Lentibacillus lacisalsi sp. nov., a moderately halophilic bacterium isolated from a saline lake in China

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An aerobic, spore-forming, moderately halophilic bacterium, strain BH260^T, was isolated from a salt lake in China. Cells of this strain were found to be motile rods with flagella. The organism grew optimally at 30–32 °C and pH 8.0 in the presence of 12–15 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate formed a clade with the type strains of the genus *Lentibacillus*. The levels of 16S rRNA gene sequence similarity for strain BH260^T with respect to *Lentibacillus salsus* KCTC 3911^T, *Lentibacillus juripiscarius* JCM 12147^T and *Lentibacillus salicampi* KCCM 41560^T were 96.0, 95.5 and 95.3 %, respectively. Chemotaxonomic data (a DNA G+C content of 44 mol%; MK-7 as the major isoprenoid quinone; A1 γ -meso-diaminopimelic acid as the cell-wall type; phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids; anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} as the major fatty acids) also supported assignment of the isolate to the genus *Lentibacillus*. On the basis of physiological and molecular properties, the isolate represents a novel species within the genus *Lentibacillus*, for which the name *Lentibacillus lacisalsi* sp. nov. is proposed. The type strain is BH260^T (=KCTC 3915^T=DSM 16462^T).

Aerobic, spore-forming, moderately halophilic, Gram-positive rods are taxonomically diverse and have been isolated from different marine environments and related habitats (Oren, 2002). They were originally assigned to the genus *Bacillus* (Ash *et al.*, 1991; Stackebrandt & Liesack, 1993; Nielsen *et al.*, 1994; Ventosa *et al.*, 1998), but molecular and chemical analyses have shown that they can be divided into several phylogenetically distinct lineages, such as the new genera *Halobacillus* (Spring *et al.*, 1996), *Gracilibacillus* (Wainø *et al.*, 1999), *Virgibacillus* (Heyndrickx *et al.*, 1999), *Filobacillus* (Schlesner *et al.*, 2001), *Oceanobacillus* (Lu *et al.*, 2001), *Tenuibacillus* (Ren & Zhou, 2005) and *Pontibacillus* (Lim *et al.*, 2005).

Recently, novel species isolated from salty habitats, such as *Lentibacillus juripiscarius* and *Lentibacillus salarius*, have

been added to the genus since the genus *Lentibacillus* was proposed for novel moderately halophilic Gram-positive rods (Yoon *et al.*, 2002; Namwong *et al.*, 2005; Jeon *et al.*, 2005). In this study, polyphasic taxonomy demonstrated that another novel Gram-positive bacterium, strain BH260^T, isolated from a salt lake in Xin-Jiang Province in China, represents a novel species of the genus *Lentibacillus*.

Strain BH260^T was isolated on marine agar 2216 (MA; Difco) with the addition of 20 % (w/v) NaCl [final concentration, 21.94 % (w/v) NaCl] at 28 °C for 3 days from soil sediment of a salt lake in China. Requirements for, and tolerance of, NaCl were determined in trypticase soy broth [TSB, containing (l⁻¹) 17.0 g casein, 3.0 g soy-bean meal, 2.5 g glucose, 5.0 g NaCl, 2.5 g dipotassium phosphate] and supplemented with modified artificial sea water [containing (l⁻¹) 0–30 % (w/v) NaCl, 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 0.64 g KCl, 1.3 g CaCl₂]. Optimum growth was tested at different temperatures (4–55 °C) on MA containing 12 % (w/v) NaCl and at different pH values (5.0–11.0) in TSB supplemented with artificial sea water containing 12 % (w/v) NaCl. Anaerobic growth was determined by using incubation in an anaerobic chamber at 30 °C

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A transmission electron micrograph of a cell of strain BH260^T is available as a supplementary figure in IJSEM Online.

for 5 days on MA containing 12 % (w/v) NaCl. Cell morphology and flagellum type were studied using light microscopy (LABOPHOT apparatus; Nikon) and transmission electron microscopy (JEM-1010 apparatus; JEOL) as described by Lee *et al.* (2005). Motility was observed at 24 and 36 h in wet mounts by using a light microscope (E600; Nikon). Endospores were stained using Schaeffer–Fulton stain (Smibert & Krieg, 1981).

Gram staining was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase activity was determined by assessing the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Oxidase activity was tested for by assessing the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Hydrolysis of aesculin, casein, starch, Tween 80, urea, hypoxanthine, tyrosine and xanthine was determined on MA, according to methods described previously (Lanyi, 1987; Smibert & Krieg, 1994). Nitrate reduction was determined according to the method of Lanyi (1987). Acid production from carbohydrates was tested for as described by Leifson (1963); all suspension media were supplemented with artificial sea water containing 12 % (w/v) NaCl. GC analysis of fatty acid methyl esters was performed using cells grown at 30 °C for 3 days on MA with the addition of 10 % (w/v) NaCl according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Analyses of peptidoglycan, polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The DNA G + C content of strain BH260^T was determined by reverse-phase HPLC (HP 1090 apparatus; Hewlett Packard) using the method of Tamaoka & Komagata (1984).

