Lentibacillus salis sp. nov., a moderately halophilic bacterium isolated from a salt lake

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A Gram-positive, moderately halophilic bacterium, designated strain BH113^T, was isolated from a salt lake located in Xinjiang Province, China. Cells of the strain were aerobic, spore-forming, motile rods with flagella. The organism grew optimally at 37 °C and pH 8.0 in the presence of 10 % (w/v) NaCl. A phylogenetic analysis based on comparisons of 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 between strain BH113^T and *Lentibacillus lacisalsi* KCTC 3915^T, *Lentibacillus juripiscarius* JCM 12147^T and *Lentibacillus kapialis* JCM 12580^T were 96.7, 96.3 and 96.1 %, respectively. The G+C content of the genomic DNA was 46.2 mol% and the major isoprenoid quinone was MK-7. The peptidoglycan type was A1γ (*meso*-diaminopimelic acid) and the major polar lipids were phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids were anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. Therefore, on the basis of physiological, chemotaxonomic and molecular properties, strain BH113^T represents a novel species within the genus *Lentibacillus*, for which the name *Lentibacillus salis* sp. nov. is proposed. The type strain is BH113^T (=KCTC 3936^T =DSM 16817^T).

Moderately halophilic, aerobic, Gram-positive rods are taxonomically diverse and have been isolated from various salty environments and related habitats, including solar salterns, saline soils and soda lakes (Oren, 2002). They are a diverse group of bacteria belonging to the genera *Bacillus* (Ventosa *et al.*, 1989, 1998), *Amphibacillus* (Niimura *et al.*, 1990), *Halobacillus* (Spring *et al.*, 1996), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Gracilibacillus* (Wainø *et al.*, 1999), *Filobacillus* (Schlesner *et al.*, 2001), *Jeotgalibacillus*, *Marinibacillus* (Yoon *et al.*, 2001), *Oceanobacillus* (Lu *et al.*, 2001), *Lentibacillus* (Yoon *et al.*, 2002), *Paraliobacillus* (Ishikawa *et al.*, 2002), *Marinilactobacillus* (Ishikawa *et al.*, 2003), *Cerasibacillus* (Nakamura *et al.*, 2004), *Tenuibacillus* (Ren & Zhou, 2005a), *Pontibacillus* (Lim *et al.*, 2005a), *Salinibacillus* (Ren & Zhou, 2005b), *Thalassobacillus* (García *et al.*, 2005), *Alkalibacillus* (Jeon *et al.*, 2005b), *Halolactibacillus* (Ishikawa *et al.*, 2005), *Caldalkalibacillus* (Xue *et al.*, 2006), *Paucisalibacillus* (Nunes *et al.*, 2006), *Terribacillus* (An *et al.*, 2007), *Halalkalibacillus* (Echigo *et al.*, 2007), *Piscibacillus* (Tanasupawat *et al.*, 2007), *Pelagibacillus* (Kim *et al.*, 2007) and *Salsuginibacillus* (Carrasco *et al.*, 2007). Recently, the moderately halophilic species *Bacillus halophilus* (Ventosa *et al.*, 1989) was reclassified within a novel genus designated *Salimicrobium* (Yoon *et al.*, 2007).

The genus *Lentibacillus* includes Gram-variable rods able to produce spherical or oval endospores. Colonies are cream-coloured, catalase- and oxidase-positive and urease-negative. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid, the predominant menaquinone is MK-7, the major polar lipids are diphosphatidylglycerol and phosphatidylglycerol, the major fatty acids are anteiso-$C_{15:0}$ and iso-$C_{16:0}$ and the G+C content of the DNA is in the range 42–49 mol%. The genus *Lentibacillus* contains eight species, *Lentibacillus salicampi* (the type species) (Yoon *et al.*, 2002), *L. juripiscarius* (Namwong *et al.*, 2005), *L. salarius* (Jeon *et al.*, 2005a), *L. lacisalsi* (Lim *et al.*, 2005b), *L. halophilus* (Tanasupawat *et al.*, 2006), *L. kapialis* (Pakdeeto *et al.*, 2007), *L. halodurans* (Yuan *et al.*, 2007) and *L. salinarum* (Lee *et al.*, 2008). In this study, a novel Gram-positive bacterium, strain BH113^T, belonging to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH113^T is AY762976.

genus *Lentibacillus* was isolated from the soil of a salt lake in Xinjiang Province, China, and its taxonomic status was established using phenotypic and chemotaxonomic properties and 16S rRNA gene sequence analysis.

