Planifilum yunnanense sp. nov., a thermophilic thermoactinomycete isolated from a hot spring

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A thermophilic actinomycete, strain LA5^T, was isolated from a hot spring in Yunnan province, People's Republic of China. The isolate grew aerobically at temperatures of 50–75 °C. Aerobic mycelia were not observed. Single spores were produced along the substrate hypha. Strain LA5^T was characterized chemotaxonomically by having MK-7 as the predominant menaquinone and iso-C_{17:0} (27.7 %) and C_{16:0} (22.4 %) as the major fatty acids. The cell wall contained meso-diaminopimelic acid. The DNA G+C content was 56.8 mol%. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain LA5^T belongs to the genus *Planifilum*. Levels of DNA–DNA relatedness between strain LA5^T and *Planifilum fimeticola* H0165^T and *Planifilum fulgidum* 500275^T were 43.6 and 52.7 %, respectively. On the basis of phenotypic properties and phylogeny, strain LA5^T represents a novel species of the genus *Planifilum*, for which we propose the name *Planifilum yunnanense* sp. nov., with the type strain LA5^T (=CCTCC AA206002^T=KCTC 13052^T).

Only one genus, *Thermoactinomyces*, was included in the family ‘*Thermoactinomycetaceae*’ before 2005. Yoon *et al.* (2005) suggested that the family ‘*Thermoactinomycetaceae*’ should be divided into four genera, *Laceyella*, *Thermoactinomyces sensu stricto*, *Thermoflavimicrobium* and *Seinonella*, based on 16S rRNA gene sequence analysis as well as chemotaxonomic and physiological characteristics of recognized *Thermoactinomyces* species. In addition, Hatayama *et al.* (2005) proposed a new genus *Planifilum* with two species.

Strain LA5^T was isolated from a hot spring in Yunnan province, People's Republic of China. It could grow at temperatures of 50–75 °C, the highest growth temperature obtained for all *Thermoactinomyces* strains reported so far, with optimum growth occurring at 60–70 °C. Colonies of 3–4 mm in diameter appeared after 48 h incubation on LB Gelrite plates at 70 °C. The colonies were cream–yellow with radial wrinkles. No aerobic mycelia were observed. Single spores were produced along the substrate hypha. Strain LA5^T did not produce any diffusible pigment during the whole incubation period. Strain LA5^T grew at pH 6–10, with optimum growth at pH 8.5.

To establish accurately the phylogenetic position of strain LA5^T, its 16S rRNA gene sequence was determined. Genomic DNA extraction and PCR amplification of 16S rRNA genes using universal primers were performed by using the method of Ausubel *et al.* (1999). The resultant sequence for strain LA5^T was aligned manually against

sequences obtained from GenBank. Phylogenetic trees were inferred using minimum-evolution, maximum-parsimony and neighbour-joining methods (Kumar *et al.*, 2004). The bootstrap values of the neighbour-joining tree were calculated based on 1000 repeats.

The results of alignment with 16S rRNA gene sequences obtained from GenBank showed that strain LA5^T had similarities of 99 and 97 % with *Planifilum fulgidum* 500275^T and *Planifilum fimeticola* H0165^T, respectively, and less than 94 % with any others used. In the phylogenetic trees constructed using three different methods, strain LA5^T and members of the genus *Planifilum* formed an independent cluster in the family ‘*Thermoactinomycetaceae*’. Strain LA5^T was most closely related to *P. fulgidum* 500275^T, as shown in the neighbour-joining tree (Fig. 1). Therefore, strain LA5^T should be classified in the genus *Planifilum*.

The DNA base composition of strain LA5^T was determined by using the thermal denaturation method (Marmur & Doty, 1962), with *Escherichia coli* K-12 as reference (DNA G+C content, 50 mol%). The DNA G+C content was calculated by using the method of De Ley (1970). The results showed that the DNA G+C content of strain LA5^T was 56.8 mol%, close to the values obtained for *P. fulgidum* 500275^T (60.0 mol%) and *P. fimeticola* H0165^T (60.3 mol%), but much higher than those of other members of the family ‘*Thermoactinomycetaceae*’, which also indicated that strain LA5^T was closely related to the genus *Planifilum*.

In order to determine further the relationship between strain LA5^T and other members of the genus *Planifilum*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LA5^T is DQ119659.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain LA5^T, members of the family 'Thermoactinomycetaceae' and *Bacillus subtilis* DSM 618. Numbers at nodes are percentage bootstrap values based on 1000 repeats (only values greater than 50 % are shown). Bar, 0.01 substitutions per nucleotide position.

phylogenetically, DNA–DNA hybridizations of strain LA5^T with *P. fulgidum* 500275^T and *P. fimeticola* H0165^T were respectively performed by using thermal denaturation and reassociation (De Ley *et al.*, 1970). A UV spectrophotometer (Lambda 35; Perkin Elmer) and temperature controller (PTP-6; Perkin Elmer) were used. Levels of DNA–DNA relatedness between strain LA5^T and *P. fimeticola* H0165^T and *P. fulgidum* 500275^T were only 43.6 and 52.7 %, respectively. Therefore, strain LA5^T represents a novel species of the genus *Planifilum*.

