Laceyella tengchongensis sp. nov., a thermophile isolated from soil of a volcano

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A thermophilic strain, designated YIM 10002^T, was isolated from a soil sample of Big Empty Volcano in Tengchong county, Yunnan province, south-west China, and a polyphasic approach was used to investigate its taxonomic position. Strain YIM 10002^T formed endospores on both aerial and substrate mycelia. Whole-cell hydrolysates contained *meso*-diaminopimelic acid, ribose, xylose and glucose. The major fatty acids were iso-C_{15:0} and anteiso-C_{15:0}. The predominant menaquinone was MK-9. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides, together with some unknown phospholipids. The G + C content of its genomic DNA was 48.6 mol%. All of these chemotaxonomic data together with morphological characters consistently assigned strain YIM 10002^T to the genus *Laceyella*. 16S rRNA gene sequence analysis showed that strain YIM 10002^T was most closely related to *Laceyella sacchari* KCTC 9790^T and *Laceyella putida* KCTC 3666^T (99.9 and 98.0 % 16S rRNA gene sequence similarity, respectively). However, strain YIM 10002^T showed relatively low DNA–DNA relatedness (34.0 and 39.0 %, respectively) with the above strains. Therefore, strain YIM 10002^T represents a novel species of the genus *Laceyella*, for which the name *Laceyella tengchongensis* sp. nov. is proposed. The type strain is YIM 10002^T (=DSM 45262^T =CCTCC AA 208050^T).

The genus *Thermoactinomyces* was first described by Tsilinsky (1899). Lacey & Cross (1989) placed the genus *Thermoactinomyces* in the family *Bacillaceae* as the only thermoactinomycete genus (Stackebrandt & Woese, 1981). The genus was later divided into four genera: *Thermoactinomyces*, *Laceyella*, *Thermoflavimicrobium* and *Seinonella* (Yoon *et al.*, 2005). Subsequently, two new genera, *Planifilum* and *Mechercharimyces*, were described and a new family, *Thermoactinomycetaceae*, was proposed (Hatayama *et al.*, 2005; Matsuo *et al.*, 2006). At the time of writing, the genus *Laceyella* comprises only two species: *Laceyella sacchari* (basonym *Thermoactinomyces sacchari* Lacey 1971) and *Laceyella putida* (basonym *Thermoactinomyces putidus* Lacey and Cross 1989) (Lacey & Vince, 1971; Lacey &

Cross, 1989; Yoon *et al.*, 2000, 2005). Members of the genus *Laceyella* are Gram-positive, aerobic, thermophilic, filamentous bacteria. Aerial and substrate mycelia are formed and sessile endospores may be produced on sporophores. The predominant menaquinone is MK-9, the major fatty acids are iso-C_{15:0} and anteiso-C_{15:0} and the DNA G + C content is 48–49 mol% (Yoon *et al.*, 2005).

Volcanoes and hot springs, for which Tengchong county is famous, are special habitats. As part of an investigation of the diversity of thermophilic micro-organisms in these environments, strain YIM 10002^T was isolated from a soil sample collected from Big Empty Volcano in Tengchong county, Yunnan province, south-west China. In this study, 2 g soil sample was suspended in 18 ml sterile water and mixed at 55 °C for 30 min. Soil particles were allowed to sediment, the liquid phase was diluted to 1 : 100 and 100 µl aliquots were spread onto the surface of isolation agar and incubated at 55 °C for a week. Strain YIM 10002^T was isolated from International *Streptomyces* Project (ISP) medium 3 (Shirling & Gottlieb, 1966). The isolate was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 10002^T is FJ426598.

A scanning electron micrograph of growth of strain YIM 10002^T and comparisons of polar lipids and fatty acids for the genus *Laceyella* are available as supplementary material with the online version of this paper.

maintained on ISP 3 agar slants at 4 °C and as suspensions of mycelium fragments in glycerol (20 %, v/v) at –80 °C.

Biomass for chemical and molecular studies was obtained by cultivation in ISP 3 broth in shaken flasks (about 150 r.p.m.) at 55 °C for 1 week. Cultural characteristics were determined after 1–2 weeks by methods used by the ISP (Shirling & Gottlieb, 1966). The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). Strain YIM 10002^T grew well on ISP 3 agar, moderately well on Czapek's, ISP 2 and ISP 4 agar, weakly on ISP 5 agar and not at all on nutrient agar. The aerial and substrate mycelia were white to white–yellow. No soluble pigment was produced. The morphological characteristics of strain YIM 10002^T were observed by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) after growth on ISP 3 agar at 55 °C for 14 days. The substrate and aerial mycelia of the isolate were well developed and formed endospores (Supplementary Fig. S1, available in IJSEM Online).

