Gracilibacillus lacisalsi sp. nov., a halophilic Gram-positive bacterium from a salt lake in China

Che Ok Jeon,¹ Jee-Min Lim,² Ho Hee Jang,³ Dong-Jin Park,² Li-Hua Xu,⁴ Cheng-Lin Jiang⁴ and Chang-Jin Kim²

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

¹Department of Life Science, Chung-Ang University, Seoul 156-756, Republic of Korea

²Korea Research Institute of Bioscience and Biotechnology, 52 Oeundong, Yusong, Daejeon 305-333, Republic of Korea

³Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea

⁴Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China

Two Gram-positive, motile, rod-shaped, halophilic bacteria showing optimum growth at 5–7 % (w/v) NaCl were isolated from a salt lake, Xin-Jiang Province, China. The strains, designated BH312^T and BH314, grew in the presence of 1–18 % (w/v) NaCl and at temperatures of 15–50 °C (optimum: 40 °C) and pH values of 5.5–10.0 (optimum: pH 7.5–8.0). The major cellular fatty acids were anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. The DNA G + C content was 38.8–39.0 mol% and the predominant lipoquinone was MK-7. The major cellular phospholipids were phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids. Phylogenetic analyses based on 16S rRNA gene sequences showed that the isolates formed a cluster with *Gracilibacillus orientalis* XH-63^T within the genus *Gracilibacillus*. The levels of 16S rRNA gene sequence similarity with closely related type strains were lower than 96.2 %. On the basis of phylogenetic, phenotypic and chemotaxonomic properties, the isolates represent a novel species of the genus *Gracilibacillus* for which the name *Gracilibacillus lacisalsi* sp. nov. is proposed. The type strain is BH312^T (=KCTC 13129^T=DSM 19029^T).

Moderately halophilic bacteria that grow optimally in media containing 3–15 % (w/v) NaCl are widely distributed in different marine environments and are made up of very heterogeneous physiological groups taxonomically, including both Gram-positive and Gram-negative microorganisms (Ventosa *et al.*, 1998; Oren, 2002; Jeon *et al.*, 2005a; Lim *et al.*, 2005). Aerobic, spore-forming, halophilic, Gram-positive rods that were originally assigned to the genus *Bacillus* are also very diverse taxonomically and they form several phylogenetically distinct lineages (Ash *et al.*, 1991; Stackebrandt & Liesack, 1993; Nielsen *et al.*, 1994; Jeon *et al.*, 2005b). Among them, the genus *Gracilibacillus* was first proposed by Wainø *et al.* (1999) to accommodate *Gracilibacillus halotolerans*, isolated from the Great Salt

Lake, Utah (USA), and *Gracilibacillus dipsosauri*, transferred from *Bacillus dipsosauri*. Currently, the genus includes only four species: *G. halotolerans*, *G. dipsosauri* (Wainø *et al.*, 1999), *G. orientalis* (Carrasco *et al.*, 2006) and *G. boracitololerans* (Ahmed *et al.*, 2007). In the course of screening sediment of Ai-Ding Lake, Xin-Jiang Province, China, to isolate halophilic bacteria, two aerobic, Gram-positive, moderately halophilic bacteria, strains BH312^T and BH314, were isolated. Ai-Ding Lake (42° 32' 10"–42° 49' 13" N 89° 10' 32"–89° 54' 32" E) is located in the most arid area of China, where evaporation is significantly higher than precipitation. The lake is the lowest point in China (154 m below sea-level) and has been a target area for the study of halophilic microorganisms for many years. Water is largely supplied from the nearby glacier of Tian-Shan Mountain. Ai-Ding Lake is a typical chloride-sulfate saline lake with a slightly alkaline pH (7.2–7.6) and a salt concentration of 20–26 % (w/v). The content of Mg²⁺ and Ca²⁺ in the brine is very low. On the basis of phylogenetic and phenotypic characteristics, strains BH312^T and BH314 have been classified as representatives of a novel species of the genus *Gracilibacillus*.

