Laceyella thermophila sp. nov., a thermophilic bacterium isolated from a hot spring

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Abstract

A novel thermophilic bacterium, designated YIM 79486T, was isolated from a sediment sample collected from Jinze hot spring in Tengchong county, Yunnan province, south-west China. Phylogenetic analysis based on 16S rRNA gene sequence analysis revealed that strain YIM 79486T should be assigned to the genus Laceyella and formed a monophyletic clade with the type strain Laceyella putida KCTC 3666T (98.7% similarity). Strain YIM 79486T formed white aerial mycelium and brown substrate mycelium. Abundant endospores were produced on short sporophores. Cell-wall peptidoglycan contained mesodiaminopimelic acid. The predominant menaquinones were MK-9 and MK-8. The genomic DNA G+C content observed for strain YIM 79486T was 47.8 mol%. Based on low DNA–DNA hybridization data, chemotaxonomic characteristics and differential physiological properties, strain YIM 79486T is considered to represent a novel species within the genus Laceyella, for which the name Laceyella thermophila sp. nov. is proposed. The type strain is YIM 79486T (=CCTC AB 2015040T=NBRC 110772T).

The genus Laceyella was originated from the genus Thermoaotinomycetes, which was first described by Tsilinsky [1]. Members of the species Laceyella are thermophilic, and form aerial and substrate mycelia. Endospores are produced on short or long sporophores. MK-9 is characterized as the predominant menaquinone as well as MK-8 also being reported for the recognized members of this genus. The major fatty acids are iso-C15:0 and anteiso-C15:0. At the time of writing, the genus comprised four recognized species, Laceyella putida [2], Laceyella sacchari [3], Laceyella tengchongensis [4] and Laceyella sediminis [5].

Strain YIM 79486T was isolated from sediment collected from the outer pool of Jinze hot spring (pH 7.0, temperature 58°C, 25.26029'N 98.27035'E), located in Tengchong county, Yunnan province, south-west China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM 79486T was affiliated to the genus Laceyella, constituting a well-supported clade with L. putida KCTC 3666T. The aim of the present study was to determine the taxonomic status of this isolate, by using a polyphasic approach.

A 2 g sediment sample was collected from the outer pool of Jinze hot spring. The procedure for isolation of bacteria from the sediment from the hot spring was carried out as described by Ming et al. [6]. Subsequently, successive 10-fold and 100-fold dilutions of the suspensions were made and 0.2 ml aliquots were spread on modified T5 [7] agar plates adjusting to pH 7.0. The isolation plates were incubated at 50°C for 7 days, and colonies were selected. One actinomycete-like thermophilic micro-organism, designated YIM 79486T, was selected and purified on modified T5 medium at pH 7.0 and 50°C. The pure cultures were preserved as glycerol suspensions (20%, v/v) at −80°C.

Genomic DNA of strain YIM 79486T was extracted, and the 16S rRNA gene was amplified and sequenced (ABI Prism3730; Applied Biosystems) as described by Li et al. [8]. The amplicons were purified using a PCR purification kit (Sangon Biotech). The nearly complete 16S rRNA gene was obtained and assembled by using the SeqMan program (DNAStar). Phylogenetic relationships based on 16S rRNA gene sequences between strain YIM 79486T and members of the family Thermoaotinomycetaceae were investigated. The 16S rRNA gene sequences of members of the family

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The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain YIM 79486T is KP232927. One supplementary table and four supplementary figures are available with the online Supplementary Material.
**Thermoactinomycetaceae** were searched for in the NCBI [9] and the EzTaxon-e server [10] databases, and retrieved from the GenBank/EMBL/DDBJ database. Multiple alignments were performed by using the **CLUSTAL X** software package [11]. Evolutionary distances were calculated by use of the Kimura two-parameter model [12, 13]. Phylogenetic trees were reconstructed with the neighbour-joining [14], maximum-parsimony [15] and maximum-likelihood [16] algorithms with the software packages **MEGA** version 6.0 [17] and **PHYLML** [18]. The 16S rRNA gene sequences of **Geobacillus stearothermophilus** DSM 22\textsuperscript{T} (AJ294817) and **Bacillus subtilis** NCDO 1769\textsuperscript{T} (X60646) were used as an outgroup. The resultant tree topology was evaluated by bootstrap analysis with 1000 replications [19].