During the screening of halophilic bacteria, strain BH113^T was isolated from a soil sample collected in the summer of 2002 from the shore of Ayakekum salt lake (37° 34′ N 89° 39′ E; 3879 m altitude) located in the Altun mountain range natural reserve in Xinjiang Uygur Autonomous Region in the west of China. The pH of the soil was in the range 8.2–9.3. Soil samples were diluted serially with 1 % (w/v) saline solution and strain BH113^T was isolated on marine agar 2216 (MA; Difco) to which 10 % (w/v) NaCl had been added (final NaCl concentration: 11.94 %, w/v), at 35 °C for 3 days. Requirements for, and tolerance of, NaCl were determined in trypticase soy broth (TSB; containing the following, l^{-1}: 17.0 g casein, 3.0 g soybean meal, 2.5 g glucose, 5.0 g sodium chloride and 2.5 g dipotassium phosphate) supplemented with modified artificial seawater (ASW; containing the following, l^{-1}: 0–30 %, w/v, NaCl; 5.94 g $MgSO_4 . 7H_2O$; 4.53 g $MgCl_2 . 6H_2O$; 0.64 g KCl; 1.3 g $CaCl_2$). Optimum growth was tested at different temperatures (4–55 °C) on MA containing 10 % (w/v) NaCl and at different pH values (5.0–11.0) in TSB supplemented with ASW containing 10 % (w/v) NaCl. Anaerobic growth was determined in an anaerobic chamber ($H_2/CO_2/N_2$, 5 : 10 : 85; Mart Microbiology) at 37 °C for 5 days on MA containing 10 % (w/v) NaCl. Cell morphology and the flagellum type were studied using light microscopy and transmission electron microscopy as described by Lee *et al.* (2006). Motility was checked at 24 and 36 h in wet mounts by using a light microscope (E600; Nikon). The presence of endospores was determined using Schaeffer–Fulton stain (Smibert & Krieg, 1981).

Gram staining was performed 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 by assessing the oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine using a Bactident Oxidase strip (Merck). Nitrate reduction and hydrolysis of aesculin, casein, starch, Tween 80, urea, hypoxanthine, tyrosine and xanthine were determined on MA according to the methods described by Cowan & Steel (1965), Lányí (1987) and Smibert & Krieg (1994). Acid production from carbohydrates was tested as described by Leifson (1963); all suspension media were supplemented with ASW containing 10 % (w/v) NaCl.

On MA medium with 10 % (w/v) NaCl, strain BH113^T formed light-yellow, low-convex, circular colonies. It showed a moderately halophilic response, growing in TSB containing 5–15 % (w/v) NaCl; optimum growth occurred in TSB with 10 % (w/v) NaCl. Growth was not observed in the absence of NaCl. Growth was observed at temperatures between 20 and 45 °C, with optimum growth occurring at 37 °C. Strain BH113^T grew at pH 7.0–9.2 in TSB containing 10 % (w/v) NaCl; optimal growth was observed at pH 8.0. Cells of strain BH113^T from early and late growth phases showed Gram-positive reactions. Cells of the isolate were strictly aerobic, short rods, 0.4–0.6 μm wide and 0.8–2.5 μm long. Cells of the isolate produced single, spherical, terminal endospores in swollen sporangia. Cell motility occurred by means of peritrichous flagella. Anaerobic growth was not observed under anaerobic conditions after 5 days at 30 °C on MA with 10 % (w/v) NaCl. In Table 1, the phenotype of strain BH113^T is summarized and compared with those of the type strains of *Lentibacillus* species.

The 16S rRNA gene was amplified by using a PCR with the primers Eubac 27F and 1492R (DeLong, 1992) and the PCR products were purified using the QIAquick PCR purification kit (Qiagen). Sequencing of the purified 16S rRNA gene was performed using an ABI PRISM BigDye Terminator cycle sequencing kit, as recommended by the manufacturer (Applied Biosystems), and five primers (337F, 785F, 1225F, 518R and 1100R). The purified sequencing-reaction mixtures were electrophoresed automatically using an Applied Biosystems model 377 automatic DNA sequencer. The resultant 16S rRNA gene sequence was compared with 16S rRNA gene sequences available from GenBank by using the BLAST program and was aligned with closely related sequences by using CLUSTAL W software (Thompson *et al.*, 1994). Sequence similarity values were computed using SIMILARITY MATRIX, version 1.1 (Ribosomal Database Project II; http://rdp.cme.msu.edu/; Cole *et al.*, 2007). Gaps at the 5′- and 3′- ends of the alignment were omitted from further analysis. Phylogenetic trees were constructed using three different methods, the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms, available in PHYLIP version 3.6 (Felsenstein, 2002). Evolutionary distance matrices were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) 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.

