Gracilibacillus saliphilus sp. nov., a moderately halophilic bacterium isolated from a salt lake

Shu-Kun Tang,^{1†} Yun Wang,^{2†} Kai Lou,² Pei-Hong Mao,³ Xiang Jin,³ Cheng-Lin Jiang,¹ Li-Hua Xu¹ and Wen-Jun Li¹

Correspondence

Wen-Jun Li
wjli@ynu.edu.cn
or
liact@hotmail.com

¹The 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 650091, PR China

²Xinjiang Institute of Microbiology, Xinjiang Academy of Agricultural Science, Urumqi, Xinjiang 830091, PR China

³Institute of Ion Beam Biotechnology, College of Physics Science and Technology, Xinjiang University, Urumqi, Xinjiang 830008, PR China

A novel Gram-positive-staining, aerobic, spore-forming, rod-shaped bacterium, designated strain YIM 91119^T, was isolated from Ebinur Lake in Xinjiang Province, north-west China. Cells were motile, produced terminal endospores and grew at pH 6.0–8.0 (optimally at pH 7.0), 4–45 °C (optimally at 28–37 °C) and 1–22 % (w/v) NaCl, (optimally at 10–15 %, w/v). Comparative 16S rRNA gene sequence analysis showed that strain YIM 91119^T belongs to the genus *Gracilibacillus*, exhibiting the highest sequence similarity with respect to the type strain of *Gracilibacillus orientalis* (97.8 %); the next most similar 16S rRNA gene sequences were those of the type strains of *Gracilibacillus boracitolerans* (96.8 %), *Gracilibacillus dipsosauri* (96.5 %) and *Gracilibacillus halotolerans* (95.8 %). DNA–DNA hybridization with *G. orientalis* AS 1.4250^T showed a relatedness of 55 %. The major fatty acids of strain YIM 91119^T were anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. The peptidoglycan type was A1_γ (directly cross-linked meso-diaminopimelic acid). The genomic DNA G + C content was 40.1 mol% and the predominant respiratory quinone was MK-7. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. On the basis of the evidence from this polyphasic study, strain YIM 91119^T represents a novel species of the genus *Gracilibacillus*, for which the name *Gracilibacillus saliphilus* sp. nov. is proposed, with YIM 91119^T (=DSM 19802^T =CCTCC AA 208015^T) as the type strain.

Ebinur Lake in Xinjiang Uygur Autonomous Region in north-west China is one of four sandstorm sources in China. As the result of the climate having become drier, and also because of disturbance from human activities, the lake has shrunk dramatically and a large area of salt marsh has been exposed. Experts in various disciplines have been attracted to Ebinur Lake to conduct ecological research (Qian *et al.*, 2007). Recent studies have reported the isolation of many novel species from the lake, all of which are extremely halophilic archaea: *Natrinema versiforme* (Xin *et al.*, 2000), *Natronorubrum aibiense* (Cui *et al.*, 2006a), *Haloterrigena longa* and *Haloterrigena limicola* (Cui

et al., 2006b) and *Haloarcula amylolytica* (Yang *et al.*, 2007). The genus *Gracilibacillus* was established by Wainø *et al.* (1999). At the time of writing, there are four recognized species: *Gracilibacillus halotolerans* (Wainø *et al.*, 1999), *Gracilibacillus dipsosauri* (Lawson *et al.*, 1996; Wainø *et al.*, 1999), *Gracilibacillus orientalis* (Carrasco *et al.*, 2006) and *Gracilibacillus boracitolerans* (Ahmed *et al.*, 2007). The genus *Gracilibacillus* was described as comprising Gram-positive, halophilic or halotolerant bacteria containing MK-7 as the predominant isoprenoid quinone and meso-diaminopimelic acid as the diagnostic diamino acid. In the course of screening for halophilic bacteria, we isolated a moderate halophile, designated strain YIM 91119^T, from Ebinur Lake. On the basis of the data presented below, it is proposed that this strain represents a novel species of the genus *Gracilibacillus*.