Samples were collected from sediment near the lake bank and packed in ice (0 °C) before transportation to the

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains BH312^T and BH314 are DQ664540 and DQ664541, respectively.

A transmission electron micrograph showing general morphology of negatively stained cells of strain BH312^T and thin-layer chromatograms of polar lipids from strain BH312^T and related strains are available as supplementary material with the online version of this paper.

laboratory. The samples were serially diluted using saline solution (0.9 %, w/v), spread on marine agar 2216 (MA; Difco) with the addition of 10 % (w/v) NaCl [final concentration of 11.94 % NaCl (w/v)] and incubated for 2 days at 35 °C. After isolation, the isolates were cultivated at 40 °C for 2 days in marine broth (MB; Difco) with the addition of 3 % (w/v) NaCl and stored at -80 °C in MB supplemented with 10 % (v/v) glycerol. Except where indicated, the isolates were routinely grown aerobically on MA with the addition of 3 % (w/v) NaCl for 2 days at 40 °C. NaCl requirements and tolerance were determined by using nutrient broth (NB: 3.0 g beef extract l⁻¹ and 5.0 g peptone l⁻¹) (Difco) with different concentrations (0–20 %, w/v) of NaCl. Optimum growth at different temperatures (5–55 °C at 5 °C intervals) and pH values (5.0–11.0 at 0.5 pH unit intervals) was tested in MB with the addition of 3 % (w/v) NaCl. Media with different pH values were prepared using appropriate biological buffers: Na₂HPO₄-NaH₂PO₄ buffer, Na₂CO₃-NaHCO₃ buffer and Na₂HPO₄-NaOH buffer were used for pH values below 8.0, pH 8.0–10.0 and pH 11.0, respectively (Gomori, 1955). Anaerobic growth was determined by incubation in an anaerobic chamber at 40 °C for 5 days on MA with the addition of 3 % (w/v) NaCl. Gram staining was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Catalase activity was determined by bubble production in 3 % (v/v) aqueous hydrogen peroxide solution. Oxidase activity was tested using a strip containing tetramethyl-*p*-phenylenediamine (Merck). Nitrate reduction and hydrolysis of starch, aesculin, urea, casein, Tween 80, hypoxanthine and xanthine were determined according to methods described previously (Lányi, 1987; Smibert & Krieg, 1994). Acid production from carbohydrates was determined as described by Leifson (1963); all suspension media were supplemented with 5 % (w/v) NaCl. Additional enzymic activities were determined using API ZYM (bioMérieux) at 40 °C as recommended by the manufacturer. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon *et al.* (2005a). For visualization of the flagella, cells were mounted on Formvar-coated copper grids (Electron Microscopy Science) and negatively stained with 2 % (w/v) uranyl acetate for 15 s, then subjected to transmission electron microscopy operating at 60 kV. Endospores were stained according to the method of Schaeffer-Fulton (Smibert & Krieg, 1994). Strains BH312^T and BH314 formed cream, smooth, convex and circular/slightly irregular colonies when grown on MA with the addition of 3 % (w/v) NaCl at 40 °C for 2 days. Cells of the isolates were motile, short rods with non-polar amphitrichous flagella after 2 days incubation at 40 °C (see Supplementary Fig. S1 in IJSEM Online). Strains BH312^T and BH314 showed the same phenotypic properties (Table 1), indicating that they may not be diverse physiologically.

For quantitative analysis of whole-cell fatty acids, strains BH312^T and BH314 were cultivated on MA containing 3.5