An almost-complete 16S rRNA gene sequence comprising 1518 nucleotides was determined for strain YIM 79486\textsuperscript{T}.

Pairwise comparison of the sequences with representatives of the family **Thermoactinomycetaceae** indicated that strain YIM 79486\textsuperscript{T} should be assigned to the genus **Laceyella**. The genus was found to be monophyletic, with strain YIM 79486\textsuperscript{T} demonstrating the closest relationship (sharing a branching node) with the type strain **L. putida** KCTC 3666\textsuperscript{T}.

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain YIM 79486\textsuperscript{T} and members of the family **Thermoactinomycetaceae**. Bootstrap percentages (≥50\%) based on 1000 resamplings are listed at the nodes. Asterisks indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods. Bar 0.01 substitutions per nucleotide position. The 16S rRNA gene sequences of strains **G. stearothermophilus** DSM 22\textsuperscript{T} (AJ294817) and **B. subtilis** NCDO 1769\textsuperscript{T} (X60646) were used as an outgroup.
(sharing 98.7 % 16S rRNA gene sequence similarity) (Fig. 1). Pairwise similarity values of 97.7, 97.2 and 97.1 % were also detected between the novel isolate and L. sacchari KCTC 9790T, L. tengchongensis YIM 10002T and L. sediminis RHA1T, respectively. The clustering of strain YIM 79486T with members of the genus Laceyella was supported by a bootstrap value of 100 %. The same branching was recovered in maximum-parsimony and maximum-likelihood phylogenetic trees with bootstrap values of 96 and 99 %, respectively (Figs S1 and S2, available in the online Supplementary Material).

All species of the genus Laceyella were used as the reference type strains in the present study. Strain YIM 79486T and all members of the genus Laceyella were cultured for determining phenotypic, physiological and chemotaxonomic characteristics. Gram staining was carried out by using the standard Gram reaction [20]. Cultural characteristics were tested on ISP 2, oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5) [21], potato-dextrose agar (PDA), Czapek’s agar and nutrient agar [22]. The colours of the aerial and substrate mycelia and soluble pigment production were determined by using the ISCC-NBS colour charts [23]. The morphological characteristics were tested by light microscopy (model BH2; Olympus) and scanning electron microscopy (XL30 ESEM-TMP, Philips–FEI) after the isolate was grown on modified T5 medium at 30 °C for 5 days. The growth conditions for production of aerial and substrate mycelia, and the size and surface ornamentation of endospores were observed. Strain YIM 79486T grew well on ISP 2, ISP 3, ISP 4, PDA and Czapek’s media with well-developed aerial and substrate mycelia, grew moderately on ISP 5 and weakly on nutrient agar (Table S1). White aerial and brown substrate mycelia were formed on all media tested. No soluble pigment was found to be produced. Abundant terminally located endospores were produced, and their growth was terminal on a short sporophore (Fig. S3).

Temperatures for growth were tested at 4, 15, 28, 30, 37, 42, 45, 50, 55, 60, 65 and 70 °C. The pH range for growth was tested from pH 4.0 to 10.0 (at intervals of 1.0 pH units) for 7 days in modified T5 broth, using the buffer system as described by Nie et al. [24]. Salt tolerance for growth was observed with 0–5% NaCl (w/v) in modified T5 agar (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 %, w/v, respectively). Ability to utilize single carbon sources was tested with Pridham and Gottlieb’s basal mineral salts medium supplemented with filter-sterilized (0.22 µm; Millipore) carbon sources. Nitrogen source utilization was observed in a basal liquid medium according to the method of Nie et al. [25]. API galleries (API 20NE and API ZYM) were used to determine metabolic properties and some enzyme activities according to the instructions of the manufacturer (bioMérieux). The Biolog GII MicroPlate system was used to supplement the carbon source utilization and chemical sensitivity tests according to the manufacturer’s instructions. Tween (20, 40, 60 and 80) degradation, H₂S production and nitrate reduction, hydrolysis of gelatin, starch, cellulose, xylan, pectin and casein, activity of urease, and milk peptonization and coagulation were observed as previously described by MacFaddin [26], Williams et al. [27] and Smibert and Krieg [28].

