Saccharibacillus kuerlensis sp. nov., isolated from a desert soil

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A taxonomic study was performed on strain HR1^T, which was isolated from a desert soil sample collected from Xinjiang Province (China). Cells were aerobic, Gram-positive-staining, pink-pigmented, sporulating rods with a single lateral flagellum. The organism can grow at 15–42 °C and pH 5.0–10.0, optimally at 30–37 °C and pH 6.0–8.0. Growth is inhibited by 6% NaCl. Analysis of almost-complete 16S rRNA gene sequence revealed that the isolate represents a distinct taxon within the genus *Saccharibacillus*; *Saccharibacillus sacchari* LMG 24085^T was the nearest relative (97.9% sequence similarity). DNA–DNA hybridization showed 29.6% genetic relatedness between strain HR1^T and *S. sacchari* LMG 24085^T. The major isoprenoid quinone was MK-7 and the predominant fatty acid was anteiso-C_{15:0} (50.3%). The G + C content of the DNA was 50.5 mol%. Therefore, based on phenotypic criteria and the phylogenetic position, strain HR1^T belongs to a previously unidentified species of the genus *Saccharibacillus*, for which the name *Saccharibacillus kuerlensis* sp. nov. is proposed. The type strain is HR1^T (=KCTC 13182^T =JCM 14865^T =CGMCC 1.6964^T).

The genus *Saccharibacillus* of the family ‘*Paenibacillaceae*’ was first proposed by Rivas *et al.* (2008) to include a Gram-variable, facultatively anaerobic, motile and rod-shaped bacterium that was negative for catalase and oxidase activities and that had anteiso-C_{15:0} as the main fatty acid and menaquinone-7 as the major respiratory menaquinone. At present, the genus comprises only one species, *Saccharibacillus sacchari*.

In our search for organisms capable of growing in desert ecosystems, a bacterial strain, designated HR1^T, was isolated from a soil sample collected from the southern desert of Kuerle (41° 46′ N 86° 07′ E), a city at the heart of Xinjiang, the most westerly province of China. The combination of phenotypic and chemotaxonomic characteristics, phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA hybridization showed that strain HR1^T is a representative of a novel species of the genus *Saccharibacillus*.

Strain HR1^T was isolated using the standard dilution-plating technique on Luria–Bertani (LB) agar medium. The isolate was cultivated routinely on LB agar at 30 °C under aerobic conditions and maintained as a glycerol suspension

(20%, w/v) at –70 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained by culturing strain HR1^T in liquid LB medium and harvesting the cells by centrifugation.

Amplification and sequencing of the 16S rRNA gene was performed as described by Cui *et al.* (2001). The sequence obtained was compared with available 16S rRNA gene sequences retrieved from GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine an approximate phylogenetic affiliation. Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 3.1 (Kumar *et al.*, 2001) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to Kimura’s two-parameter model; Kimura, 1980, 1983) and clustering were based on the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods. Bootstrap analysis (1000 replications) was used to evaluate the topology of the neighbour-joining tree (Felsenstein, 1985).

The almost-complete 16S rRNA gene sequence of strain HR1^T (1513 bp) revealed the highest similarity to *S. sacchari* LMG 24085^T (97.9%); the sequence similarities to members of other genera of the family ‘*Paenibacillaceae*’ with validly published names were lower than 93.4%.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HR1^T is EU046270.

Phylogenetic trees obtained with different algorithms were identical with respect to the position of strain HR1^T, which formed a distinct branch with its closest relative *S. sacchari* LMG 24085^T. A phylogenetic tree based on the neighbour-joining algorithm is shown in Fig. 1.

Genomic DNA was extracted and purified according to Yoon *et al.* (1996) and the DNA G+C content was determined using the thermal denaturation method (Mandel & Marmur, 1968). The G+C content of the DNA was 50.5 mol%, which is slightly lower than the value recorded for *S. sacchari* LMG 24085^T (57.8 mol%; Rivas *et al.*, 2008). DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain HR1^T and *S. sacchari* LMG 24085^T by applying the optical renaturation rate method as described by De Ley *et al.* (1970) with the modifications described by Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were computed with the program TRANSFER.BAS (Jahnke, 1992). The DNA–DNA relatedness between strain HR1^T and its closest relative *S. sacchari* LMG 24085^T was 29.6%. According to Stackebrandt & Goebel (1994) and Wayne *et al.* (1987), the values for 16S rRNA gene similarity (<97%) and DNA–DNA relatedness (<70%) support the genomic distinction of strain HR1^T from *S. sacchari* LMG 24085^T.