or 5.0 % (w/v) NaCl and on MH agar (Carrasco *et al.*, 2006) containing 10 % (w/v) NaCl for 2 days at 35 °C. GC analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Analyses of polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The DNA G+C content of the isolates was determined by reverse-phase HPLC (Hewlett Packard series II 1090) using the method of Tamaoka & Komagata (1984). The fatty acid profiles of the strains were characterized by the presence of saturated fatty acids such as anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0} as the major fatty acids. The fatty acid profiles of the isolates were similar to those of other related type strains in the genus *Gracilibacillus* (Table 2). The major isoprenoid quinone was menaquinone-7 (MK-7). Strain BH312^T contained phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids as the major polar lipids, but phosphatidylethanolamine was not detected in this strain, which had a similar polar lipid pattern to those of *Gracilibacillus halotolerans* NN^T and *Gracilibacillus dipsosauri* DD1^T (see Supplementary Fig. S2 in IJSEM Online). The genomic DNA G+C contents of strains BH312^T and BH314 were 39.0 and 38.8 mol%, respectively. The major fatty acid profile, the major lipoquinone and the major polar lipids of strains BH312^T and BH314 were typical of those of members of the genus *Gracilibacillus* (Wainø *et al.*, 1999; Carrasco *et al.*, 2006; Ahmed *et al.*, 2007). Typical phenotypic and chemotaxonomic properties of strain BH312^T and BH314 are compared with those of phylogenetically related relatives in Table 1. Some of them are in accordance with characteristics of members of the genus *Gracilibacillus*, whereas others enable the differentiation of strains BH312^T and BH314 from closely related *Gracilibacillus* species.

Sequencing of the 16S rRNA genes was carried out as described previously (Lane, 1991). The resulting 16S rRNA gene sequences of strains BH312^T and BH314 were compared with available 16S rRNA gene sequences from GenBank using the BLAST program (NCBI; <http://www.ncbi.nlm.nih.gov/BLAST/>) to determine the approximate phylogenetic affiliation. Gene sequences were aligned with those of closely related species by using the software CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different methods, i.e. neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Evolutionary distance matrices were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the NJ method. Using the FASTA3 program in EBI, 16S rRNA gene sequence comparisons were made between the novel strains and other members of the genus *Gracilibacillus* for similarity calculations. To evaluate the stability of the phylogenetic tree, bootstrap analysis (1000 replications) was performed with the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE in the PHYLIP package.

Table 1. Characteristics of *Gracilibacillus lacisalsi* sp. nov. and some related species

Species/strains: 1, *Gracilibacillus lacisalsi* sp. nov. (this study); 2, *Gracilibacillus boracitolterans* T-16X^T (Ahmed *et al.*, 2007); 3, *Gracilibacillus orientalis* XH-63^T (Carrasco *et al.*, 2006); 4, *Gracilibacillus dipsosauri* DD1^T (Wainø *et al.*, 1999); 5, *Gracilibacillus halotolerans* NN^T (Wainø *et al.*, 1999). +, Positive; −, negative; ND, no data. All species/strains are positive for acid production from D-glucose and D-mannitol.

Characteristic	1	2	3	4	5
Flagellation	Non-polar amphitrichous	Monotrichous	ND	ND	Peritrichous
Spore shape*	S	S/E	S	S	E
Spore position†	T	T/ST	T	T	T
Catalase	+	+	+	+\$	+
Oxidase	+	+	−	+	+
Nitrate reduction	+	−	−	+	+
Anaerobic growth	−	−\$	−	+	−
Hydrolysis of:					
Aesculin	+	+\$	+	+	+
Casein	−	−\$	−	−	−
Starch	−	−\$	+	+	+
Tween 80	−	−\$	−	−\$	+
Urea	−	−	−	−	+
Temperature range (°C)	15–50	16–37	4–45	28–50	6–50
Optimum growth temperature (°C)	40	25–28	37	35–37\$	47
NaCl range for growth (% w/v)	0.5–18	0–11	1–20	0–15\$	0–20
Optimum NaCl concentration for growth (% w/v)	5–7	0.5–3.0	10	2.0–3.0\$	0
Acid production from:					
Glycerol	+	−\$	+	+	ND
Maltose	+	+	+\$	+\$	ND
Major fatty acids	anteiso-C _{15:0} , iso-C _{15:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , iso- C _{15:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , anteiso-C _{17:0}
Major polar lipids‡	DPG, PG	DPG, PG	DPG, PG, PE	DPG, PG	DPG, PG
DNA G + C content (mol%)	38.8–39.0	35.8	37.1	39.4	38

*E, Ellipsoidal; S, spherical.

†T, Terminal; ST, subterminal.

