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

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A thermophilic bacterium, designated strain RHA1T, 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 RHA1T 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-C15:0 (62.39%) and anteiso-C15:0 (17.55%). The predominant menaquinone was MK-9. The G+C content of the genomic DNA of strain RHA1T was 47.9 mol%. Based on DNA–DNA hybridization data, chemotaxonomic characteristics and differential physiological properties, strain RHA1T 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 RHA1T (=DSM 45263T=CCTCC AA 208058T).

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, chemotrophic, 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–white substrate mycelia, bearing single endospores on short sporophores. The predominant menaquinone was MK-9. The cell-wall peptidoglycan contained meso-diaminopimelic acid, but no characteristic sugars. The major fatty acids are iso-C15:0 and anteiso-C15:0, 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).

During long-term investigations on the diversity of micro-organisms from particular habitats, a thermophilic strain, designated RHA1T, 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 μI 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 RHA1T, 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 RHA1T grown on ISP 3 agar medium for 5 days at 55 °C.

The physiological features and biochemical characteristics of strain RHA1T were examined as described by...
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-C₁₅:0 (62.39 %) and anteiso-C₁₅:0 (17.55 %). A detailed fatty acid profile of strain RHA¹ was given in Table 2; it was similar to those of L. sacchari DSM 43356ᵀ, L. putida DSM 44608ᵀ and L. tengchongensis YIM 10002ᵀ.

Extraction of genomic DNA, and PCR amplification and sequencing of the 16S rRNA gene of strain RHA¹ were carried out according to Li et al. (2007). The almost-complete 16S rRNA gene sequence (1467 bp) of strain RHA¹ was determined. The 16S rRNA gene sequence of strain RHA¹ 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 1769ᵀ 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 showed that it was affiliated to the family Thermoactinomycetaceae. Fig. 2 shows the phylogenetic position of strain RHA¹ within the radiation of species of the genus Laceyella. Strain RHA¹ occupied a distinct branch with L. sacchari DSM 43356ᵀ, L. tengchongensis YIM 10002ᵀ and L. putida DSM 44608ᵀ, 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, 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 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 and its closest phylogenetic neighbours, L. sacchari DSM 43356ᵀ, L. tengchongensis YIM 10002ᵀ and L. putida DSM 44608ᵀ, 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 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...
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 RHA1T and its closest phylogenetic neighbours, demonstrate that strain RHA1T represents a novel species of the genus *Laceyella*, for which the name *Laceyella sediminis* sp. nov. is proposed.

**Description of *Laceyella sediminis* sp. nov.**


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
Laceyella sediminis sp. nov.

The type strain is RHA1\textsuperscript{T} (=DSM 45263\textsuperscript{T} = CCTCC AA 208058\textsuperscript{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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References


charimyces asporophogenes sp. nov., antitumour substance-producing


