Thermocatellispora tengchongensis gen. nov., sp. nov., a new member of the family Streptosporangiaceae

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A novel Gram-positive, aerobic, spore-forming, thermophilic actinomycete, designated strain YIM 77521T, was isolated from a sandy soil sample collected at Rehai National Park, Tengchong, Yunnan province, south-west China. The strain formed branched substrate mycelia and no fragmentation was found. Masses of short, straight or irregular chains of three to eight warty ornamented spores were borne from aerial mycelia. The strain contained meso-diaminopimelic acid in the cell wall and the whole-cell sugars contained mannose, galactose, glucose and ribose. The predominant menaquinones were MK-9(H4), MK-9(H6) and MK-9(H8). The diagnostic polar lipids were diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxyphosphatidylmonomethylethanolamine and N-acetylglucosamine-containing phospholipids. The major fatty acids were iso-C16:0, C17:0 10-methyl and C18:0. The DNA G+C content of strain YIM 77521T was 73.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 77521T fell within the radiation of the suborder Streptosporangineae and formed a distinct monophyletic lineage adjacent to the family Streptosporangiaceae with a high bootstrap value. On the basis of combined data from the phenotypic and phylogenetic analyses, strain YIM 77521T represents a novel genus and species within the family Streptosporangiaceae, for which the name Thermocatellispora tengchongensis gen. nov., sp. nov. is proposed. The type strain is YIM 77521T (=DSM 45615T =CCTCC AA 2011013T).

The suborder Streptosporangineae was originally proposed by Stackebrandt et al. (1997) based on phylogenetic and signature nucleotide analysis and was emended by Zhi et al. (2009). At the time of writing, this suborder comprised only three families, namely, Streptosporangiaceae, Nocardiopsaceae and Thermomonosporaceae, with Streptosporangiaceae as the type family. The family Streptosporangiaceae was described for the redefined maduromycete group (Goodfellow et al., 1990). The chemotaxonomic properties of the genera classified in this family are more or less similar except for the menaquinone compositions of members of the genus Herbidospora (Table 1). Members of the family

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 77521T is JN315666.

Four supplementary figures are available with the online version of this paper.
Table 1. Morphological features and chemotaxonomic characteristics of strain YIM 77521<sup>T</sup> and members of genera in the family Streptosporangiaceae

Taxa: 1, YIM 77521<sup>T</sup>; 2, Sphaerisporangium; 3, Nonomuraea; 4, Streptosporangium; 5, Acrocarpospora; 6, Herbidospora; 7, Microbispora; 8, Microtetraspora; 9, Planobispora; 10, Planomonospora; 11, Planotetraspora; 12, Thermopolyspora. Data were taken from this study and previous studies (Greiner-Mai et al., 1987; Goodfellow et al., 1990; Kudo et al., 1993; Tamura et al., 2000; Stackebrandt et al., 2001; Tamura & Sakane, 2004; Ara & Kudo, 2007; Goodfellow et al., 2005; Cao et al., 2009). The major constituents of the cell wall for all taxa represented here were alanine, glutamic acid, glucosamine and meso-diaminopimelic acid (Lechevalier & Lechevalier, 1970a), constituting a cell-wall type III. Phospholipid types: PII, phosphatidylethanolamine; PIV, glucosamine (with phosphatidylethanolamine and phosphatidylmethylethanolamine variable) (Lechevalier et al., 1977). Whole organism sugar patterns of actinomycetes containing meso-diaminopimelic acid: A, arabinose and galactose; B, madurose; C, no diagnostic sugar; D, arabinose and xylose (Lechevalier & Lechevalier, 1970b). For all taxa, the fatty acid type, including saturated, unsaturated, iso-, variable and methyl-branched fatty acids (Kroppenstedt, 1985), was 3c. +, Present; −, absent.

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<td>Morphology of sporangium formation on aerial hyphae</td>
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<td>Straight chains of smooth surfaced spores</td>
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<td>Smooth surfaced spores in characteristic longitudinal pairs</td>
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<td>Spore chains typically containing four smooth surfaced spores</td>
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<td>Cylindrical to clavate spore vesicles containing longitudinal pairs of spores</td>
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<td>Cylindrical to clavate spore vesicles containing single spores</td>
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<td>Spore vesicles containing four spores</td>
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<td>Hooked or irregular spiral chains of four to ten warty to spiny ornamented spores</td>
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Motile spores: 1, −; 2, +; 3, +; 4, +; 5, +; 6, +; 7, +; 8, +; 9, +; 10, +; 11, +; 12, +

Whole cell-wall sugar pattern: 1, A; 2, B, C; 3, A, B, C; 4, A, B, C; 5, A, B, C; 6, A, B, C; 7, A, B, C; 8, A, B, C; 9, A, B, C; 10, A, B, C; 11, A, B, C; 12, A, B, C