‡DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

\$Data from this study.

DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain BH312^T and strain BH314 using the fluorometric microplate method (Ezaki *et al.*, 1989).

A tree constructed using the NJ method showed that strains BH312^T and BH314 formed a phyletic line with *G. orientalis* XH-63^T with a 66 % bootstrap value within the genus *Gracilibacillus* (Fig. 1). The overall tree topology of the NJ tree was supported by trees built using the ML and MP algorithms (data not shown). Comparative 16S rRNA gene sequence analysis showed that strain BH312^T was most closely related to *G. orientalis* XH-63^T, *G. boracitolterans* T-16X^T, *G. dipsosauri* DD1^T and *G. halotolerans* NN^T with similarities of 96.2, 95.5, 95.1 and 94.2 %, respectively, values that are generally sufficient to define a novel species (Rosselló-Mora & Amann, 2001; Stackebrandt *et al.*, 2002). The 16S rRNA gene sequence similarity between strains BH312^T and BH314 was 97.9 %. Therefore, DNA–DNA

hybridization was used to evaluate the genomic DNA relatedness between strains BH312^T and BH314; the value obtained was 83 %. It was concluded that the DNA–DNA relatedness values were sufficiently high for strains BH312^T and BH314 to be classified as members of a single species (Stackebrandt *et al.*, 2002). Phenotypic properties such as flagellation, spore shape and spore position also supported the view that the isolates were distinguishable from closely related *Gracilibacillus* species (Table 1). On the basis of the above results, it is proposed that strains BH312^T and BH314 should be placed in the genus *Gracilibacillus* as representatives of a novel species, for which the name *Gracilibacillus lacisalsi* sp. nov. is proposed.

Description of *Gracilibacillus lacisalsi* sp. nov.

Gracilibacillus 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).

Table 2. Fatty acid profiles (%) of strains BH312^T and BH314 (*Gracilibacillus lacisalsi* sp. nov.) and related type strains grown on various media

Strains: 1, BH312^T (this study); 2, BH314 (this study); 3, *Gracilibacillus boracitolterans* T-16X^T (Ahmed *et al.*, 2007); 4, *Gracilibacillus orientalis* XH-63^T (Carrasco *et al.*, 2006); 5, *Gracilibacillus dipsosauri* DD1^T (Wainø *et al.*, 1999); 6, *Gracilibacillus halotolerans* NN^T (Wainø *et al.*, 1999). tr, Trace amount; ND, not detected. Strains were grown as indicated on MA containing 3.5 or 5.0 % (w/v) NaCl and/or on MH agar (Carrasco *et al.*, 2006) containing 10.0 % (w/v) NaCl for 2 days at 35 °C.

Fatty acid	1			2		3	4	5	6
	3.5 % MA	5.0 % MA	MH	5.0 % MA	MH	3.5 % MA	MH	MH	MH
Straight-chain									
C _{14:0}	1.27	1.4	0.9	ND	0.91	0.8	ND	tr	tr
C _{15:0}	4.15	10.9	2.2	8.19	1.91	2.0	ND	tr	tr
C _{16:0}	10.96	9.8	12.1	7.94	16.30	5.6	4.9	7.21	9.05
C _{17:0}	2.25	4.4	1.6	4.81	0.76	tr	3.2	ND	ND
C _{18:0}	1.40	1.0	0.7	1.39	1.61	tr	ND	tr	tr
Branched									
iso-C _{13:0}	ND	0.5	ND	ND	ND	ND	ND	ND	ND
iso-C _{14:0}	1.14	1.1	ND	ND	ND	0.6	1.0	tr	8.96
iso-C _{15:0}	14.42	15.1	26.6	14.43	24.74	14.5	8.5	17.57	4.19
anteiso-C _{15:0}	39.34	36.7	28.3	39.42	27.66	50.8	51.9	39.12	56.15
iso-C _{16:0}	4.07	2.4	2.1	2.83	1.52	1.8	3.3	4.67	7.05
iso-C _{17:0}	3.25	2.5	7.2	3.10	6.68	2.0	2.4	7.25	ND
anteiso-C _{17:0}	17.39	12.3	17.3	16.51	16.56	18.4	20.6	24.19	14.59
iso-C _{11:0} 3-OH	ND	0.6	ND	ND	ND	ND	ND	ND	ND
Unsaturated									
C _{16:1} ω11c	tr	ND	1.0	1.37	0.97	tr	1.89	ND	ND