Growth of strain YIM 10002^T was tested at 4, 10, 15, 20, 28, 37, 40, 45, 55, 65, 70 and 75 °C on ISP 3 agar. The pH range for growth was investigated at ISP 3 agar at pH 4.0–10.0 (at intervals of 1 pH unit) using the following buffer systems: 0.1 M citric acid/0.1 M sodium citrate (pH 4.0–5.0), 0.1 M KH₂PO₄/0.1 M NaOH (pH 6.0–8.0) and 0.1 M NaHCO₃/

0.1 M Na₂CO₃ (pH 9.0–10.0). The media and procedures used to determine physiological features and the carbon source utilization pattern were those described by Williams *et al.* (1989). Strain YIM 10002^T grew well at 55 °C, but not below 28 °C or above 70 °C, showing that the strain was thermophilic, and at pH 6.0–8.0. The above morphological features of strain YIM 10002^T are consistent with those of members of the genus *Laceyella* described by Yoon *et al.* (2005). Strain YIM 10002^T could be distinguished easily from the two described *Laceyella* species by using a battery of phenotypic properties (Table 1).

Isomers of diaminopimelic acid and whole-cell sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983). Polar lipids were extracted and examined by two-dimensional TLC and identified using procedures described by Minnikin *et al.* (1984). Menaquinones were extracted according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). For fatty acid analysis, cells were collected after growth in tryptic soy broth (Difco) in shaken flasks (about 150 r.p.m.) at 55 °C for 2 days. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). Strain YIM 10002^T contained *meso*-diaminopimelic acid as the cell-wall diamino acid and ribose, xylose and glucose as the major whole-cell sugars. The menaquinones were MK-9 (87 %) and MK-8 (13 %). The phospholipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol

Table 1. Differentiating characteristics of strain YIM 10002^T and its closest phylogenetic neighbours

All data were obtained in this study. +, Positive; v, variable; –, negative.

Characteristic	Strain YIM 10002 ^T	<i>L. sacchari</i> DSM 43356 ^T	<i>L. putida</i> DSM 44608 ^T
Soluble pigment	–	Yellow–brown	Greyish yellow
Melanin production	–	v	+
Gelatin liquefaction	+	–	+
Temperature range for growth (°C)	28–70	35–65	30–65
Degradation of:			
Hypoxanthine	+	–	–
Starch	–	+	+
Utilization of:			
D-Fructose	–	+	–
Glycine	–	+	+
Lactose	+	+	–
L-Lysine	+	–	–
D-Mannitol	+	+	–
L-Proline	–	–	+
L-Rhamnose	+	–	–
Trehalose	+	+	–
Whole-cell sugars*	Rib, Xyl, Glc	Xyl, Ara, Glc	Xyl, Ara, Glc
Phospholipids†	DPG, PE, PG, PI, PIM, PL	DPG, PE, PME, PI, PIM, PL	DPG, PE, PG, PI, PIM, PL
Menaquinones	MK-9, MK-8	MK-9, MK-8, MK-10	MK-9, MK-8

*Ara, Arabinose; Glc, glucose; Rib, ribose; Xyl, xylose.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmonomethylethanolamine; PL, unknown phospholipid.

mannosides and some unknown phospholipids. Strain YIM 10002^T had a cellular fatty acid profile that contained major amounts of branched fatty acids and minor amounts of straight-chain and unsaturated fatty acids: iso-C_{15:0} (57.63 %), anteiso-C_{15:0} (13.79 %), iso-C_{16:0} (8.88 %), iso-C_{14:0} (7.11 %), iso-C_{17:0} (3.62 %), anteiso-C_{17:0} (1.83 %), C_{16:0} (1.61 %), iso-C_{13:0} (1.32 %), summed feature 5 (iso-C_{17:1} I and/or anteiso-C_{17:1} B; 1.18 %), C_{14:0} (1.16 %), C_{18:1} *cis*9 (1.02 %) and C_{17:0} (0.85 %) (Supplementary Table S1). On the whole, the chemotaxonomic properties for strain YIM 10002^T were consistent with those of members of the genus *Laceyella* (Yoon *et al.*, 2005), but the strain differed from *L. sacchari* DSM 43356^T by having ribose as a whole-cell sugar (Table 1) and phosphatidylglycerol as a phospholipid (Supplementary Fig. S2). Genomic DNA was extracted and purified according to the method described by Marmur (1961) and, using the HPLC method (Mesbah *et al.*, 1989), the DNA G+C content of strain YIM 10002^T was 48.6 mol%.