Predominant menaquinones: 1, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 2, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 3, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 4, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 5, MK-10<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 6, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 7, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 8, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 9, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 10, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 11, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>; 12, MK-9<sub>(H<sub>4</sub>, H<sub>6</sub>, H<sub>8</sub>)</sub>

Phospholipid type: 1, PIV; 2, PIV; 3, PIV; 4, PIV; 5, PIV; 6, PIV, PII; 7, PIV; 8, PIV; 9, PIV; 10, PIV; 11, PIV; 12, PIV

DNA G+C content (mol%): 1, 73.3; 2, 67–72; 3, 64–69; 4, 69–71; 5, 68–69; 6, 69–71; 7, 71–73; 8, 69–71; 9, 70–71; 10, 72; 11, 71; 12, 77
*Streptosporangiaceae* can be distinguished at the genus level by their morphological features, including the existence of spore vesicles (sporangia) and the number of spores per spore vesicle or chain. Members of the family *Streptosporangiaceae* have a type III cell wall, a whole-cell sugar pattern of type B or C, a fatty acid pattern of type 3c, MK-9(H₂, H₄, H₆) as the major menaquinone and a phospholipid profile of type PIV. During a study of the diversity of thermophilic actinomycetes in geothermal areas at Rehai National Park, a novel actinomycete strain, designated YIM 77521ᵀ, was isolated and purified. Based on a polyphasic taxonomic study of the isolate, which included phylogenetic, genotypic and phenotypic approaches, it is proposed that strain YIM 77521ᵀ represents a novel species in a new genus within the family *Streptosporangiaceae*.

Strain YIM 77521ᵀ was isolated from a sandy soil sample from a geothermal area, collected at Rehai National Park, Tengchong, Yunnan province, south-west China. The strain was isolated using the dilution plate method with Czapek’s agar (Waksman, 1961) and incubation at 55 °C for 2 weeks. The purified isolate was routinely cultured on International *Streptomyces* Project medium 2 (ISP 2) agar (Shirling & Gottlieb, 1966) at 45 °C and maintained as 20 % (w/v) glycerol suspensions at −80 °C. Biomass for most of the chemical and molecular studies was obtained by cultivation in shake flasks (~200 r.p.m.) using ISP 2 liquid medium at 45 °C for 1 week, except for fatty acid analysis where strain YIM 77521ᵀ was cultivated at 45 °C using Tryptic Soy Broth (Difco) medium for 1 week.

The cultural characteristics of the strain were determined using 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-glucose (dextrose) agar and nutrient agar (Waksman, 1961) following growth at 45 °C for 7, 14, 21 and 28 days. The colony colour was determined by means of the ISCC-NBS colour charts (Kelly, 1964). The Gram reaction was performed according to Gregersen (1978) by using KOH for cell lysis. The morphological properties of strain YIM 77521ᵀ were observed using light microscopy (BH-2; Olympus) and scanning electron microscopy (QUANTA200; FEI) after growth for 7 days at 45 °C on ISP 3 agar.

Strain YIM 77521ᵀ was an aerobic, Gram-reaction-positive, non-motile actinomycete that formed extensively branched substrate mycelia. Strain YIM 77521ᵀ grew well on ISP 2, ISP 3, ISP 4 and Czapek’s agar and moderately well on potato–glucose agar but poorly on ISP 5 and nutrient agar. Orange–yellow soluble pigments were produced on Czapek’s agar. The colour of colonies on test media were orange–yellow on ISP 3, ISP 4 and ISP 5 media, and yellowish brown on ISP 2, Czapek’s agar, nutrient agar and potato–glucose agar. Among the media tested, strain YIM 77521ᵀ only developed white aerial mycelia on ISP 3 medium. Masses of short, straight or irregular spore chains of three to eight warty ornamented spores (1.0–1.5 μm in diameter) were borne from aerial mycelia (Fig. 1a, b).

Growth was tested at 10, 15, 20, 28, 37, 45, 50–60 (at intervals of 1 °C) and 65 °C on ISP 2 agar. Other physiological and biochemical tests were performed at 45 °C. For NaCl tolerance experiments, ISP 2 medium was used as the basal medium, supplemented with 0–10 % (w/v) NaCl (at intervals of 1 %). The pH range for growth was investigated at pH 4–10 at intervals of 1 pH unit with the buffer system described by Xu et al. (2005). Carbon and nitrogen source utilization was assessed by using the media and methods of Gordon et al. (1974). Catalase activity was detected based on bubble formation in 3 % (v/v) H₂O₂ solution. Oxidase activity was determined from the oxidation of 1 % p-aminodimethylaniline oxalate. Hydrolysis of starch, gelatin, urea, cellulose and Tweens 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965). Other physiological and biochemical tests were performed as described by Gordon et al. (1974). Strain YIM 77521ᵀ grew at 28–58 °C (optimum 45–50 °C), at pH 6–8 (optimum pH 7) and in up to 2 % (w/v) NaCl. The isolate gave positive oxidase and catalase reactions. Milk was coagulated and peptonized but H₂S was not produced. Other physiological and biochemical characteristics of strain YIM 77521ᵀ are presented in the species description below.

