Saccharopolyspora tripterygii sp. nov., an endophytic actinomycete isolated from the stem of *Tripterygium hypoglaucum*

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An endophytic actinomycete, designated strain YIM 65359^T, was isolated from a surface-sterilized stem sample of *Tripterygium hypoglaucum* collected from Yunnan province, south-west China. The morphological and chemotaxonomic properties of the new isolate were consistent with those of members of the genus *Saccharopolyspora*. Analysis of 16S rRNA gene sequences revealed that the new isolate was most closely related to '*Saccharopolyspora endophytica*' YIM 61095 (98.6%), *Saccharopolyspora flava* AS4.1520^T (97.6%) and *Saccharopolyspora spinosa* DSM 44228^T (97.0%). The results of DNA–DNA hybridizations (57.5%, 44.9% and 48.5%, respectively) with the above micro-organisms, in combination with differences in the biochemical and physiological characteristics, suggested that strain YIM 65359^T should be classified as a novel species of the genus *Saccharopolyspora*. The name *Saccharopolyspora tripterygii* sp. nov. is proposed for this novel species, with YIM 65359^T (=CCTCC AA 208062^T=DSM 45269^T) as the type strain.

The genus *Saccharopolyspora* was proposed in 1975 by Lacey & Goodfellow (1975) to describe actinomycetes from sugar cane bagasse which grew at 40 °C and produced white aerial mycelia with bead-like chains of spores enclosed in a characteristic hairy sheath. Members of this genus are aerobic, Gram-positive, non-acid-fast organisms with substrate hyphae that either fragment into rod-shaped elements, do not fragment or are partially transformed into chains of spores (Korn-Wendisch *et al.*, 1989). They lack mycolic acids, but contain *meso*-diaminopimelic acid, arabinose and galactose in the cell wall and possess predominantly tetrahydrogenated menaquinones with nine isoprene units (Embley *et al.*, 1987; Goodfellow *et al.*, 1989). The DNA G+C contents of members of the genus are in the range 66–77 mol% (Goodfellow *et al.*, 1989). In this study, we describe the phenotypic and genotypic properties of a novel species of the genus *Saccharopolyspora*.

Strain YIM 65359^T was isolated during a search for endophytic microbes from pharmaceutical plants with

the prospect that they might produce novel bioactive products. The novel strain was isolated from the surface-sterilized stem of samples of *Tripterygium hypoglaucum*, which were collected from Yunnan province, south-west China. The isolation procedures were performed as described by Li *et al.* (2009). The purified strain was routinely cultured on YIM 38 medium [4 g malt extract, 4 g yeast extract, 4 g glucose, vitamin mixture (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] at 28 °C and stored as glycerol suspension (20%, v/v) at –70 °C.

Cultural characteristics were observed on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5) (Shirling & Gottlieb, 1966), Czapek's agar, potato agar and nutrient agar (Waksman, 1961) media following growth at 28 °C after 7, 14, 21 and 28 days. Colour determination was performed by using colour chips from the ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964). Morphological properties were examined using a light microscope (BH-2; Olympus) and a scanning electron microscope (JSM 5600LV; JEOL) after 20–30 days incubation on YIM 38 medium at 28 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 65359^T is FJ214364.

A detailed fatty acid composition for strain YIM 65359^T is presented in Supplementary Table S1, available with the online version of this paper.

Physiological tests such as growth at different temperatures (4, 10, 15, 20, 28, 37, 45, 55 and 65 °C), pH values (4.0–10.0) and NaCl concentrations [1, 3, 5, 7, 10, 12, 15 and 20 % (w/v)] were examined by growing the novel strain on YIM 38 medium as the basal medium. Hydrolysis of starch, gelatin and Tweens 20, 40 and 80 was determined as described by Smibert & Krieg (1994). Carbon source utilization tests and other phenotypic characteristics were conducted using the media and methods of Gordon *et al.* (1974).

Strain YIM 65359^T grew well on ISP 2, ISP 4, ISP 5 and potato agar media, with moderate growth on ISP 3, Czapek's agar and nutrient agar media. White aerial mycelia developed well on most of the media tested. The vegetative mycelia were well developed and soft orange–yellow to moderate orange–yellow in colour on all media tested. Soluble pigments were not produced on any of the media. Strain YIM 65359^T exhibited morphological properties characteristic of members of the genus *Saccharopolyspora*, forming extensively branched substrate mycelia that did not fragment and aerial mycelia developed well with long spore chains. All spores were non-motile and smooth-surfaced (Fig. 1). Detailed physiological results are given in Table 1 and in the species description.