Strain YIM 91119^T was isolated from a soil sample (GPS coordinates for sampling site: 45° 04' 16" N 82° 35' 24" E);

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 91119^T is EU784646.

The polar lipid and cellular fatty acid compositions of strain YIM 91119^T are available as supplementary material with the online version of this paper.

the pH was 7.2 and the major ions were Na⁺ (4.2 %), Mg²⁺ (0.2 %), Ca²⁺ (0.4 %), Cl⁻ (2.1 %) and SO₄²⁻ (3.0 %). For isolation, serial dilutions of the sample were spread on modified ISP5 medium (containing 1 g L-asparagine, 10 g glycerol, 5 g yeast extract, 1 g K₂HPO₄, 5 g KNO₃, 100 g NaCl and 15 g agar, per litre distilled water). Colonies were picked and repeatedly restreaked onto modified ISP5 medium, until purity was confirmed. After the strain had grown to late-exponential phase on modified ISP5 medium, its cell morphology and flagellation were investigated using a transmission electron microscope (H-800; Hitachi). The Gram reaction was determined using the KOH lysis method (Cerny, 1978). Cell motility was confirmed by the presence of turbidity throughout a tube containing semisolid medium (Leifson, 1960). Methyl red and Voges–Proskauer tests were performed as described previously (Smibert & Krieg, 1981). The temperature range for growth was determined by incubating cells for 1 week on modified ISP5 medium at the following temperatures: 0, 4, 10, 15, 20, 28, 37, 45 and 55 °C. The pH growth range was investigated between pH 4.0 and pH 10.0 (in increments of 1 pH unit), using the buffer system described by Xu *et al.* (2005). NaCl concentrations between 0 and 30 % (w/v) were tested, using modified ISP5 as the basal medium. Catalase activity

was determined by assessing bubble production after the addition of a drop of 3 % H₂O₂. Oxidase activity was determined by assessing the oxidation of tetramethyl-*p*-phenylenediamine. Hydrolysis of casein, starch and Tweens 40 and 80 were determined as described by Cowan & Steel (1965). Metabolic properties and enzyme activities were determined by means of the API 20E, API 50 CHB and API ZYM systems (bioMérieux) according to the manufacturer's instructions. The utilization of different compounds as sole carbon or nitrogen and energy sources was tested as described by Carrasco *et al.* (2006). The sensitivity of strain YIM 91119^T to antibiotics was tested using Sensi-Discs after incubation for 72 h on modified ISP5 medium. *G. orientalis* AS 1.4250^T (obtained from the China General Microbiological Culture Collection Center, Academia Sinica, Beijing) was used as a reference strain for phenotypic and DNA–DNA hybridization studies. The morphological, cultural and physiological properties of strain YIM 91119^T are given in Table 1 and in the species description.

Peptidoglycan was purified and the cell-wall amino acids and peptides in cell-wall hydrolysates were analysed by means of two-dimensional ascending TLC on cellulose plates with the solvent systems of Schleifer & Kandler

Table 1. Characteristics that serve to differentiate strain YIM 91119^T and closely related previously described species

Strains: 1, YIM 91119^T; 2, *G. orientalis* CCM 7326^T (data from Carrasco *et al.*, 2006); 3, *G. boracitolterans* T-16X^T (Ahmed *et al.*, 2007); 4, *G. dipsosauri* DSM 11125^T (Deutch, 1994; Lawson *et al.*, 1996); 5, *G. halotolerans* DSM 11805^T (Wainø *et al.*, 1999). All of the strains produce spores in a terminal position. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Pigmentation	Creamy white	Cream	Light pink to red	White	Creamy white
Spore shape*	S	S	S/E	S	E
NaCl concentration for growth (% w/v)					
Range	1–22	1–20	0–11	0–15	0–20
Optimum	10–15	10	0.5–3.0	3	0
pH range	6.0–8.0	5.0–9.0	6.0–9.7	ND	5.0–10.0
Temperature range (°C)	4–45	4–45	16–37	28–50	6–50
Nitrate reduction	+	–	–	+	+
Oxidase activity	+	–	+	+	+
H ₂ S production	–	–	–	–	+
Voges–Proskauer	+	ND	+	–	–
Hydrolysis of:					
Gelatin	–	+	–	+	+
Urea	+	–	–	–	+
Tween 80	–	–	ND	ND	+
Polar lipids†	DPG, PG, PE, PI, APL, PGL, GL	DPG, PG, PE, PL, APL	DPG, PG	DPG, PG, PL	DPG, PG, PL
Major fatty acid(s) (>10 %)	i-C _{15:0} , ai-C _{15:0} , ai-C _{17:0}	i-C _{15:0} , ai-C _{17:0}	i-C _{15:0} , ai-C _{15:0} , ai-C _{17:0}	i-C _{15:0} , ai-C _{15:0} , ai-C _{17:0}	ai-C _{15:0}
DNA G + C content (mol%)	40.1	37.1	35.8	39.4	38