For quantitative analysis of the cellular fatty acid content, cells were harvested after 2 days growth at 30 °C on tryptic soy agar (Merck) and fatty acid methyl esters were

prepared and identified by following the instructions of the Microbial Identification system (MIDI) as described by Sasser (1990). The type strain of the closely related species *S. sacchari* (LMG 24085^T) was also cultured under the same nutrient and temperature conditions and characterized concurrently with strain HR1^T. Determination of the cell-wall peptidoglycan was performed using the methods of Schleifer & Kandler (1972). Polar lipids were extracted, examined by two-dimensional TLC and identified following Minnikin *et al.* (1984). Menaquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. The cellular fatty acids of strain HR1^T are listed in Table 1 and compared with *S. sacchari* LMG 24085^T. Anteiso-C_{15:0}, the major fatty acid found in *S. sacchari* LMG 24085^T (Rivas *et al.*, 2008), was also the major fatty acid component of strain HR1^T, comprising 50.3% of the total. The peptidoglycan type was *meso*-diaminopimelic acid, in line with all other members of the family 'Paenibacillaceae', which includes the genus *Saccharibacillus*. The predominant isoprenoid quinone was MK-7, which supports the affiliation of strain HR1^T to the genus *Saccharibacillus*; in addition, a small amount of MK-6 was detected. This profile is slightly different from that of *S. sacchari* LMG 24085^T, in which small amounts of both MK-6 and an unidentified menaquinone were detected (Rivas *et al.*, 2008). The polar lipids of strain HR1^T are diphosphatidylglycerol, phosphatidylglycerol and an unknown phosphoglycolipid. Glycolipids and aminophospholipids, which occur in *S. sacchari* LMG 24085^T,

Fig. 1. Phylogenetic tree obtained by neighbour-joining analysis based on 16S rRNA gene sequences, showing the position of strain HR1^T among phylogenetically related species. Numbers on branch nodes are bootstrap values (1000 resamplings). Bootstrap values >50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

Table 1. Fatty acid profiles of strain HR1^T and *S. sacchari* LMG 24085^T

Data were taken from present study. Values are percentages of total fatty acids. —, Not detected; fatty acids amounting to less than 1.0 % in both strains are not listed.

Fatty acid	Strain HR1 ^T	<i>S. sacchari</i> LMG 24085 ^T
iso-C _{13:0} 3-OH	—	3.3
iso-C _{14:0}	0.4	8.1
iso-C _{15:1} F	—	3.1
anteiso-C _{15:1} A	—	1.1
iso-C _{15:0}	1.3	2.1
anteiso-C _{15:0}	50.3	44.4
C _{16:1} ω11c	9.0	—
C _{16:1} ω7c alcohol	2.3	—
iso-C _{16:0}	0.5	12.4
C _{16:0}	2.8	12.0
iso-C _{17:1} ω10c	1.7	—
iso-C _{17:0}	0.5	1.6
anteiso-C _{17:0}	4.5	4.0
C _{18:1} ω9c	5.9	1.0
C _{18:1} ω5c	—	1.4
C _{18:0}	1.2	1.7
Summed feature 3*	1.4	0.7
Summed feature 4*	16.8	—

*Summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B.

and phosphatidylethanolamine, which occurs in members of other genera of the family 'Paenibacillaceae', were not detected. However, strain HR1^T contained the unknown phosphoglycolipid that is present in *S. sacchari* LMG 24085^T but has not been reported in other genera. This polar lipid profile provided decisive evidence that strain HR1^T belongs to the genus *Saccharibacillus* and is different from other related species.

Morphological features were examined by using light microscopy (model BH 2; Olympus) and transmission electron microscopy (Hitachi-7650) after 24 h growth on LB agar at 30 °C. For transmission electron microscopy observation, cells were negatively stained with 1 % (w/v) phosphotungstic acid and, after air drying, Gram-staining and endospore formation were investigated as described by Smibert & Krieg (1994). Growth at different temperatures and pH values was investigated as described by Xu *et al.* (2005), using LB broth as the basal medium. NaCl tolerance was tested using LB broth supplemented with 0, 1, 2, 3, 5 and 6 % NaCl, respectively. Growth was determined on cetrimide agar, Simmons' citrate agar, MacConkey agar and nutrient agar. All tests were incubated at 30 °C for 2 days. Antibiotic sensitivity tests were performed using the diffusion method on LB agar at 30 °C with filter-paper discs (8 mm diameter) containing one of the following antibiotics: amoxicillin (10 µg),