The almost-complete 16S rRNA gene sequence (1518 nt) of strain BH113^T was obtained and used for an initial BLAST search in GenBank and phylogenetic analysis. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BH113^T formed a distinct line within the genus *Lentibacillus* and was located in a clade with the type strain of *L. lacisalsi* in the neighbour-joining analysis (Fig. 1). The topologies of the phylogenetic trees obtained using the maximum-likelihood and maximum-parsimony algorithms were similar to that obtained using the neighbour-joining analysis, i.e. strain BH113^T was positioned in a clade with the type strains of *L. juripiscarius*, *L. kapialis*, *L. salicampi*, *L. halophilus*, *L. salinarum*, *L. halodurans and L. salarius* (data not shown). The levels of 16S rRNA gene sequence similarity between strain BH113^T and *L. lacisalsi*

Table 1. Characteristics that serve to differentiate strain BH113^T from type strains of related members of the genus *Lentibacillus*

Strains: 1, BH113^T; 2, *L. lacisalsi* KCTC 3915^T (data from Lim *et al.*, 2005b); 3, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 4, *L. kapialis* JCM 12580^T (Pakdeeto *et al.*, 2007); 5, *L. salarius* KCTC 3911^T (Jeon *et al.*, 2005a); 6, *L. halodurans* DSM 18342^T (Yuan *et al.*, 2007); 7, *L. salicampi* JCM 11462^T (Yoon *et al.*, 2002); 8, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006); 9, *L. salinarum* AHS-1^T (Lee *et al.*, 2008). All strains were catalase-positive, urease-negative rods. +, Positive; −, negative; W, weak; NA, no data available; ai, anteiso; i, iso.

Characteristic	1	2	3	4	5	6	7	8	9
Spore shape	Spherical	Spherical	Oval	Spherical	Spherical/ oval	Spherical/oval	Spherical/ oval	Spherical	Oval
Pigmentation	Light yellow	−	−	Red	−	−	−	−	−
Motility	+	+	−	−	+	−	+	+	+
Colony diameter (mm)	0.2–0.3	NA	0.9–3.9	1.2–3.0	NA	4–5	1.0–2.0	0.2–0.6	0.5–1.2
Cell size (μm)	0.4–0.6 × 0.8–2.5	0.4–0.6 × 1.2–3.0	0.4–0.5 × 1.5–6.0	0.2–0.4 × 0.8–2.5	0.2–0.3 × 1.5–3.0	0.5 × 1.5–2.5	0.4–0.7 × 2.0–4.0	0.4–0.6 × 1.0–3.0	0.7–1.2 × 2.0–4.0
Temperature for growth (°C)									
Range	20–45	15–40	10–45	15–45	15–50	22–45	15–40	15–42	15–45
Optimum	37	30–32	37	37	30–35	30	30	30–37	37–40
NaCl concentration for growth (%, w/v)									
Range	5–15	5–25	3–30	5–30	1–20	5–30	2–23	12–30	3–24
Optimum	10	12–15	10	15	12–14	8–12	4–8	20–26	10–12
pH for growth									
Range	7.0–9.2	7.0–9.5	5.0–9.0	5.0–9.0	6.0–8.0	6.0–9.0	6.0–8.0	6.0–8.0	6.0–9.5
Optimum	8.0	8.0	7.0	7.0	7.0–7.5	7.0–7.5	NA	7.0–7.5	6.5–7.0
Oxidase activity	+	+	+	+	−	+	+	+	+
Nitrate reduction	+	+	+	+	+	−	+	−	+
Hydrolysis of:									
Aesculin	−	−	−	−	+	−	−	−	+
Casein	−	−	+	−	−	−	+	−	−
Tween 80	−	−	+	−	−	−	+	−	−
Acid production from:									
L-Arabinose	−	+	−	−	+	NA	−	−	−
Cellobiose	−	NA	−	−	NA	−	W	−	−
D-Fructose	+	+	+	+	+	+	−	−	−
D-Galactose	−	NA	−	+	NA	−	W	−	W
D-Glucose	W	−	+	+	+	+	+	−	+
Lactose	−	−	−	−	+	−	−	−	−
Maltose	−	−	−	−	+	−	−	−	−
D-Mannitol	W	−	−	+	W	−	−	−	−
D-Mannose	−	−	−	W	+	+	W	−	−
D-Ribose	−	+	+	+	+	−	−	−	+
Salicin	−	−	−	−	−	−	W	−	−
Sucrose	−	NA	W	W	NA	−	−	−	−
Trehalose	+	−	−	−	W	−	−	−	−
D-Xylose	+	W	+	−	+	−	−	−	−
Major fatty acids	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, $C_{16:0}$
Polar lipids*	PG, DPG	PG, DPG	PG, DPG, GL	PG, DPG	PG, DPG	PG, DPG, GL	PG, DPG	PG, DPG, GL	PG, DPG, GL
DNA G+C content (mol%)	46.2	44	43.4	41.6	43	43.4	42.4	42.4	49.0