The diagnostic isomers of diaminopimelic acid were determined by TLC (Hasegawa et al., 1983; Lechevalier &
The whole-cell sugar pattern analysis was performed according to the procedures described by Tang et al. (2009). The menaquinones were extracted and purified as described by Collins et al. (1977) and analysed by HPLC (Groth et al., 1997). Polar lipids were extracted and examined by using procedures as described previously (Collins & Jones, 1980; Minnikin et al., 1979). The analysis of the whole-cell fatty acid composition was performed by using standard methods (Sasser, 1990) with the Sherlock Microbial Identification System (version 6.1; MIDI database: TSB6). The G+C content of the genomic DNA was determined by using reversed-phase HPLC (Mesbah et al., 1989).

The whole-cell hydrolysate of the novel isolate contained meso-diaminopimelic acid as the diagnostic diamino acid and the whole-cell sugars detected were mannose, galactose, glucose and ribose. The predominant menaquinones (>10 % of total menaquinones) were MK-9(H4) (52.8 %), MK-9(H6) (17.5 %) and MK-9(H8) (13.4 %), with MK-10(H4) (6.4 %), MK-9(H2) (4.5 %), MK-10(H4) (3.9 %) and MK-10(H6) (1.5 %) as minor components. The polar lipids comprised diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, hydroxyphosphatidylmethylethanolamine, N-acetylglucosamine-containing phospholipids, phosphatidylinositol, phosphatidylinositolmannosides and some unknown lipids (Fig. S1). The detailed fatty acid profile (>0.5 % of total fatty acids) of strain YIM 77521T was iso-C16:0 (24.93 %), C17:0 10-methyl (22.3 %), C18:0 12:0 (12.96 %), C17:0 9c (9.63 %), C16:0 (6.96 %), C18:0 10-methyl (6.27 %), iso-C18:0 3:0 (3.34 %), C17:0 10c (2.16 %), C17:1 10c 1 (1.77 %), C18:1 10c 9c (1.01 %), C16:1 0 (0.96 %), C19:0 1 (0.91 %), C20:4 06e6, 06c12, 015c (0.86 %), C17:0 2-0H (0.86 %), C14:0 (0.83 %), summed feature 9 (0.72 %), summed feature 3 (0.54 %) and iso-C15:0 (0.50 %). The fatty acid pattern corresponds to fatty acid type 3c of Kroppe (1985). The G+C content of the genomic DNA was 73.3 mol%.

Genomic DNA and 16S rRNA gene sequences of strain YIM 77521T were obtained as described by Li et al. (2007). The resulting 16S rRNA gene sequence was compared with those available in GenBank using BLAST searches to determine an approximate phylogenetic affiliation. Multiple alignments with sequences of the most closely related actinobacteria were carried out using CLUSTAL_X (Thompson et al., 1997) software. Sequence similarity values were computed using the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007). Gaps at the 5' and 3' ends of the alignment were omitted for further analyses. The phylogenetic analysis was performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms and a phylogenetic tree was reconstructed by using the software packages MEGA version 4.0 (Tamura et al., 2007) and PHYLIP version 3.6 (Felsenstein, 2002). Evolutionary distance matrices were calculated according to the algorithm of Kimura's two-parameter model for the neighbour-joining method (Kimura, 1980).

The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

For the phylogenetic analysis, almost complete 16S rRNA gene sequences were determined for strain YIM 77521T consisting of 1526 nt. BLAST results for the 16S rRNA gene sequence of strain YIM 77521T indicated that this strain was most closely related to the type strains of members of the genera Sphaerisporangium (95.5–97.1 % sequence similarity), Nonomuraea (95.6–97.0 %), Streptosporangium (95.8–96.5 %) and Planotetraspora (95.4–96.2 %) as well as the species Sinosporangium album (95.7 %) and Thermopolyspora flexuosa (94.1 %). The neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of all these reference taxa showed that the novel isolate fell within the radiation of the suborder Streptosporangiineae and formed a distinct monophyletic lineage adjacent to the family Streptosporangiaceae with a high bootstrap value of 84 % (Fig. 2). The topology of the tree generated with the three different kinds of tree-making algorithms was similar (Figs S2, S3). Phylogenetic analysis based on the 16S rRNA gene sequences of all the genera in the suborder Streptosporangiineae also confirmed the position of strain YIM 77521T (Fig. S4).