The chemotaxonomic characteristics of strain LA5^T were determined using cells cultured in LB medium, supplemented with starch. Cell walls were analysed for principal amino acids by using the method of Lechevalier & Lechevalier (1970). Whole-cell sugars were determined using GC and MS (GC-MS model HP-6890; Hewlett Packard) according to the methods of Saddler *et al.* (1991) and Takahashi & Egusa (1992). Cellular fatty acids were analysed using HP-6890 GC, equipped with a hydrogen flame-ionization detector (FID) and HP Chemstation (version A 5.01), based on the method of Collins (1985). The results revealed the presence of *meso*-diaminopimelic acid, alanine and glutamic acid, indicating that the cell wall chemotype was type III, consistent with all other members of the family 'Thermoactinomycetaceae'. MK-7 was the predominant menaquinone. Whole-cell sugars were found to be glucose and ribose, without any diagnostic sugars. The predominant fatty acids were iso-C_{17:0} (27.7 %), C_{16:0} (22.4 %), iso-C_{16:0} (8.8 %) and anteiso-C_{17:0} (8.6 %), which were different from those of the type strains of species of all five genera in the family 'Thermoactinomycetaceae' (Table 1).

Utilization of sugars and other substrates was tested using the following medium (g l⁻¹): (NH₄)₂SO₄, 1.5; KH₂PO₄, 0.5; K₂HPO₄·3H₂O, 1.5; MgSO₄·7H₂O, 0.2; NaCl, 0.5; yeast extract, 0.02; at 65 °C and pH 8.5. Strain LA5^T could utilize many sugars, organic acids and amino acids as sole carbon sources and showed a greater range of substrate utilization than *P. fimeticola* H0165^T and *P. fulgidum* 500275^T (Table 1). Strain LA5^T grew on LB, potato dextrose agar, Czapek and starch-casein, but only weak growth was observed on glycerol-asparagine and Gause's synthetic medium.

Description of *Planifilum yunnanense* sp. nov.

Planifilum yunnanense (yun.nan.en'se. N.L. neut. adj. *Yunnanense* referring to Yunnan, the southern Province, P. R. China, where the sample was collected).

Colonies are rough and cream–yellow with radial wrinkles. Growth occurs at 50–75 °C (optimum growth at 60–70 °C) and pH 6.0–10.0 (optimum growth at pH 8.5). Casein, gluten and starch are degraded. Predominant fatty acids are iso-C_{17:0} (27.7 %) and C_{16:0} (22.4 %). The DNA G+C content of the type strain is 56.8 mol%.

The type strain, LA5^T (=CCTCC AA206002^T=KCTC 13052^T), was isolated from a hot spring in Yunnan Province, P. R. China.

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Table 1. Phenotypic characteristics of strain LA5^T and recognized *Planifilum* species

Strains: 1, *P. fimeticola* H0165^T; 2, *P. fulgidum* 500257^T; 3, strain LA5^T (*Planifilum yunnanense* sp. nov.). +, Positive reaction; –, negative reaction; NO, not observed. All strains can use the following substrates as carbon sources: casein, gluten and starch, α -cyclodextrin, D-fructose, maltose, D-mannitol, D-raffinose, D-melibiose, turanose, D-gluconic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketovaleric acid, malonic acid, propionic acid, acetic acid, *cis*-aconitic acid, D-alanine and L-alanine.

Characteristic	1	2	3
Aerial mycelium*	NO	NO	NO
Optimum growth temperature (°C)*	55–63	60–65	60–70
DNA G + C content (mol%)*	60.3	60.0	56.8
Predominant menaquinone*	MK-7	MK-7	MK-7
Major fatty acids*	iso-C _{16:0} , iso-C _{17:0} , anteiso-C _{17:0}	iso-C _{17:0} , anteiso-C _{17:0} , iso-C _{15:0}	iso-C _{17:0} , C _{16:0} , iso-C _{16:0} , anteiso-C _{17:0}
Carbon source utilization:			
D-Raffinose	+	–	+
Trehalose	+	–	–
Gentiobiose	–	–	+
Lactose	+	–	+
Sucrose	–	+	+
D-Arabinose	+	+	–
D-Xylose	–	+	+
L-Fucose	+	–	+
D-Galactose	+	–	+
D-Mannose	+	–	–
D-Psicose	–	+	+
myo-Inositol	–	–	+
i-Erythritol	–	+	+
D-Sorbitol	+	–	+
Xylitol	+	+	–
N-Acetyl-D-glucosamine	–	–	+
Methyl β -D-glucoside	–	+	+
Succinic acid monomethyl ester	–	–	+
D-Galacturonic acid	–	–	+
α -Hydroxybutyric acid	–	+	–
p-Hydroxyphenylacetic acid	–	–	+
Itaconic acid	–	+	+
Quinic acid	–	+	–
Succinic acid	–	–	+
L-Aspartic acid	–	+	+
L-Glutamic acid	–	–	+
L-Leucine	–	–	+
L-Ornithine	–	+	+
L-Proline	–	+	+
L-Threonine	+	–	+
L-Tyrosine	+	–	–
Inosine	+	–	+
Uridine	–	–	+

*Data for *P. fimeticola* and *P. fulgidum* are from Hatayama *et al.* (2005).

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