*E, Ellipsoidal; S, spherical.

†APL, Aminophospholipid; DPG, diphosphatidylglycerol; GL, unknown glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PI, phosphatidylinositol; PL, phospholipid.

(1972). For fatty acid analysis, cells of strain YIM 91119^T were cultured on tryptic soy agar (Difco) containing 10 % NaCl at 37 °C for 48 h. Analysis of the cellular fatty acid pattern was performed as described by Sasser (1990), using the Microbial Identification System (MIDI). Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and were separated using HPLC (Kroppenstedt, 1982). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984). The peptidoglycan contained directly cross-linked *meso*-diaminopimelic acid, type A3 (peptidoglycan type A1_γ; http://www.dsmz.de/microorganisms/main.php?content_id=35), which is characteristic of the genus *Gracilibacillus*. The cellular fatty acid composition of strain YIM 91119^T was anteiso-C_{15:0} (32.9 %), iso-C_{15:0} (22.2 %), anteiso-C_{17:0} (16.5 %), C_{16:0} (7.2 %), iso-C_{17:0} (6.1 %), C_{15:0} (3.6 %) and C_{17:0} (3.0 %). The predominant menaquinone was menaquinone-7 (MK-7). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Supplementary Fig. S1, available in IJSEM Online). All of these features are consistent with the chemotaxonomic description of the genus *Gracilibacillus* (Wainø *et al.*, 1999).

Genomic DNA was extracted and purified according to the method described by Li *et al.* (2007). The 16S rRNA gene was amplified by means of a PCR with primers Eubac 27F and 1492R (DeLong, 1992) and the PCR products were purified using a Sangon PCR purification kit. The purified PCR product was sequenced using the ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 310; Applied Biosystems). The resulting sequence data were analysed with the GenBank programs BLASTN and BLASTX. Sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997) and the alignment was corrected manually. Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Phylogenetic analyses were performed using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A neighbour-

joining phylogenetic tree was constructed from K_{nuc} values (Kimura, 1980) by using MEGA, version 3.0 (Kumar *et al.*, 2004). The topology of the phylogenetic tree was evaluated using the bootstrap resampling method, with 1000 replicates (Felsenstein 1985).

An almost-complete 16S rRNA gene sequence (1501 bp) was obtained for strain YIM 91119^T. Fig. 1 shows the relationship between the novel strain and its closest phylogenetic relatives. The levels of 16S rRNA gene sequence similarity between strain YIM 91119^T and the type strains of species from the genus *Gracilibacillus* were as follows: 97.8 % (*G. orientalis*), 96.8 % (*G. boracitolterans*), 96.5 % (*G. dipsosauri*) and 95.8 % (*G. halotolerans*). In the neighbour-joining phylogenetic tree, strain YIM 91119^T clustered with *G. orientalis* XH-63^T (Fig. 1). The results of the 16S rRNA gene sequence comparisons clearly demonstrated that strain YIM 91119^T was a member of the genus *Gracilibacillus*. The topologies of the phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the neighbour-joining tree (data not shown).