Members of the family Streptosporangiaceae are mainly defined by their chemotaxonomic characteristics. The chemotaxonomic characteristics of strain YIM 77521T combined with the phylogenetic analysis results indicated that the strain YIM 77521T represents a member of the family Streptosporangiaceae. In addition, it could also be readily distinguished from previously described organisms classified in this family. Strain YIM 77521T formed short spore chains of warty spores on aerial mycelia; this morphological characteristic is very similar to that seen in members of the genus Nonomuraea but clearly differed from members of the genus Sphaerisporangium, which form globose sporangia on aerial hyphae. The chemical characteristics of strain YIM 77521T also differentiated it from members of the genus Sphaerisporangium, which contain MK-9(H4, H6) as the major menaquinones and display a type B whole-organism sugar pattern. Members of the genus Nonomuraea contain MK-9(H4, H6) as the predominant menaquinones and a type B or C whole-cell sugar pattern. Some members of Nonomuraea also contain >10 % MK-9(H8). Strain YIM 77521T appeared to share similar phenotypic characteristics, including morphology, predominant isoprenoid quinone and sugar pattern, with members of the genus Nonomuraea. However, strain YIM 77521T could be differentiated from species of the genus Nonomuraea in that it contained >10 % MK-9(H8). The differences between strain YIM 77521T and members of the genera classified in the family Streptosporangiaceae are shown in Table 1. In addition, phylogenetic analysis indicated that strain YIM 77521T was not affiliated with any other recognized genus of the family Streptosporangiaceae based on the distinctness of its 16S rRNA gene sequence and phylogenetic position. Based on these data, strain YIM
Thermocatellispora tengchongensis gen. nov., sp. nov.

Description of Thermocatellispora tengchongensis gen. nov., sp. nov.

Thermocatellispora [Ther.mo.ca.tel.li.spora] gen. nov., sp. nov.

Thermocatellispora tengchongensis (teng.chong.en’s.isis). N.L. fem. adj. tengchongensis of or pertaining to Tengchong county, Yunnan province, south-west China, where the type strain was collected).

General morphological and chemotaxonomic characters are included in the genus description. Colonies grow well on ISP 2, ISP 3, ISP 4 and Czapek’s agar, moderately well on potato–glucose agar, but poorly on ISP 5 and nutrient agar. Orange–yellow soluble pigment is produced on Czapek’s agar. Grows at 28–58 °C, at pH 6–8 (optimum pH 7) and in 0–2 % (w/v) NaCl. Positive for oxidase, catalase, milk coagulation, milk peptonization, nitrate reduction and starch hydrolysis. Negative for gelatin liquefaction, urea and cellulose hydrolysis and H2S production. Tween 60 is hydrolysed but Tween 20, 40 and 80 are not hydrolysed. Sucrose, maltose, xylose, xylitol, myo-inositol, galactose, lactose, mannose, fructose and arabinose are utilized as sole carbon sources but cellobiose, mannitol, raffinose, rhamnose, trehalose, succin acid and sodium malate are not.
Grows well on asparagines, ornithine, proline, hydroxyproline, alanine, threonine, tyrosine, valine, lysine, phenylalanine, histidine, serine, xanthine and hypoxanthine as sole nitrogen sources. Arginine, methionine, glycine and glutamic acid are not utilized. The diagnostic amino acid of the peptidoglycan is \textit{meso}-diaminopimelic acid. Whole-cell hydrolysates contain mannose, galactose, glucose and ribose. The polar lipids consist of diphosphatidylglycerol, phosphatidylmethylthanolamine, phosphatidylethanolamine, hydroxyphosphatidylmethylthanolamine, \textit{N}-acetylglucosamine-containing phospholipids, phosphatidylinositol and phosphatidylinositolmannosides, as well as one unknown lipid, one unknown phosphoglycolipid, two unknown glycolipids and three unknown phospholipids. The predominant menaquinones are MK-9(H$_4$) (52.8%), MK-9(H$_6$) (17.5%) and MK-9(H$_8$) (13.4%). The major fatty acids are iso-C$_{16.0}$ (24.93% of the total), C$_{17.0}$ 10-methyl (22.30%) and C$_{18.0}$ (12.96%).

The type strain, YIM 77521$^T$ (=DSM 45615$^T$ =CCTCC AA 2011013$^T$), was isolated from a sandy soil sample collected at Rehai National Park, Tengchong, Yunnan Province, south-west China. The DNA G+C content of the type strain is 73.3 mol%.

\section*{Acknowledgements}

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\section*{References}