ampicillin (10 µg), carbenicillin (100 µg), cefamezin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), ofloxacin (5 µg), penicillin G (10 IU), polymyxin B (30 µg), rifampicin (5 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg). After 2 days of incubation, the diameters of the inhibition zones were measured. Acid production from carbohydrates was determined using the medium and methods described by Yamaguchi & Yokoe (2000). Single carbon-source utilization was determined as described by Liu *et al.* (2008). Oxidase activity was tested by determining the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by determining the production of oxygen bubbles in a 5 % (v/v) aqueous hydrogen peroxide solution. Phenylalanine deaminase activity and hydrolysis of Tweens 80 and 20, aesculin, DNA and CM-cellulose were investigated as described by Smibert & Krieg (1994). Urease activity and hydrolysis of casein, gelatin, tyrosine and starch were determined as described by Cowan & Steel (1965). Some other biochemical characteristics were tested using API 20E kits (bioMérieux) according to the instructions of the manufacturer. Cells of strain HR1^T stained Gram-positive and were motile, sporulating rods with a single lateral flagellum, about 0.4–0.5 µm wide and 0.8–1.4 µm long (Fig. 2). Colonies were convex, almost-circular, opaque, non-glossy and pink after 48 h of incubation at 30 °C. Phenotypic features of strain HR1^T are given in the species description and in Table 2.

In the light of the combined phylogenetic, biochemical and morphological data, strain HR1^T should be classified as the type strain of a novel species within the genus *Saccharibacillus*, for which the name *Saccharibacillus kuerlensis* sp. nov. is proposed.

Fig. 2. Transmission electron micrograph of a cell of strain HR1^T grown on LB agar for 24 h at 30 °C, showing the single lateral flagellum. Bar, 500 nm.

Table 2. Differentiating characteristics of HR1^T and *S. sacchari* LMG 24085^TData for *S. sacchari* LMG 24085^T were taken from Rivas *et al.* (2008).

Characteristic	Strain HR1 ^T	<i>S. sacchari</i> LMG 24085 ^T
Growth at:		
8 °C	—	+
42 °C	+	—
Tolerance of 5 % NaCl	+	—
Catalase	+	—
Arginine dihydrolase	+	—
Hydrolysis of CM-cellulose	—	+
Utilization of:		
D-Xylose	+	—
N-Acetyl-D-glucosamine	+	—
L-Arabinose	+	—
Acid production from:		
D-Mannose	+	—
Maltose	+	—
D-Mannitol	+	—
DNA G + C content (mol%)	50.5	57.8

Description of *Saccharibacillus kuerlensis* sp. nov.

Saccharibacillus kuerlensis (ku.er.len'sis. N.L. masc. adj. *kuerlensis* pertaining to Kuerle, a city of Xinjiang Province in the north-west of China where the type strain was isolated).

Gram-positive-staining, aerobic, rod-shaped cells, 0.4–0.5 µm wide and 0.8–1.4 µm long, motile by a single lateral flagellum. Spores are oval and subterminal and occur in unswollen sporangia. After 2 days incubation on LB agar, colonies are 1.0–2.0 mm in diameter, convex, almost-circular, opaque, non-glossy and pink-pigmented. Grows at 15–42 °C (optimum, 30–37 °C), pH 5.0–10.0 (optimum, pH 6.0–8.0) and 0–5 % NaCl. Good growth occurs on nutrient agar, but not on Simmons' citrate agar, MacConkey agar or cetrimide agar. Nitrate is reduced to nitrite. Tween 20 and aesculin are hydrolysed, but starch, casein, gelatin, DNA, Tween 80, tyrosine and CM-cellulose are not. Positive for catalase, arginine dihydrolase, β-galactosidase and the methyl red reaction. Negative for oxidase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, urease, indole and hydrogen sulphide production, phenylalanine deaminase and the Voges–Proskauer reaction. Acid is produced from D-glucose, D-lactose, trehalose, D-fructose, D-xylose, L-rhamnose, maltose, raffinose, melibiose, cellobiose, melezitose, D-mannose, D-ribose, D-galactose, sucrose, turanose, D-mannitol, inositol, amygdalin, salicin and dextrin. Utilizes L-arabinose, L-arabitol, adonitol, acetate, inulin, gluconate and N-acetyl-D-glucosamine, but not L-sorbose, xylitol, D-sorbitol, i-erythritol, glycerol, methyl α-glucoside or tartrate. Resistant to streptomycin, but sensitive to amoxicillin, ampicillin, carbenicillin, cefazolin, ceftazidime,

chloramphenicol, erythromycin, gentamicin, kanamycin, ofloxacin, penicillin G, polymyxin B, rifampicin, tetracycline and vancomycin. The peptidoglycan type is *meso*-diaminopimelic acid. The main phospholipid is diphosphatidylglycerol and MK-7 is the major isoprenoid quinone. The DNA G+C content of the type strain is 50.5 mol%. The fatty acid profile is shown in Table 1.

The type strain, HR1^T (=KCTC 13182^T =JCM 14865^T =CGMCC 1.6964^T), was isolated from a desert soil sample collected from Xinjiang Province, China.

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