-C_{17:0} and iso-C_{15:0}. The DNA G + C content of the type strain is 71.9 mol%.

The type strain, YIM 61503^T (=DSM 45237^T =CCTCC AA 208023^T), was isolated from surface-sterilized stems of *Maytenus austroyunnanensis* collected from a tropical rainforest of Xishuangbanna, Yunnan Province, south-west China.

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