*PG, Phosphatidylglycerol; DPG, diphosphatidylglycerol; GL, unknown glycolipid(s).

KCTC 3915^T, *L. juripiscarius* JCM 12147^T, *L. kapialis* JCM 12580^T, *L. salarius* KCTC 3911^T, *L. halodurans* DSM 18342^T, *L. salinarum* AHS-1^T, *L. salicampi* JCM 11462^T and *L. halophilus* JCM 12149^T were 96.7, 96.3, 96.1, 96.0, 95.7, 95.7, 95.5 and 94.3 %, respectively. Furthermore, strain BH113^T showed 93.8–95.4 % 16S rRNA gene sequence similarity with respect to members of the genus *Virgibacillus*. These levels of sequence similarity were

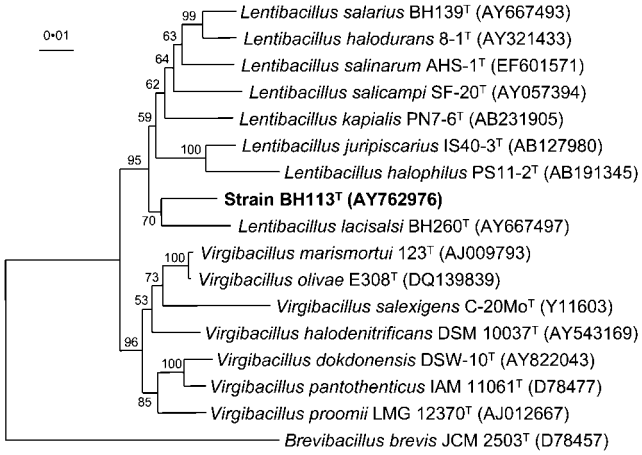

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain BH113^T and related taxa. Bootstrap percentages (based on 1000 replicates) are shown, where more than 50 %. Accession numbers are given in parentheses. *Brevibacillus brevis* JCM 2503^T was used as the outgroup. Bar, 0.01 changes per nucleotide position.

sufficient to indicate that strain BH113^T is a member of a novel species (Rosselló-Mora & Amann, 2001; Stackebrandt *et al.*, 2002).

GC analysis of fatty acid methyl esters was performed, starting with cells grown at 37 °C for 3 days on MA with 10 % (w/v) NaCl, according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Analyses of the peptidoglycan, polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The DNA G+C content of strain BH113^T was determined by using reversed-phase HPLC according to the method of Tamaoka & Komagata (1984).

The major isoprenoid quinone of strain BH113^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-$C_{15:0}$ (37.7 %), iso-$C_{16:0}$ (24.8 %) and anteiso-$C_{17:0}$ (15.0 %) as the major fatty acids, in common with the type strains of *Lentibacillus* species. Although this fatty acid profile was similar to that of *L. lacisalsi* KCTC 3915^T, which is currently the closest relative on the basis of 16S rRNA gene sequences, the fatty acid compositions of the two micro-organisms were somewhat different (Table 2). Analysis of the cell-wall peptidoglycan indicated that the diagnostic diamino acid of strain BH113^T was *meso*-diaminopimelic acid (A1γ type). This peptidoglycan type is characteristic of *Lentibacillus* species (Yoon *et al.*, 2002). The major polar lipids of the strain BH113^T were diphosphatidylglycerol and phosphatidylglycerol, as in *L. lacisalsi* KCTC 3915^T. The genomic DNA G+C content of strain BH113^T was 46.2 mol%. In their emended description of the genus *Lentibacillus*, Jeon *et al.* (2005a) gave a range of G+C content of 42–44 mol%. However, the genomic DNA G+C content of *L. salinarum* is 49 mol% (Lee *et al.*, 2008), suggesting a wider range of G+C content within the genus. The genomic DNA G+C content of strain BH113^T was therefore within this extended range for the genus *Lentibacillus*, and the other chemotaxonomic properties, including the major fatty acid profile, the major isoprenoid quinone and the cell-wall type, were typical of those of the genus *Lentibacillus* (Table 1).

