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

Jae-Chan Lee,¹ Wen-Jun Li,^{2,3} Li-Hua Xu,² Cheng-Lin Jiang² and Chang-Jin Kim¹

Correspondence
Chang-Jin Kim
changjin@kribb.re.kr

¹Functional Metabolomics Research Center, KRIBB, Daejeon 305-806, Republic of Korea

²The Key Laboratory for Microbial Resources of the Ministry of Education, PR China, and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China

³Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China

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), *Paucisolibacillus* (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 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 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 (H₂/CO₂/N₂, 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), Lányi (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. salarii* (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} ω7c 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 (A1 γ 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.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program (Ministry of Science and Technology, Republic of Korea) and by a grant from the KRIIBB Research Initiative Program.

References

- An, S.-Y., Asahara, M., Goto, K., Kasai, H. & Yokota, A. (2007). *Terribacillus saccharophilus* gen. nov., sp. nov. and *Terribacillus halophilus* sp. nov., spore-forming bacteria isolated from field soil in Japan. *Int J Syst Evol Microbiol* 57, 51–55.
- Carrasco, I. J., Márquez, M. C., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2007). *Salsuginibacillus kocurii* gen. nov., sp. nov., a moderately halophilic bacterium from soda-lake sediment. *Int J Syst Evol Microbiol* 57, 2381–2386.
- Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & Tiedje, J. M. (2007). The ribosomal database project (RDP-II):

introducing *myRDP* space and quality controlled public data. *Nucleic Acids Res* 35 (Database issue), D169–D172.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* 89, 5685–5689.

Echigo, A., Fukushima, T., Mizuki, T., Kamekura, M. & Usami, R. (2007). *Halalkalibacillus halophilus* gen. nov., sp. nov., a novel moderately halophilic and alkaliphilic bacterium isolated from a non-saline soil sample in Japan. *Int J Syst Evol Microbiol* 57, 1081–1085.

Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

García, M. T., Gallego, V., Ventosa, V. & Mellado, E. (2005). *Thalassobacillus devorans* gen. nov., sp. nov., a moderately halophilic, phenol-degrading, Gram-positive bacterium. *Int J Syst Evol Microbiol* 55, 1789–1795.

Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, G. & Logan, N. A. (1998). *Virgibacillus*: a new genus to accommodate *Bacillus panthothenticus* (Proom and Knight 1995). Emended description of *Virgibacillus panthothenticus*. *Int J Syst Bacteriol* 48, 99–106.

Ishikawa, M., Ishizaki, S., Yamamoto, Y. & Yamasato, K. (2002). *Paraliobacillus ryukyuensis* gen. nov., sp. nov., a new Gram-positive, slightly halophilic, extremely halotolerant, facultative anaerobe isolated from a decomposing marine alga. *J Gen Appl Microbiol* 48, 269–279.

Ishikawa, M., Nakajima, K., Yanagi, M., Yamamoto, Y. & Yamasato, K. (2003). *Marinilactibacillus psychrotolerans* gen. nov., sp. nov., a halophilic and alkaliphilic marine lactic acid bacterium isolated from marine organisms in temperate and subtropical areas of Japan. *Int J Syst Evol Microbiol* 53, 711–720.

Ishikawa, M., Nakajima, K., Itamiya, Y., Furukawa, S., Yamamoto, Y. & Yamasato, K. (2005). *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis* sp. nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a phylogenetic lineage in *Bacillus* rRNA group 1. *Int J Syst Evol Microbiol* 55, 2427–2439.

Jeon, C. O., Lim, J.-M., Lee, J.-C., Lee, G. S., Lee, J.-M., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2005a). *Lentibacillus salarius* sp. nov., isolated from saline sediment in China, and emended description of the genus *Lentibacillus*. *Int J Syst Evol Microbiol* 55, 1339–1343.

Jeon, C. O., Lim, J.-M., Lee, J.-M., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2005b). Reclassification of *Bacillus haloalkaliphilus* Fritze 1996 as *Alkalibacillus haloalkaliphilus* gen. nov., comb. nov. and the description of *Alkalibacillus salilacus* sp. nov., a novel halophilic bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* 55, 1891–1896.

Kim, Y.-G., Hwang, C. Y., Yoo, K. W., Moon, H. T., Yoon, J.-H. & Cho, B. C. (2007). *Pelagibacillus goriensis* gen. nov., sp. nov., a moderately halotolerant bacterium isolated from coastal water off the east coast of Korea. *Int J Syst Evol Microbiol* 57, 1554–1560.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–207.

Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 19, 1–67.

Lee, J. C., Lim, J. M., Park, D. J., Jeon, C. O., Li, W. J. & Kim, C. J. (2006). *Bacillus seohaeanensis* sp. nov., a halotolerant bacterium that contains L-lysine in its cell wall. *Int J Syst Evol Microbiol* 56, 1893–1898.