Cells form cream, smooth, convex and circular/slightly irregular colonies. Cells are Gram-positive, spore-forming, strictly aerobic rods (approximately 0.2–1.0 µm wide and 1.0–2.2 µm long) that are motile by means of non-polar amphitrichous flagella. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. A spherical endospore is formed terminally in swollen sporangium. Growth occurs at 15–50 °C (optimum: 40 °C), pH 5.5–10.0 (optimum:

pH 7.5–8.0) and in 1–18 % (w/v) NaCl (optimum: 5–7 %). Aesculin is hydrolysed. Hydrolysis of casein, starch, hypoxanthine, Tween 80, xanthine and urea is not observed. Acids are produced from D-glucose, galactose, sucrose, melibiose, maltose, raffinose, D-fructose, D-mannitol, D-mannose, glycerol, L-arabinose and α-D-lactose, but not from inositol. Produces esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-glucosidase, but not alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. Leucine arylamidase and β-glucosidase activities are weak. Major isoprenoid quinone is MK-7. Predominant cellular fatty acids are anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. Contains phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids. DNA G + C content is 38.8–39.0 mol% (HPLC).

The type strain is BH312^T (=KCTC 13129^T=DSM 19029^T), isolated from Ai-Ding Lake, Xin-Jiang Province, China.

Fig. 1. Neighbour-joining tree showing phylogenetic relationships based on 16S rRNA gene sequences of strains BH312^T and BH314 and other related taxa. Bootstrap values (percentages of 1000 replicates) greater than 50 % are shown. *Brevibacillus brevis* JCM 2503^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, and by a grant from the hub network establishment for security of foreign biological resources of the global joint R&D program, Republic of Korea.

References

- Ahmed, I., Yokota, A. & Fujiwara, T. (2007). *Gracilibacillus boracitolerans* sp. nov., a highly boron-tolerant and moderately halotolerant bacterium isolated from soil. *Int J Syst Evol Microbiol* **57**, 796–802.
- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* as revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.
- Carrasco, I. J., Márquez, M. C., Yanfen, X., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2006). *Gracilibacillus orientalis* sp. nov., a novel moderately halophilic bacterium isolated from a salt lake in Inner Mongolia, China. *Int J Syst Evol Microbiol* **56**, 599–604.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (2002). PHYLIP (phylogeny inference package) version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.
- Jeon, C. O., Lim, J.-M., Lee, J.-M., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2005a). 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.
- Jeon, C. O., Lim, J.-M., Lee, J.-C., Lee, G. S., Lee, J.-M., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2005b). *Lentibacillus salarii* sp. nov., isolated from saline sediment in China, and emended description of the genus *Lentibacillus*. *Int J Syst Evol Microbiol* **55**, 1339–1343.
- 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.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- 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. (2005). *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.
- Nielsen, P., Rainey, F. A., Outtrup, H., Priest, F. G. & Fritze, D. (1994). Comparative 16S rDNA sequence analysis of some alkaliphilic bacilli and the establishment of a sixth rRNA group within the genus *Bacillus*. *FEMS Microbiol Lett* **117**, 61–66.
- Oren, A. (2002). Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *J Ind Microbiol Biotechnol* **28**, 56–63.
- Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.
- 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.
- Stackebrandt, E. & Liesack, W. (1993). Nucleic acids and classification. In *Handbook of New Bacterial Systematics*, pp. 152–189. Edited by M. Goodfellow & A. G. O'Donnell. London: Academic Press.
- 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.
- 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., Márquez, M. C., Garabito, M. J. & Arahál, D. R. (1998). Moderately halophilic gram-positive bacterial diversity in hypersaline environments. *Extremophiles* **2**, 297–304.
- 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.