Table 2. Cellular fatty acid compositions of strain BH113^T and type strains of related members of the genus *Lentibacillus*

Strains: 1, BH113^T; 2, *L. lacisalsi* KCTC 3915^T (data from Lim *et al.*, 2005b); 3, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 4, *L. kapialis* JCM 12580^T (Pakdeeto *et al.*, 2007); 5, *L. salarius* KCTC 3911^T (Jeon *et al.*, 2005a); 6, *L. halodurans* DSM 18342^T (Yuan *et al.*, 2007); 7, *L. salicampi* JCM 11462^T (Yoon *et al.*, 2002); 8, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006); 9, *L. salinarum* AHS-1^T (Lee *et al.*, 2008). –, Not detected or no data available.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated									
$C_{14:0}$	–	–	0.2	0.2	0.2	–	–	0.9	0.6
$C_{15:0}$	–	–	0.1	0.2	0.3	–	–	0.6	1.2
$C_{16:0}$	3.5	1.3	0.8	0.7	1.0	2.2	1.4	3.0	3.4
Unsaturated									
$C_{16:1}\omega7c$ alcohol	–	1.5	0.6	–	–	–	0.5	–	0.4
Branched									
iso-$C_{14:0}$	13.9	5.7	10.2	14.8	13.9	3.1	12.0	0.4	3.6
iso-$C_{15:0}$	3.6	8.0	4.9	6.9	16.5	20.7	3.4	3.1	1.6
anteiso-$C_{15:0}$	37.7	50.8	45.1	38.3	25.3	42.4	38.6	58.0	54.0
iso-$C_{16:0}$	24.8	12.0	20.2	23.4	26.5	7.7	30.1	1.9	7.2
iso-$C_{17:0}$	–	1.9	0.5	0.9	4.4	5.3	0.7	0.8	0.7
anteiso-$C_{17:0}$	15.0	18.2	16.7	14.1	11.5	17.2	13.4	27.5	27.0

Therefore, on the basis of the results of our polyphasic study, strain BH113^T represents a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus salis* sp. nov. is proposed.

Description of *Lentibacillus salis* sp. nov.

Lentibacillus salis (sal′is. L. gen. n. *salis* of salt).

Cells are approximately 0.4–0.6 μm wide and 0.8–2.5 μm long and are strictly aerobic, Gram-positive, motile rods with peritrichous flagella. Single, terminal, spherical endospores are formed in swollen sporangia. Colonies are light yellow, low-convex and circular on MA supplemented with 10 % (w/v) NaCl. Growth occurs at 20–45 °C (optimally at 37 °C), pH 7.0–9.2 (optimally at pH 8.0) and 5–15 % (w/v) NaCl (optimally at 10 %). Nitrate is reduced to nitrite. Hydrolysis of urea, L-tyrosine, hypoxanthine, casein, starch, Tween 80, aesculin, gelatin, DNA and xanthine is not observed. D-Glucose, trehalose, D-xylose, D-mannitol and D-fructose are utilized as carbon sources, D-ribose, rhamnose, glycerol, lactose, adonitol, arbutin and D-mannose are utilized weakly and maltose, D-arabinose, raffinose, salicin and melibiose are not utilized. Acids are produced from adonitol, arbutin, D-fructose, trehalose and D-xylose, produced weakly from D-glucose and D-mannitol and not produced at all from cellobiose, D-galactose, glycerol, lactose, maltose, D-mannose, melibiose, raffinose, D-ribose, salicin or sucrose. The major isoprenoid quinone is MK-7. The cell wall contains *meso*-diaminopimelic acid (A1γ type). The predominant polar lipids are phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The DNA G+C content of the type strain is 46.2 mol% (by HPLC).

The type strain, BH113^T (=KCTC 3936^T =DSM 16817^T), was isolated from the soil of Ayakekum salt lake in Xinjiang Province, China.

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