Sequencing and assembly of the 16S rRNA gene was carried out as described previously (Bakermans & Madsen, 2002). The resultant 16S rRNA gene sequence was compared with available 16S rRNA gene sequences from GenBank by using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) and was aligned with closely related 16S rRNA gene sequences with CLUSTAL W software (Thompson *et al.*, 1994). Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II, <http://35.8.164.52/html/>; Cole *et al.*, 2003). Phylogenetic trees were constructed using three different methods: neighbour-joining, maximum-likelihood and maximum-parsimony algorithms available in PHYLIP software, version 3.6 (Felsenstein, 2002). Evolutionary distance matrices were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 80) for the neighbour-joining method. To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1000 replications) was performed with the SEQBOOT, DNADIST, NEIGHBOR and CONSENSE programs in the PHYLIP package.

Strain BH260^T on MA medium supplemented with 12 % (w/v) NaCl formed cream-coloured, low-convex and circular/slightly irregular colonies and the colony morphologies were consistent at various salinities of NaCl. Strain

BH260^T showed halophilic properties, growing in TSB containing 5–25 % (w/v) NaCl; optimum growth occurred in TSB with 12–15 % (w/v) NaCl. Growth was not observed in the absence of NaCl. Growth was observed at temperatures between 15 and 40 °C, with optimum growth at 30–32 °C. Strain BH260^T grew at pH values in the range 7.0–9.5 in 12 % (w/v) NaCl-containing TSB; optimal growth was observed at pH 8.0. Cells of strain BH260^T from early and late growth phases showed obvious Gram-positive reactions. The cells of the isolate were strictly aerobic, short rods, 0.4–0.6 µm wide and 1.2–3.0 µm long (see the transmission electron micrograph available as a supplementary figure in IJSEM Online). Cells of the isolate produced single spherical terminal endospores within swollen sporangia. Cell motility was facilitated by means of flagella on the sides of the cells. Anaerobic growth was not observed under anaerobic conditions after 5 days at 30 °C on MA with 12 % (w/v) NaCl.

The major respiratory lipoquinone of strain BH260^T was MK-7. The fatty acid profile of the strain was characterized by the presence of branched and saturated fatty acids such as anteiso-C_{15:0} (50.78 %), anteiso-C_{17:0} (18.20 %) and iso-C_{16:0} (11.95 %) as the major fatty acids, in common with the type strains of the genus *Lentibacillus*; their fatty acid compositions were somewhat different (Table 1). Cell-wall peptidoglycan analysis indicated A1γ type, meso-diaminopimelic acid as the diagnostic diamino acid. This peptidoglycan type is characteristic of *Lentibacillus* species (Yoon *et al.*, 2002). The major polar lipids of the test

Table 1. Cellular fatty acid compositions of strain BH260^T and related taxa

Strains: 1, strain BH260^T; 2, *L. salarius* KCTC 3911^T; 3, *L. salicampi* KCCM 41560^T; 4, *L. juripiscarius* JCM 12147^T; 5, *Virgibacillus marismortui* DSM 12325^T; 6, *V. carmonensis* LMG 20964^T; 7, *V. necropolis* DSM 14866^T. Data are from Jeon *et al.* (2005), Yoon *et al.* (2002), Namwong *et al.* (2005), Arahall *et al.* (1999, 2000), Heyrman *et al.* (2003) and this study. Data are expressed as percentages of total fatty acids; fatty acids representing less than 0.5 % are not included. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7
Straight-chain							
C _{16:0}	1.34	0.99	1.4	ND	1.1	1.13 ± 0.16	1.30
Branched							
iso-C _{14:0}	5.70	13.91	12.0	5–13	3.5	3.50 ± 0.36	2.95
iso-C _{15:0}	8.03	16.52	3.4	3–18	34.4	4.56 ± 0.74	4.18
anteiso-C _{15:0}	50.78	25.34	38.6	38–54	31.4	65.54 ± 1.40	71.50
iso-C _{16:0}	11.95	26.47	30.1	13–30	7.6	3.65 ± 0.36	3.63
iso-C _{17:0}	1.91	4.38	0.7	ND	7.6	ND	ND
anteiso-C _{17:0}	18.20	11.51	13.4	13–18	10.9	9.43 ± 0.28	9.33
Unsaturated							
C _{16:1ω7c}	1.47	ND	0.5	ND	1.0	5.01 ± 0.59	2.45
alcohol							

strain were diphosphatidylglycerol and phosphatidylglycerol. The genomic DNA G+C content of strain BH139^T was 44 mol%, a value that falls within the defined range for the genus *Lentibacillus* (Jeon *et al.*, 2005). The major fatty acid profile, the major lipoquinone, the cell-wall type and the DNA G+C content of strain BH260^T were typical of the genus *Lentibacillus* (Table 2).

Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BH260^T is located in a clade that includes the type strains of *L. salarius*, *L. juripiscarius* and *Lentibacillus salicampi* in the neighbour-joining analysis (Fig. 1). The topologies of phylogenetic trees obtained using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the tree constructed using neighbour-joining analysis (data not shown). The levels of 16S rRNA gene sequence similarity for strain BH260^T with respect to *Lentibacillus salsus* KCTC 3911^T, *L. juripiscarius*

JCM 12147^T and *L. salicampi* KCCM 41560^T were 96.0, 95.5 and 95.3 %, respectively – values that are generally sufficient to define a novel species (Rosselló-Mora & Amann, 2001; Stackebrandt *et al.*, 2002).

On the basis of the polyphasic taxonomic evidence, we classify strain BH260^T as a novel *Lentibacillus* species, for which the name *Lentibacillus lacisalsi* sp. nov. is proposed.

Description of *Lentibacillus lacisalsi* sp. nov.

Lentibacillus lacisalsi (la.ci.sal'si. L. masc. n. *lacus* lake; L. adj. *salsus* -a -um salted, salt; N.L. gen. n. *lacisalsi* of a salt lake).

Cells are approximately 0.4–0.6 µm wide and 1.2–3.0 µm long, strictly aerobic, motile rods with peritrichous flagella. Colonies are cream-coloured, low-convex and circular/slightly irregular on MA supplemented with 10 % (w/v) NaCl. Growth occurs at 15–40 °C (optimum, 30–32 °C),

Table 2. Characteristics of strain BH260^T and some other related species

Strains: 1, strain BH260^T; 2, *L. salarius* KCTC 3911^T; 3, *L. salicampi* KCCM 41560^T; 4, *L. juripiscarius* JCM 12147^T; 5, *V. marismortui* DSM 12325^T; 6, *V. carmonensis* LMG 20964^T; 7, *V. necropolis* DSM 14866^T. Data are from Jeon *et al.* (2005), Yoon *et al.* (2002), Namwong *et al.* (2005), Arahal *et al.* (1999, 2000), Heyman *et al.* (2003) and this study. Symbols: +, positive; –, negative; W, weak; NA, not available.

Characteristic	1	2	3	4	5	6	7
Sporangia							
Spore shape*	S	S/O	S/O	O	E	E(S)	E
Spore position†	T	T	T	T	T/ST	ST	C/ST
Motility	+	+	+	–	+	+	+
Temperature range (°C)	15–40	15–50	15–40	10–45	15–50	10–40	10–40
NaCl range (% w/v)	5–25	1–20	2–23	3–30	5–25	NA	NA
Oxidase	+	–	+	+	+	NA	NA
Nitrate reduction	+	+	NA	+	–	+	+
Hydrolysis							
Aesculin	–	+	–	NA	+	W	–
Casein	–	–	+	+	+	+	+
Tween 80	–	–	+	+	–	NA	NA
Urea	–	–	–	–	+	NA	NA
Acid production from:							
D-Glucose	–	+	–	+	+	–	W
D-Fructose	+	+	–	+	+	–	W
D-Mannitol	–	W	–	–	–	–	–
D-Ribose	+	+	–	+	NA	NA	+
D-Xylose	W	+	–	+	–	NA	+
Maltose	–	+	–	–	+	NA	NA
D-Trehalose	–	W	–	–	–	–	W
D-Arabinose	W	W	–	–	–	–	–
D-Mannose	–	+	–	–	–	–	W
Cell-wall type‡	<i>m</i> -DAP	NA	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	NA	NA
DNA G+C content (mol%)	44	43	44	43	40.7	38.9	37.3

*E, Ellipsoidal; S, spherical; O, oval.

†C, Central; T, terminal; ST, subterminal.

‡*m*-DAP, *meso*-Diaminopimelic acid.

Fig. 1. Neighbour-joining tree showing phylogenetic relationships based on 16S rRNA gene sequences of strain BH260^T and other related taxa. Bootstrap values are presented as percentages of 1000 replicates; only values above 50% are shown. *Brevibacillus brevis* JCM 2503^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

pH 7.0–9.5 (optimum, pH 8.0) and 5–25% (w/v) NaCl (optimum, 12–15%). Nitrate is reduced to nitrite. Hydrolysis of urea, L-tyrosine, hypoxanthine, casein, starch, Tween 80, aesculin and xanthine is not observed. Acids produced from D-fructose, D-ribose, D-xylose and L-arabinose but not from glycerol, D-glucose, maltose, D-trehalose, α -D-lactose, D-mannitol, D-mannose, rhamnose, adonitol, raffinose, arbutin, D-salicin or D-melibiose. The major isoprenoid quinone is MK-7. Cell wall contains *meso*-diaminopimelic acid (A1 γ type). The predominant polar lipids are phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}. The DNA G + C content is 44 mol% (by HPLC).

The type strain, BH260^T (= KCTC 3915^T = DSM 16462^T), was isolated from a salt lake in China.

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