The genomic G+C content of the DNA of strain YIM 91119^T was determined according to the method of Mesbah *et al.* (1989), using DNA from *Escherichia coli* K-12 as a control. DNA–DNA hybridization was performed fluorometrically using the method of Ezaki *et al.* (1989), with photobiotin-labelled DNA probes and microdilution wells. The genomic G+C content of the DNA of strain YIM 91119^T was 40.1 mol%. The genomic relatedness between strain YIM 91119^T and *G. orientalis* AS 1.4250^T was 55 %, which is lower than the cut-off point (70 %) recommended by Wayne *et al.* (1987) for assigning strains to the same species.

On the basis of the phenotypic (Table 1), chemotaxonomic (Table 1 and Supplementary Table S1), phylogenetic (Fig. 1) and DNA–DNA hybridization data indicating differences between the strain isolated from Lake Ebinur and previously described *Gracilibacillus* species, YIM 91119^T represents a novel species of the genus, for which we propose the name *Gracilibacillus saliphilus* sp. nov.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain YIM 91119^T with respect to previously described species. Numbers at branching points refer to bootstrap percentages (based on 1000 resamplings); only values above 50 % are shown. The sequence of *Bacillus subtilis* NCDO 1769^T was used as an outgroup. Bar, 1 % sequence divergence.

Description of *Gracilibacillus saliphilus* sp. nov.

Gracilibacillus saliphilus (sa.li'phi.lus. L. n. *sal*, *salis* salt; Gr. adj. *philos* loving; N.L. masc. adj. *saliphilus* salt-loving).

Cells are Gram-positive-staining, aerobic, thin rods, $0.7\text{--}0.9 \times 2.0\text{--}10.0\text{ }\mu\text{m}$ (Fig. 2). Cells are motile and spherical endospores are produced in a terminal position. Colonies are circular, creamy white, convex, opaque and 0.5–1 mm in diameter on modified ISP5 medium. Moderately halophilic, growing over a wide range (1–22 %, w/v) of NaCl concentrations (optimally at 10–15 %, w/v). No growth occurs in the absence of NaCl. Growth occurs at 4–45 °C and pH 6.0–8.0; optimal growth occurs at 28–37 °C and pH 7.0. Positive for oxidase and catalase and in the methyl red and Voges–Proskauer tests. Indole and H₂S are not produced. Negative (in API 20E tests) for ONPG, lysine and ornithine decarboxylases, arginine dihydrolase and tryptophan deaminase. Nitrate is reduced. Aesculin, gelatin, starch and urea are hydrolysed, but casein, gelatin and Tweens 40 and 80 are not hydrolysed. Acids are produced (in API 50 CHB tests) from *N*-acetylglucosamine, aesculin, amygdalin, D- and L-arabinose, arbutin, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, methyl α -D-glucopyranoside, D-glucose, glycerol, glycogen, lactose, D-lyxose, maltose, D-mannose, D-mannitol, melibiose, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, raffinose, L-rhamnose, D-ribose, sucrose, salicin, L-sorbose, starch, D-tagatose, trehalose, turanose, methyl β -D-xylopyranoside and D-xylose. In the API ZYM system, results are positive for alkaline phosphatase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, β -galactosidase and β -glucosidase, but negative for α -chymotrypsin, cystine arylamidase, esterase (C4), α -galactosidase, α -glucosidase, β -glucuronidase, *N*-

acetyl- β -glucosaminidase, α -fucosidase, leucine arylamidase, lipase (C14), α -mannosidase, acid phosphatase, trypsin and valine arylamidase. L-Arginine, cellobiose, glycine and L-lysine are utilized as sole carbon, nitrogen and energy sources, but the following substrates are not: acetate, adenine, aspartic acid, citrate, dulcitol, L-histidine, hypoxanthine, inositol, propionate, L-proline, L-serine, L-threonine, L-tyrosine, xanthine and xylitol. Resistant to the following antibiotics: amikacin (30 μg), ampicillin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), novobiocin (30 μg), streptomycin (10 μg), tobramycin (10 μg) and vancomycin (30 μg). Susceptible to amoxicillin (10 μg), chloramphenicol (30 μg), lincomycin (2 μg), neomycin (10 μg), netilmicin sulfate (30 μg), norfloxacin (10 μg), penicillin (10 U), rifampicin (5 μg), trimethoprim sulfonamides (1.25 μg) and tetracycline (30 μg). The predominant isoprenoid quinone is MK-7. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-inositol, four unknown phosphoglycolipids, an unknown aminophospholipid and unknown glycolipids (Supplementary Fig. S1). The cellular fatty acid composition includes (in decreasing abundance) anteiso-C_{15:0}, iso-C_{15:0}, anteiso-C_{17:0}, C_{16:0}, iso-C_{17:0}, C_{15:0} and C_{17:0}. The DNA G + C content of the type strain is 40.1 mol%.