The cellular fatty acid composition was determined as described by Sasser (1990), using the Microbial Identification System (MIDI). For other chemotaxonomic analysis, freeze-dried cells were obtained from cultures grown in YIM 38 broth for 1 week at 28 °C. The isomer of diaminopimelic acid and the sugars of the whole-cell hydrolysates were determined using TLC as described by Stanek & Roberts (1974). Phospholipids were identified according to published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were extracted (Collins *et al.*, 1977) and separated 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).

Fig. 1. Scanning electron micrograph of spore chains of strain YIM 65359^T after growth on YIM 38 medium at 28 °C for 28 days. Bar, 2 μm.

Table 1. Distinguishing characteristics of strain YIM 65359^T and the type strains of its closest phylogenetic relatives

Strains: 1, YIM 65359^T; 2, '*S. endophytica*' YIM 61095; 3, *S. flava* AS4.1520^T; 4, *S. spinosa* DSM 44228^T. Data for all species are from the present study unless indicated. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Hydrolysis of:				
Gelatin	–	–	–	+
Urea	+	–	+	+
Starch	–	+	+	–
Tween 80	–	+	+	+
H ₂ S production	–	–	–	+
Nitrate reduction	–	+	+	+
Highest NaCl tolerance (% w/v)	12	15	7	12
Growth temperature (°C)	10–37	20–45	20–37	15–37
Growth pH	7.0–8.0	5.0–9.0	7.0–8.0	7.0–8.0
Utilization as sole carbon sources:				
Aesculin	+	+	+	–
D-Arabinose	+	–	–	+
Cellobiose	–	+	+	+
Dulcitol	–	–	–	+
Inositol	–	+	+	+
D-Mannose	–	+	–	–
L-Rhamnose	–	+	+	+
D-Ribose	+	+	–	+
Sodium acetate	+	–	+	+
Sodium oxalate	–	+	+	–
D-Sorbitol	–	+	+	+
Sucrose	–	+	+	+
Utilization as sole nitrogen sources:				
Adenine	+	+	+	–
L-Alanine	–	+	+	+
L-Arginine	+	–	–	+
Glycine	–	–	+	+
L-Tyrosine	+	+	–	+
Xanthine	–	+	+	–
DNA G+C content (mol%)*	70.5	66.2	67.0	ND

*Data from previous studies except for strain YIM 65359^T (Mertz & Yao, 1990; Lu *et al.*, 2001; Qin *et al.*, 2008).

Strain YIM 65359^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan. Whole-cell hydrolysates contained arabinose, galactose, glucose and ribose. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine and phosphatidylcholine were the major phospholipids, with moderate amounts of phosphatidylinositol, phosphatidylinositol mannosides and one unknown phospholipid. The predominant menaquinone was MK-9(H₄) (87.5 %) and MK-9(H₂) was also detected as a minor component. The fatty acid profile was composed of iso-C_{16:0} (31.29 %), anteiso-C_{17:0} (20.31 %), iso-C_{15:0} (14.70 %), iso-C_{17:0} (10.19 %), C_{16:0}

9 methyl (7.00 %), C_{17:1}cis9 (2.96 %), anteiso-C_{15:0} (2.87 %), iso-C_{16:1} H (2.77 %), C_{16:1}cis9 (1.94 %), iso-C_{14:0} (1.73 %), iso-C_{13:0} (0.60 %), C_{18:1}cis9 (0.55 %), C_{15:1} B (0.54 %), C_{17:0} (0.54 %), C_{17:0} 10 methyl (0.53 %), C_{17:1} anteiso B/iso I (0.52 %), C_{16:0} (0.50 %) and anteiso-C_{17:1} C (0.47 %). A detailed fatty acid profile of the novel strain compared with those of closely related strains is presented in Supplementary Table S1 (available in IJSEM Online). The G+C content of the genomic DNA from strain YIM 65359^T was 70.5 mol%.

Genomic DNA extraction, amplification and 16S rRNA gene sequencing were performed as described previously by Li *et al.* (2007). The resulting 16S rRNA gene sequence was aligned with corresponding sequences of representatives of the genus *Saccharopolyspora* (retrieved from the GenBank/EMBL/DBJ database) using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining (Saitou & Nei, 1987) tree-making algorithm from MEGA version 4.0 (Tamura *et al.*, 2007). The PHYLIP version 3.6 software package was used to construct the maximum-likelihood (Felsenstein, 1981) tree. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The almost-complete 16S rRNA gene sequence of strain YIM 65359^T (1418 bp) was generated. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 65359^T belonged to the genus *Saccharopolyspora* (Fig. 2). Highest sequence similarities were found with '*Saccharopolyspora endophytica*' YIM 61095 (98.6 %), followed by *Saccharopolyspora flava* AS4.1520^T (97.6 %) and *Saccharopolyspora spinosa* DSM 44228^T (97.0 %). Genomic relatedness between strains YIM 65359^T and '*S. endophytica*' YIM 61095, *S. flava* AS4.1520^T and *S. spinosa* DSM 44228^T was determined by using DNA–DNA hybridization according to the fluorometric micro-well method (Ezaki *et al.*, 1989; He *et al.*, 2005). The experiments were performed with three replications. The levels of DNA–DNA relatedness of strain YIM 65359^T to '*S. endophytica*' YIM 61095, *S. flava* AS4.1520^T and *S. spinosa* DSM 44228^T were determined to be 57.5 % (mean value, SD 4.5 %), 44.9 % (mean value, SD 3.5 %) and 48.5 % (mean value, SD 3.0 %), respectively. These data supported the finding that strain YIM 65359^T represents a separate genomic species.