Extraction of genomic DNA and PCR amplification and sequencing of the 16S rRNA gene of strain YIM 10002^T were performed as described by Li *et al.* (2007). A multiple alignment with sequences most closely related to the genus *Laceyella* and calculations of sequence similarity were performed using EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analyses were performed using three tree-making algorithms: neighbour joining (Saitou & Nei, 1987), maximum likelihood (Felsenstein, 1981) and maximum parsimony (Fitch, 1971). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. DNA–DNA relatedness between strain YIM 10002^T and *L. sacchari* DSM 43356^T and *L. putida* DSM 44608^T was determined in triplicate according to the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992).

An almost-complete 16S rRNA gene sequence (1398 bp) of strain YIM 10002^T was obtained. 16S rRNA gene sequence similarities between strain YIM 10002^T and *L. sacchari* KCTC 9790^T and *L. putida* KCTC 3666^T were 99.9 and

98.0 %, respectively. Strain YIM 10002^T showed <95.2 % sequence similarity with other members of the family *Thermoactinomycetaceae*. In the phylogenetic tree based on the neighbour-joining algorithm (Fig. 1), strain YIM 10002^T formed a cluster with *L. sacchari* KCTC 9790^T and *L. putida* KCTC 3666^T that was supported with a high bootstrap value (99 %). The topologies of the maximum-likelihood and maximum-parsimony trees were similar to that of the neighbour-joining tree (data not shown). The results of 16S rRNA gene sequence analysis clearly demonstrated that strain YIM 10002^T was a member of the genus *Laceyella*. DNA–DNA relatedness between strain YIM 10002^T and *L. sacchari* DSM 43356^T and *L. putida* DSM 44608^T was 34.0 and 39.0 %, respectively, values that are far below the 70 % cut-off value recommended for the assignment of a strain to a novel genomic species (Wayne *et al.*, 1987).

Strain YIM 10002^T exhibited morphological characteristics that were typical of the genus *Laceyella* and the phylogenetic data clearly linked this strain to the genus *Laceyella*. However, comparison of the phenotypic characteristics of strain YIM 10002^T and its closest phylogenetic neighbours revealed significant differences between them, including production of soluble pigment and melanin, degradation of hypoxanthine and starch, temperature range for growth and utilization of L-lysine and glycine. Hence, on the basis of phylogeny, DNA–DNA relatedness and chemotaxonomic and phenotypic characters, we consider that strain YIM 10002^T represents a novel species, for which the name *Laceyella tengchongensis* sp. nov. is proposed.

Description of *Laceyella tengchongensis* sp. nov.

Laceyella tengchongensis (teng.chong.en'sis. N.L. fem. adj. *tengchongensis* pertaining to Tengchong county, Yunnan province, south-west China, where the type strain was collected).

Gram-positive, aerobic, thermophilic, filamentous actinomycete. Substrate and aerial mycelia are well developed and form endospores (0.7–0.8 µm). Aerial and substrate mycelia are white to yellow–white. No soluble pigment is produced. Positive for gelatin liquefaction and milk peptonization and coagulation and negative for nitrate reduction and H₂S and

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequences, reconstructed from evolutionary distances with the neighbour-joining method, showing the position of strain YIM 10002^T within the family *Thermoactinomycetaceae*. Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. *Bacillus subtilis* NCDO 1769^T was used as an outgroup. Bar, 1 % sequence divergence.

melanin production. Casein, hypoxanthine, gelatin and L-tyrosine are degraded, but adenine, xanthine, starch and urea are not. L-Fucose, lactose, mannitol and L-rhamnose are utilized as carbon sources, but D-arabinose, D-fructose, D-galactose, maltose, D-mannose, raffinose, D-ribose and D-xylose are not. L-Arginine, L-asparagine, L-cysteine, L-lysine and L-threonine are used as nitrogen sources, but adenine, glycine, L-hydroxyproline, L-proline, L-serine, L-valine and xanthine are not. The cell wall contains *meso*-diaminopimelic acid as the diagnostic amino acid of peptidoglycan. The whole-cell sugars are ribose, xylose and glucose. The predominant menaquinone is MK-9. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and some unknown phospholipids. The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. The DNA G + C content of the type strain is 48.6 mol%.

The type strain is YIM 10002^T (=DSM 45262^T =CCTCC AA 208050^T), isolated from a soil sample collected from Big Empty Volcano in Tengchong county, Yunnan province, south-west China.

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