The type strain, YIM 91119^T (=DSM 19802^T =CCTCC AA 208015^T), was isolated from a saline soil collected from Ebinur Lake in Xinjiang Province, north-west China.

Acknowledgements

This research was supported by the National Basic Research Program of China (no. 2004CB719601), the National Natural Science Foundation of China (30600001, 30870005), the Open Project Program of the Extremophiles Lab. of Xinjiang (no. XJYS0203-2005-01) and the Youth Technological Innovation Foundation of Xinjiang Academy of Agricultural Science (no. 2007Q07). W.-J. L. was supported by the Program for New Century Excellent Talents in University.

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.
- 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.
- Cerny, G. (1978). Studies on the aminopeptidase test for the distinction of gram-negative from gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 113–122.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Cui, H.-L., Tohty, D., Feng, J., Zhou, P.-J. & Liu, S.-J. (2006a). *Natronorubrum aibiense* sp. nov., an extremely halophilic archaeon isolated from Aibi salt lake in Xin-Jiang, China, and emended

Fig. 2. Electron micrograph of cells of strain YIM 91119^T, showing rod-shaped cells. Bar, 500 nm.

- description of the genus *Natronorubrum*. *Int J Syst Evol Microbiol* **56**, 1515–1517.
- Cui, H.-L., Tohty, D., Zhou, P.-J. & Liu, S.-J. (2006b). *Haloterrigena longa* sp. nov. and *Haloterrigena limicola* sp. nov., extremely halophilic archaea isolated from a salt lake. *Int J Syst Evol Microbiol* **56**, 1837–1840.
- DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.
- Deutch, C. E. (1994). Characterization of a novel salt-tolerant *Bacillus* sp. from the nasal cavities of desert iguanas. *FEMS Microbiol Lett* **121**, 55–60.
- 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. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- 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.
- Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* **5**, 2359–2367.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lawson, P. A., Deutch, C. E. & Collins, M. D. (1996). Phylogenetic characterization of a novel salt-tolerant *Bacillus* species: description of *Bacillus dipsosauri* sp. nov. *J Appl Bacteriol* **81**, 109–112.
- Leifson, E. (1960). *Atlas of Bacterial Flagellation*. London: Academic Press.
- Li, W.-J., Xu, P., Schumann, P., Zhang, Y.-Q., Pukall, R., Xu, L.-H., Stackebrandt, E. & Jiang, C.-L. (2007). *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Qian, Y.-B., Wu, Z.-N., Yang, Q., Zhang, L.-Y. & Wang, X.-Y. (2007). Ground-surface conditions of sand-dust event occurrences in the southern Junggar Basin of Xinjiang, China. *J Arid Environ* **70**, 49–62.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC News* **20**, 16.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, 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. Philips. Washington, DC: American Society for Microbiology.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- 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.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.
- Xu, P., Li, W.-J., Tang, S.-K., Zhang, Y.-Q., Chen, G.-Z., Chen, H.-H., Xu, L.-H. & Jiang, C.-L. (2005). *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family 'Oxalobacteraceae' isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.
- Yang, Y., Cui, H.-L., Zhou, P.-J. & Liu, S.-J. (2007). *Haloarcula amylytica* sp. nov., an extremely halophilic archaeon isolated from Aibi salt lake in Xin-Jiang, China. *Int J Syst Evol Microbiol* **57**, 103–106.