Besides the genotypic evidence, strain YIM 65359^T could also be distinguished from its closest relatives by additional phenotypic characteristics. '*S. endophytica*' YIM 61095 produces pink diffusible pigments when grown on ISP 5, Czapek's agar and potato agar media (Qin *et al.*, 2008), while *S. spinosa* DSM 44228^T also produces brown soluble pigments on some media (Mertz & Yao, 1990), whereas strain YIM 65359^T did not produce soluble pigments on any of the media tested. The substrate mycelium of *S. flava* AS4.1520^T fragments into rod-shaped elements (Lu *et al.*, 2001), but this was not found in strain YIM 65359^T.

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain YIM 65359^T and other members of the genus *Saccharopolyspora*. Bootstrap values (expressed as percentages of 1000 replications) >50 % are given at the nodes. Asterisks indicate the clades that were conserved when the maximum-likelihood method was used to construct the phylogenetic tree. Bar, 1 nt substitution per 100 nt.

Furthermore, as shown in Table 1, many differences between strains YIM 65359^T and '*S. endophytica*' YIM 61095, *S. flava* AS4.1520^T and *S. spinosa* DSM 44228^T were detected during the determination of phenotypic properties, such as differences in the hydrolysis of gelatin, urea, starch and Tween 80, in the production of H₂S, in nitrate reduction, in the utilization of sole carbon and nitrogen sources, in tolerance to NaCl, in the temperature and pH value ranges for growth and in the compositions of fatty acids (Table 1 and Supplementary Table S1). On the basis of the data presented in this study, we suggest that strain YIM 65359^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora tripterygii* sp. nov., is proposed.

Description of *Saccharopolyspora tripterygii* sp. nov.

Saccharopolyspora tripterygii (trip.te.ry'gi.i. N.L. n. *Tripterygium* a botanical genus name; N.L. gen. n. *tripterygii* of *Tripterygium*, the plant genus from which this species was isolated).

Forms extensively branched substrate mycelia and aerial mycelia differentiate into long spore chains. Spores are elliptical or short rods (0.5–0.8 × 1.0–1.5 µm). Spore surface is smooth. Aerial mycelia are white, vegetative mycelia are orange–yellow. No soluble pigment is produced. Growth occurs at 10–37 °C and pH 7.0–8.0. The NaCl tolerance range is up to 12 % (w/v). Catalase is produced. Negative for the production of H₂S and melanin, for nitrate reduction and for milk coagulation

and peptonization. Urea and Tweens 20 and 40 are hydrolysed, but Tween 80, cellulose, gelatin and starch are not hydrolysed. Utilizes D-arabinose, D-fructose, D-galactose, glucose, D-lactose, maltose, D-mannitol, raffinose, D-ribose, sodium acetate, trisodium citrate and D-xylose as sole carbon sources. Acid is produced from amygdalin and aesculin. Cellobiose, dulcitol, inositol, D-mannose, L-rhamnose, sodium oxalate, D-sorbitol and sucrose are not utilized. Adenine, L-arginine, L-asparagine, L-hydroxyproline, hypoxanthine, L-lysine, L-phenylalanine, L-serine, L-tyrosine and L-valine can be used as sole nitrogen sources, but not L-alanine, glycine or xanthine. The diagnostic amino acid in the peptidoglycan is meso-diaminopimelic acid. Arabinose, galactose, glucose and ribose are present in whole-cell hydrolysates. Phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannosides and one unknown phospholipid. The predominant menaquinone is MK-9(H₄). The major fatty acids (>10 %) are iso-C_{16:0}, anteiso-C_{17:0}, iso-C_{15:0} and iso-C_{17:0}.

The type strain, YIM 65359^T (=CCTCC AA 208062^T = DSM 45269^T), was isolated from a surface-sterilized stem sample of *Tripterygium hypoglaucum* collected from Yunnan province, south-west China. The G+C content of the genomic DNA of the type strain is 70.5 mol%.

Acknowledgements

This research was supported by the National Basic Research Program of China (No. 2004CB719601) and the Ministry of Science and Technology, P. R. China (2006DFA33550). W.-J.L. was also supported by the Program for New Century Excellent Talent in University (NCET).

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