Milk coagulation and peptonization of strain YIM 79486T was positive. Gelatin, ascesulin and pectin were hydrolysed.

| Table 1. Differential characteristics of strain YIM 79486T and other members of the genus Laceyella |
|-----------------------------|-------|-------|-------|-------|-------|
| Characteristic               | 1     | 2     | 3     | 4     | 5     |
| Growth temperature (°C)     | 37–65 | 30–65 | 35–65 | 28–70 | 28–65 |
| Urea hydrolysis              | +     | +     | +     | –     | –     |
| Degradation of Tweens        | –     | +     | +     | –     | –     |
| Gelatin liquefaction         | +     | +     | –     | +     | +     |
| N-Acetylglucosaminidase activity | –     | –     | –     | +     | +     |
| Whole-cell sugars            | Rib,  | Xylan, | Xylan, | Rib,  | Rib,  |
|                            | Glc,  | Ara,   | Ara,   | Xyl,  | Glc,  |
| Phospholipid components      | DPG,  | DPG,  | DPG,  | DPG,  | DPG,  |
|                            | PE,    | PE,    | PE,    | PE,    | PE,    |
|                            | PG,    | PG,    | PG,    | PG,    | PG,    |
|                            | PL,    | PL,    | PL,    | PL,    | PL,    |
|                            | PIM,   | PIM,   | PIM,   | PIM,   | PIM,   |
|                            | PL,    | PL,    | PL,    | PL,    | PL,    |
| Menaquinones                | MK-,   | MK-9,  | MK-9,  | MK-9,  | MK-9,  |
|                            | MK-8,  | MK-8,  | MK-8,  | MK-8,  | MK-8,  |
| DNA G+C content (mol%)      | 47.8   | 49.0   | 48.0   | 48.6   | 47.9   |

Strains: 1, YIM 79486T; 2, L. putida KCTC 3666T; 3, L. sacchari KCTC 9790T; 4, L. tengchongensis YIM 10002T; 5, L. sediminis RHA1T. +, Positive; –, negative. All data were obtained from this study under identical growth conditions. The result for degradation of Tween was the same for all forms of the molecule. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, phospholipid; AL, aminolipid.
Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 8 consists of anteiso-C<sub>15</sub>:0 (26.4 %), anteiso-C<sub>17</sub>:0 (23.2 %), iso-C<sub>17</sub>:0 (15.9 %) and anteiso-C<sub>17</sub>:0 (13.4 %). The detailed fatty acid profile of strain YIM 79486<sup>T</sup> is given in Table 2. The polar lipid profile of strain YIM 79486<sup>T</sup> consisted of diphosphatidylglycerol, phosphatidyglycerol, phosphatidylethanolamine, phosphatidylglycinol, phosphatidylglycerol mannoside, one unidentified aminolipid, three unidentified polar lipids and two unidentified lipids (Fig. S4). The genomic DNA G+C content of strain YIM 79486<sup>T</sup> was 47.8 mol%.

Due to the high 16S rRNA gene sequence similarity values, DNA–DNA relatedness was performed using the fluorometric micro-well method [40, 41]. DNA–DNA hybridization between strain YIM 79486<sup>T</sup> and the type strain <i>L. putida</i> KCTC 3666<sup>T</sup> was performed at the optimal hybridization temperature (37 °C) with eight replications. DNA–DNA relatedness values between strain YIM 79486<sup>T</sup> and the type strains <i>L. putida</i> KCTC 3666<sup>T</sup>, <i>L. sacchari</i> KCTC 9790<sup>T</sup>, <i>L. tengchongensis</i> YIM 10002<sup>T</sup> and <i>L. sediminis</i> RHA1<sup>T</sup> were 53.4±1.9 %, 50.5±1.7 %, 48.8±0.7 % and 48.4±0.6 %, respectively, which were notably lower than the threshold value (70 %) for the recognition of genomic species [42–44].

