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

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A thermophilic bacterium, designated strain RHA1^T, was isolated from a sediment sample collected from a hot spring in Tengchong county, Yunnan province, south-west China, and was characterized by using a polyphasic approach. Based on its phenotypic and phylogenetic characteristics, strain RHA1^T was affiliated to the genus *Laceyella*. The strain formed white aerial and yellow–white substrate mycelia, bearing single endospores on short sporophores. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid. Whole-cell hydrolysates contained ribose and glucose. The major fatty acids were iso-C_{15:0} (62.39 %) and anteiso-C_{15:0} (17.55 %). The predominant menaquinone was MK-9. The G + C content of the genomic DNA of strain RHA1^T was 47.9 mol%. Based on DNA–DNA hybridization data, chemotaxonomic characteristics and differential physiological properties, strain RHA1^T is considered to represent a novel species of the genus *Laceyella*, for which the name *Laceyella sediminis* sp. nov. is proposed; the type strain is RHA1^T (=DSM 45263^T=CCTCC AA 208058^T).

The genus *Laceyella* within the family *Bacillaceae* was proposed by Yoon *et al.* (2005), but was subsequently placed in the family *Thermoactinomycetaceae* by Matsuo *et al.* (2006) based on data from a polyphasic taxonomic study. Members of the genus *Laceyella* are aerobic, chemo-organotrophic, Gram-positive and thermophilic filamentous bacteria. Substrate and aerial mycelia are formed, and the aerial mycelium is white. Sessile endospores may be produced on sporophores. Greyish-yellow or yellow–brown diffusible pigment may be produced. The predominant menaquinone is MK-9. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid, but no characteristic sugars. The major fatty acids are iso-C_{15:0} and anteiso-C_{15:0}, and the DNA G + C content of the type strains of recognized *Laceyella* species is in the range 48–49 mol% (Yoon *et al.*, 2005). At the time of writing, the genus comprised three recognized species: *Laceyella sacchari* (basonym *Thermoactinomyces sacchari*; Lacey & Vince, 1971; Yoon *et al.*, 2000, 2005), *Laceyella putida* (basonym *Thermoactinomyces putidus*; Lacey & Cross, 1989; Yoon *et al.*, 2005) and *Laceyella tengchongensis* (Zhang *et al.*, 2010).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RHA1^T is FJ422144.

Two supplementary figures are available with the online version of this paper.

During long-term investigations on the diversity of micro-organisms from particular habitats, a thermophilic strain, designated RHA1^T, was isolated from a sediment sample of a hot spring (55 °C, pH 6.5). In this study, 1 g sediment was suspended in 50 ml liquid DSM 88 medium and incubated at 55 °C with shaking (140 r.p.m.) for 24 h. Samples of 100 µl were then spread onto the surface of isolation plates containing soluble starch (1.0 %, w/v) and incubated at 55 °C for 24 h. After three purification steps, one strain, designated RHA1^T, was picked from the isolation plate and maintained on DSM 88 medium agar slants at 4 °C and as glycerol suspensions (20 %, v/v) at –80 °C.

Cultures grown on International *Streptomyces* Project (ISP) 3 agar medium (Shirling & Gottlieb, 1966) for 1–7 days at 55 °C were observed by light microscopy (Olympus BH-2). The colours of substrate and aerial mycelia and any soluble pigments produced were determined with reference to Kelly (1964). Aerial and substrate mycelia were abundant, well developed, non-fragmented and white; endospores were formed (Fig. 1). Scanning electron micrographs (Philip XL30 ESEM-TMP) were taken of mature spores in aerial mycelia of strain RHA1^T grown on ISP 3 agar medium for 5 days at 55 °C.

The physiological features and biochemical characteristics of strain RHA1^T were examined as described by

Fig. 1. Scanning electron micrographs of mature spores in aerial mycelia of strain RHA1^T grown on ISP 3 agar medium for 5 days at 55 °C. Representative measurements are given in (b). Bars: (a) 5 µm; (b) 1 µm.

Goodfellow (1971) and Williams *et al.* (1983) and are detailed in Table 1 and in the species description. Growth of strain RHA1^T occurred at 28–65 °C (optimum 55 °C), at pH 5.0–9.0 (optimum pH 7.0) and in the presence of 0–1 % (w/v) NaCl (optimum 0%). The phenotypic properties of strain RHA1^T clearly distinguished it from recognized *Laceyella* species (Tables 1 and 2).