- Lee, S.-Y., Choi, W.-Y., Oh, T.-K. & Yoon, J.-H. (2008). *Lentibacillus salinarum* sp. nov., isolated from a marine solar saltern in Korea. *Int J Syst Evol Microbiol* **58**, 45–49.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Lim, J.-M., Jeon, C. O., Song, S. M. & Kim, C.-J. (2005a). *Pontibacillus chungwhensis* gen. nov., sp. nov., a moderately halophilic Gram-positive bacterium from a solar saltern in Korea. *Int J Syst Evol Microbiol* **55**, 165–170.
- Lim, J.-M., Jeon, C. O., Song, S. M., Lee, J.-C., Ju, Y. J., Xu, L. H., Jiang, C.-L. & Kim, C.-J. (2005b). *Lentibacillus lacisalsi* sp. nov., a moderately halophilic bacterium isolated from a saline lake in China. *Int J Syst Evol Microbiol* **55**, 1805–1809.
- Lu, J., Nogi, Y. & Takami, H. (2001). *Oceanobacillus iheyensis* gen. nov., sp. nov., a deep-sea extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya Ridge. *FEMS Microbiol Lett* **205**, 291–297.
- Nakamura, K., Haruta, S., Ueno, S., Ishii, M., Yokota, A. & Igarashi, Y. (2004). *Cerasibacillus quisquiliarum* gen. nov., sp. nov., isolated from a semi-continuous decomposing system of kitchen refuse. *Int J Syst Evol Microbiol* **54**, 1063–1069.
- Namwong, S., Tanasupawat, S., Smitnont, T., Visessanguan, W., Kudo, T. & Itoh, T. (2005). Isolation of *Lentibacillus salicampi* strains and *Lentibacillus juripiscarius* sp. nov. isolated from fish sauce in Thailand. *Int J Syst Evol Microbiol* **55**, 315–320.
- Niimura, Y., Koh, E., Yanagida, F., Suzuki, K., Komagata, K. & Kozaki, M. (1990). *Amphibacillus xylanus* gen. nov., sp. nov., a facultatively anaerobic sporeforming xylan-digesting bacterium which lacks cytochrome, quinone, and catalase. *Int J Syst Bacteriol* **40**, 297–301.
- Nunes, I., Tiago, I., Pires, A. L., da Costa, M. S. & Verissimo, A. (2006). *Paucisalibacillus globulus* gen. nov., sp. nov., a Gram-positive bacterium isolated from potting soil. *Int J Syst Evol Microbiol* **56**, 1841–1845.
- Oren, A. (2002). Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *J Ind Microbiol Biotechnol* **28**, 56–63.
- Pakdeeto, A., Tanasupawat, S., Thawai, C., Moonmangmee, S., Kudo, T. & Itoh, T. (2007). *Lentibacillus kapialis* sp. nov., from fermented shrimp paste in Thailand. *Int J Syst Evol Microbiol* **57**, 364–369.
- Ren, P. G. & Zhou, P. J. (2005a). *Tenuibacillus multivorans* gen. nov., sp. nov., a moderately halophilic bacterium isolated from saline soil in Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 95–99.
- Ren, P.-G. & Zhou, P.-J. (2005b). *Salinibacillus aidingensis* gen. nov., sp. nov. and *Salinibacillus kushneri* sp. nov., moderately halophilic bacteria isolated from a neutral saline lake in Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 949–953.
- Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.
- Schlesner, H., Lawson, P. A., Collins, M. D., Weiss, N., Wehmeyer, U., Völker, H. & Thomm, M. (2001). *Filobacillus milensis* gen. nov., sp. nov., a new halophilic spore-forming bacterium with Orn–D–Glu-type peptidoglycan. *Int J Syst Evol Microbiol* **51**, 425–431.
- Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Microbiology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Spring, S., Ludwig, W., Marquez, M. C., Ventosa, A. & Schleifer, K.-H. (1996). *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov., and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int J Syst Bacteriol* **46**, 492–496.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A., Kämpfer, P., Maiden, M. C., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tanasupawat, S., Pakdeeto, A., Namwong, S., Thawai, C., Kudo, T. & Itoh, T. (2006). *Lentibacillus halophilus* sp. nov., from fish sauce in Thailand. *Int J Syst Evol Microbiol* **56**, 1859–1863.
- Tanasupawat, S., Namwong, S., Kudo, T. & Itoh, T. (2007). *Piscibacillus salipiscarius* gen. nov., sp. nov., a moderately halophilic bacterium from fermented fish (pla-ra) in Thailand. *Int J Syst Evol Microbiol* **57**, 1413–1417.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Ventosa, A., García, M. T., Kamekura, M., Onishi, H. & Ruiz-Berraquero, M. (1989). *Bacillus halophilus* sp. nov., a moderately halophilic *Bacillus* species. *Syst Appl Microbiol* **12**, 162–165.
- Ventosa, A., Nieto, J. J. & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.
- Wainø, M., Tindall, B. J., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.
- Xue, Y., Zhang, X., Zhou, C., Zhao, Y., Cowan, D. A., Heaphy, S., Grant, W. D., Jones, B. E., Ventosa, A. & Ma, Y. (2006). *Caldalkalibacillus thermarum* gen. nov., sp. nov., a novel alkalithermophilic bacterium from a hot spring in China. *Int J Syst Evol Microbiol* **56**, 1217–1221.
- Yoon, J.-H., Weiss, N., Lee, K.-C., Lee, I.-S., Kang, K. H. & Park, Y.-H. (2001). *Jeotgalibacillus alimentarius* gen. nov., sp. nov., a novel bacterium isolated from jeotgal with L-lysine in the cell wall, and reclassification of *Bacillus marinus* Rüger 1983 as *Marinibacillus marinus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 2087–2093.
- Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2002). *Lentibacillus salicampi* gen. nov., sp. nov., a moderately halophilic bacterium isolated from a salt field in Korea. *Int J Syst Evol Microbiol* **52**, 2043–2048.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2007). Reclassification of *Marinococcus albus* Hao et al. 1985 as *Salimicrobium album* gen. nov., comb. nov. and *Bacillus halophilus* Ventosa et al. 1990 as *Salimicrobium halophilum* comb. nov., and description of *Salimicrobium luteum* sp. nov. *Int J Syst Evol Microbiol* **57**, 2406–2411.
- Yuan, S., Ren, P., Liu, J., Xue, Y., Ma, Y. & Zhou, P. (2007). *Lentibacillus halodurans* sp. nov., a moderately halophilic bacterium isolated from a salt lake in Xin-Jiang, China. *Int J Syst Evol Microbiol* **57**, 485–488.