Phylogenetic analysis based on the 16S rRNA gene sequences demonstrated that strain YIM 79486<sup>T</sup> belonged to the genus <i>Laceyella</i>, which was supported by biochemical, chemotaxonomic and morphological characteristics that included predominant menaquinones, profiles of major fatty acids and polar lipids, production of aerial and substrate mycelia, and formation of endospores. Strain YIM 79486<sup>T</sup> formed a distinct branch with the type strain <i>L. putida</i> KCTC 3666<sup>T</sup>, indicated that it was a potential novel candidate of the genus <i>Laceyella</i>. However, differences were detected in the major chemotaxonomic properties and the minor components of fatty acids. Significant differences in fatty acids were found between the novel species and other species of genus <i>Laceyella</i>. In spite of possessing a major fatty acid profile including iso-C<sub>15</sub>:0 and anteiso-C<sub>15</sub>:0 representative of this genus, iso-C<sub>17</sub>:0 and anteiso-C<sub>17</sub>:0 were also detected as major fatty acids of strain YIM 79486<sup>T</sup>. Meanwhile, the novel candidate YIM 79486<sup>T</sup> could be distinguished by several phenotypic characteristics (Table 1). Therefore, based on the phylogenetic analysis, morphological, physiological and chemotaxonomic characterization, strain YIM 79486<sup>T</sup> should be considered to represent a new species of the genus <i>Laceyella</i>.
novel species within the genus *Laceyella*, for which the name *Laceyella thermophila* sp. nov. is proposed.

**DESCRIPTION OF LACEYELLA THERMO PHILA SP. NOV.**

*Laceyella thermophila* [ther.mo’phi.la. Gr. n. thermē heat; N. L. fem. adj. philē loving; N.L. fem. adj. thermophila heat loving].

Cells are Gram-stain-positive, aerobic, thermophilic and form white aerial and brown substrate mycelia on several growth media. Terminally located endospores are produced on short sporophores up to 2 μm long. No soluble pigment is produced on all media. Growth occurs at 37–65 °C (optimally at 50 °C). The pH range for growth is pH 6.0–8.0 (optimal pH 7.0–7.5), and the NaCl concentration range for growth is 0–0.5% (w/v) (optimum 0%, w/v). Positive for milk coagulation and peptonization, and hydrolysis of gelatin, aesculin and pectin. Negative for degradation of Tweens 20, 40, 60 and 80, cellulose, xylan and casein. Nitrate is not reduced. H₂S is not produced. Activities of urease, alkaline phosphatase, esterase (C4) and naphthol-AS-BI-phosphatase are positive, but esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, β-fucosidase and α-mannosidase activities are negative. Utilizes acetacetic acid, D-fructose, D-fructose 6-phosphate, D-fucose, L-fucose, D-galactose, D-galacturonic acid, L-galactonic acid lactone, gentiobiose, L-glucuronic acid, α-ketogluaric acid, D-glucose, maltose, D-mannose, melibiose, pyruvate, L-threonase, D-sorbitol and D-turanose but not acetic acid, γ-aminobutyric acid, D-arabitol, bromosuccinic acid, cellobiose, citric acid, dextrin, formic acid, D-gluconic acid, D-glucose 6-phosphate, glycerol, p-hydroxyphenylacetic acid, α-hydroxybutyric acid, β-hydroxy-D,L-butric acid, inosine, α-keto-butyric acid, L-lactic acid, D-lactic acid methyl ester, lactose, L-malic acid, D-malic acid, D-mannitol, methyl β-D-glucoside, 3-methyl glucol, mucic acid, myo-inositol, propionic acid, quinic acid, raffinose, D-glucaric acid, D-salicin, sucrose, stachyose or trehalose as the sole carbon source. Able to use L-alanine, L-arginine, L-asparagine, glucuronamide, glyceryl-L-proline, L-proline and L-valine as nitrogen sources but not L-glutamic acid, L-histidine, L-cystine, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, glycine, L-isoleucine, L-lysine, N-acetyl-β-D-mannosamine, L-methionine, N-acetylneuraminic acid, L-phenylalanine, L-proglutamic acid, D-serine, L-serine, L-threonine or L-tyrosine. Cell-wall peptidoglycan contains meso-diaminopimelic acid. Cell-wall sugars are ribose and glucose. The predominant menaquinones are MK-9 and MK-8. Major fatty acids are iso-C₁₅:0 anteiso-C₁₅:0 iso-C₁₇:0 and anteiso-C₁₇:0. The polar lipid profile comprises dihexadecylglycerol phosphate, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, one unidentified aminolipid, three unidentified polar lipids and two unidentified lipids.

The type strain, YIM 79486T (=CCTC AB 2015040T=NBRC 110772T), was isolated from a sediment sample collected from the outer pool of Jinze hot spring in Tengchong county, Yunnan province, south-west China. The genomic DNA G+C content of the type strain is 47.8 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