For chemical and molecular systematic studies, biomass of strain RHA1^T and the type strains of *L. sacchari*, *L. putida* and *L. tengchongensis* was obtained after incubation in DSM 88 broth at 55 °C for 3 days in shaken flasks (about 150 r.p.m.). Amino acids and sugars of whole-cell hydrolysates were analysed according to the procedures described by Hasegawa *et al.* (1983). Polar lipids were extracted and examined by two-dimensional TLC and were identified by using the procedures described by Minnikin *et al.* (1984) (see Supplementary Fig. S1 in IJSEM Online). Menaquinones were extracted according to Minnikin *et al.* (1984) and were analysed by HPLC (Kroppenstedt, 1982). Cellular fatty acid assays were performed according to the standard protocol of the Microbial Identification System (MIDI) (Sasser, 1990) (Sherlock version 6.1; MIDI database TSBA6), according to the manufacturer's instructions. Whole-cell hydrolysates of strain RHA1^T contained *meso*-diaminopimelic acid, ribose and glucose. The phospholipids comprised disphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides and one unknown phospholipid.

The predominant menaquinone was MK-9; MK-8 was detected as a minor component. The cellular fatty acids included branched, straight-chain and unsaturated components (Kroppenstedt, 1985). The major fatty acids were iso-C_{15:0} (62.39 %) and anteiso-C_{15:0} (17.55 %). A detailed fatty acid profile of strain RHA1^T is given in Table 2; it was similar to those of *L. sacchari* DSM 43356^T, *L. putida* DSM 44608^T and *L. tengchongensis* YIM 10002^T.

Extraction of genomic DNA, and PCR amplification and sequencing of the 16S rRNA gene of strain RHA1^T were carried out according to Li *et al.* (2007). The almost-complete 16S rRNA gene sequence (1467 bp) of strain RHA1^T was determined. The 16S rRNA gene sequence of strain RHA1^T was compared with available sequences from GenBank by using the program BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine an approximate phylogenetic affiliation. The program CLUSTAL X (Thompson *et al.*, 1997) was used for multiple alignment and three algorithms, neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Guindon & Gascuel, 2003), were used to construct phylogenetic trees; *Bacillus subtilis* NCDO 1769^T was used as an outgroup. The topology of the trees generated with these methods was evaluated by the bootstrap resampling method of Felsenstein (1985) based on 1000 resamplings.

A BLAST analysis of the 16S rRNA gene sequence of strain RHA1^T showed that it was affiliated to the family *Thermoactinomycetaceae*. Fig. 2 shows the phylogenetic position of strain RHA1^T within the radiation of species of the genus *Laceyella*. Strain RHA1^T occupied a distinct branch with *L. sacchari* DSM 43356^T, *L. tengchongensis* YIM 10002^T and *L. putida* DSM 44608^T, with which it shared 99.8, 99.6 and 97.8 % 16S rRNA gene sequence similarity, respectively. This position was also supported in the tree generated with the maximum-likelihood algorithm with a bootstrap value of 100 % (Supplementary Fig. S2, available in IJSEM Online).

For determination of the G+C content of strain RHA1^T, DNA was obtained according to the method described by Marmur (1961) and the value was determined by the HPLC method (Mesbah *et al.*, 1989). The DNA G+C content of strain RHA1^T was 47.9 mol%. DNA–DNA hybridization was carried out by the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). Levels of DNA–DNA relatedness between strain RHA1^T and its closest phylogenetic neighbours, *L. sacchari* DSM 43356^T, *L. tengchongensis* YIM 10002^T and *L. putida* DSM 44608^T, were 60, 47 and 44 %, respectively, values below the 70 % threshold for the delineation of genomic species (Stackebrandt & Goebel, 1994).

Based on the combination of morphological, physiological, chemotaxonomic and phylogenetic data discussed here, it is evident that strain RHA1^T should be affiliated to the genus *Laceyella*. Differences in several phenotypic characteristics can be used to distinguish the isolate from recognized *Laceyella* species. For example, strain RHA1^T

Table 1. Differential characteristics between strain RHA1^T and the type strains of recognized species of the genus *Laceyella*

Strains: 1, RHA1^T; 2, *L. sacchari* DSM 43356^T; 3, *L. tengchongensis* YIM 10002^T; 4, *L. putida* DSM 44608^T. All data are from this study. v, Variable.

Characteristic	1	2	3	4
Soluble pigment	—	Yellow–brown	—	Greyish–yellow
Melanin	—	v	—	+
Degradation of:				
Hypoxanthine	+	—	+	—
Starch	+	+	—	+
Gelatin liquefaction	+	—	+	+
Temperature range (°C)	28–65	35–65	28–70	30–65
Utilization of:				
D-Mannitol	—	+	+	—
L-Rhamnose	—	—	+	—
Raffinose	—	+	—	—
Maltose	+	+	—	+
L-Lysine	—	—	+	—
L-Cysteine	—	+	+	—
L-Threonine	—	—	+	+
Glycine	+	+	—	+
L-Serine	—	+	—	+
Adenine	+	—	—	—
L-Valine	+	—	—	+
Xanthine	+	—	—	+
L-Proline	+	—	—	+
Chemotaxonomic data:				
Whole-cell sugars*	Rib, Glu	Xyl, Ara, Glu	Rib, Xyl, Glu	Xyl, Ara, Glu
Phospholipid components†	DPG, PE, PG, PI, PIM, PL	DPG, PE, PME, PI, PIM, PL	DPG, PE, PG, PI, PIM, PL	DPG, PE, PG, PI, PIM, PL
Menaquinones	MK-9, MK-8	MK-9, MK-8, MK-10	MK-9, MK-8	MK-9, MK-8
DNA G + C content (mol%)	47.9	48.0	48.6	49.0

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

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

Table 2. Cellular fatty acid profiles of strain RHA1^T and type strains of recognized species of the genus *Laceyella*

Strains: 1, RHA1^T; 2, *L. sacchari* DSM 43356^T; 3, *L. tengchongensis* DSM 45262^T; 4, *L. putida* DSM 44608^T. All data are from this study; cultures were grown under identical conditions. Values are percentages of the total fatty acids.

Fatty acid	1	2	3	4
Straight-chain				
C _{14:0}	2.24	1.27	1.16	1.12
C _{16:0}	2.62	1.47	1.61	1.05
C _{17:0}	0.53	0.91	0.85	1.06
Branched-chain				
iso-C _{13:0}	0.83	1.30	1.32	1.90
iso-C _{14:0}	2.38	3.28	7.11	9.89
iso-C _{15:0}	62.39	66.61	57.63	50.66
anteiso-C _{15:0}	17.55	12.02	13.79	18.34
iso-C _{16:0}	4.18	4.36	8.88	9.68
iso-C _{17:0}	4.43	4.15	3.62	3.53
anteiso-C _{17:0}	1.82	0.89	1.18	1.77

and the type strains of *L. sacchari*, *L. tengchongensis* and *L. putida* showed different results for gelatin liquefaction, starch hydrolysis, nitrate reduction and melanin production. They could also be distinguished based on cell-wall sugars and DNA G + C content. The above data, together with low levels of DNA–DNA relatedness (<70 %) between strain RHA1^T and its closest phylogenetic neighbours, demonstrate that strain RHA1^T represents a novel species of the genus *Laceyella*, for which the name *Laceyella sediminis* sp. nov. is proposed.

Description of *Laceyella sediminis* sp. nov.

Laceyella sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment).

Cells are Gram-positive, aerobic, thermophilic and filamentous. White aerial and yellow–white substrate mycelia are produced, bearing single endospores on short sporophores. No soluble pigments are produced on any of the media tested. Growth occurs at 28–65 °C (optimum 55 °C), at pH 5.0–9.0 (optimum pH 7.0) and in the

Fig. 2. Neighbour-joining phylogenetic dendrogram based on nearly complete 16S rRNA gene sequences showing the position of strain RHA1^T among its closest neighbours. Numbers at nodes are bootstrap values (percentages of 1000 replications); only values >50 % are shown. The sequence of *Bacillus subtilis* NCDO 1769^T was used as an outgroup. Bar, 0.01 inferred substitutions per site.

presence of 0–1 % (w/v) NaCl (optimum 0 %). Positive for gelatin liquefaction, degradation of starch, and milk peptonization and coagulation, but negative for nitrate reduction, and H₂S and melanin production. Utilizes lactose, trehalose, maltose and gelatin as carbon sources, but not D-mannitol, L-rhamnose, raffinose, D-mannose, D-xylose or D-ribose. Degrades L-proline, xanthine, L-valine, adenine, glycine, L-asparagine and L-arginine, but not L-lysine, L-cysteine, L-threonine, L-serine or L-hydroxyproline. The diagnostic amino acid in the peptidoglycan is *meso*-diaminopimelic acid. Hydrolysates of the cell-wall contain ribose and glucose. The predominant menaquinone is MK-9. The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}.

The type strain is RHA1^T (=DSM 45263^T = CCTCC AA 208058^T), isolated from a sediment sample of a hot spring collected from Tengchong county, Yunnan province, south-west China. The DNA G + C content of the type strain is 47.9 mol%.

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