Terribacillus aidingensis sp. nov., a moderately halophilic bacterium

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Three Gram-positive, moderately halophilic bacteria, designated Y17-61^T, IA7 and DB2, were isolated from sediments of Aiding salt lake in the Xinjiang region of China. Cells of the strains were rod-shaped, motile by means of peritrichous flagella and produced ellipsoidal spores. Colonies were pale yellow in colour. The strains grew optimally at 30–37 °C, pH 6–7 and 3–7 % (w/v) NaCl. The diamino acid in the murein was *meso*-diaminopimelic acid and the major quinone system was MK-7. The major cellular fatty acids were anteiso-C_{15:0} and anteiso-C_{17:0}. The DNA G + C content was 44.6–45.0 mol%. 16S rRNA gene sequence analysis revealed that strains Y17-61^T, IA7 and DB2 were closely related to members of the genus *Terribacillus* and showed 96.8–97.6, 96.4–97.2 and 95.4–95.5 % 16S rRNA gene sequence similarity with *Terribacillus halophilus* 002-051^T, *Terribacillus saccharophilus* RB589 and *Terribacillus goriensis* CL-GR16^T, respectively. DNA–DNA relatedness among the isolates was 88–92 % and strain Y17-61^T shared 24, 18 and 18 % DNA–DNA relatedness with *T. halophilus* JCM 21760^T, *T. saccharophilus* JCM 21759^T and *T. goriensis* DSM 18252^T, respectively. On the basis of phenotypic and phylogenetic distinctiveness, the three isolates should be placed in the genus *Terribacillus* as representatives of a novel species, for which the name *Terribacillus aidingensis* sp. nov. is proposed. The type strain is Y17-61^T (=CGMCC 1.8913^T =NBRC 105790^T).

Gram-positive, rod-shaped, spore-forming, moderately halophilic bacteria have been isolated from various saline or hypersaline environments (Ventosa *et al.*, 1998; Kim *et al.*, 2007). They were originally placed in the genus *Bacillus* (Claus & Berkeley, 1986). Subsequently, molecular and chemotaxonomic analyses have shown that they form several phylogenetically distinct lineages within the family *Bacillaceae* and many new genera and novel species of this kind of bacteria have been reported. To date, the spore-forming, moderately halophilic bacteria that have been described include *Halobacillus* (Spring *et al.*, 1996; Chen *et al.*, 2009a), *Virgibacillus* (Heyndrickx *et al.*, 1998; Chen *et al.*,

2009b; Niederberger *et al.*, 2009), *Gracilibacillus* (Wainø *et al.*, 1999; Tang *et al.*, 2009), *Oceanobacillus* (Lu *et al.*, 2001; Namwong *et al.*, 2009), *Filobacillus* (Schlesner *et al.*, 2001), *Jeotgalibacillus* (Yoon *et al.*, 2001), *Marinibacillus* (Yoon *et al.*, 2001), *Lentibacillus* (Yoon *et al.*, 2002), *Paraliobacillus* (Ishikawa *et al.*, 2002), *Halolactibacillus* (Ishikawa *et al.*, 2005), *Pontibacillus* (Lim *et al.*, 2005; Chen *et al.*, 2009c), *Thalassobacillus* (García *et al.*, 2005), *Tenuibacillus* (Ren & Zhou, 2005a), *Salinibacillus* (Ren & Zhou, 2005b), *Salsuginibacillus* (Carrasco *et al.*, 2007a), *Piscibacillus* (Tanasupawat *et al.*, 2007), *Terribacillus* (An *et al.*, 2007), *Sediminibacillus* (Carrasco *et al.*, 2008), *Aquisalibacillus* (Márquez *et al.*, 2008) and *Bacillus* (Garabito *et al.*, 1997; Arahál *et al.*, 1999; Carrasco *et al.*, 2007b; Xue *et al.*, 2008).

At present, the genus *Terribacillus* includes three species. *Terribacillus saccharophilus* and *Terribacillus halophilus* were isolated from field soil in Japan (An *et al.*, 2007), and *Terribacillus goriensis* DSM 18252^T was isolated from coastal water off the east coast of Korea (Kim *et al.*, 2007; Krishnamurthi & Chakrabarti, 2008). The main phenotypic

Abbreviation: *m*-DAP, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Y17-61^T, DB2 and IA7 are FJ386524, GQ304893 and GQ304894, respectively.

A micrograph showing a cell of strain Y17-61^T and a table of fatty acid compositions of strain Y17-61^T and its closest phylogenetic neighbours are available with the online version of this paper.

characteristics of the genus *Terribacillus* are the formation of ellipsoidal endospores, gelatin liquefaction and the absence of H₂S and indole production and nitrate reduction. The DNA G+C content is 43.0–46.0 mol%, the major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0} and MK-7 is the major component of the quinone system (An *et al.* 2007; Krishnamurthi & Chakrabarti, 2008).

Three moderately halophilic bacterial strains, designated YI7-61^T, IA7 and DB2, were isolated from sediments of Aiding salt lake in Xinjiang, China (42° 32'–49' 10"–13" N 89° 10'–54' 32" E). At the time of sampling, the sediments were 17 °C, 12.1–15.4 % (w/v) NaCl and pH 7.1–7.3. For isolation, the samples were suspended in sterilized water supplemented with 5 % NaCl, serially diluted and spread on plates of complex isolation and maintenance medium described previously (Liu *et al.*, 2005). For cultivation of the isolates for characterization, the isolation medium was adjusted to pH 7.0 and incubation was at 35 °C, unless indicated otherwise. Phylogenetic analysis of the 16S rRNA gene sequences showed that the isolates belonged to the genus *Terribacillus*. The reference strains *T. saccharophilus* JCM 21759^T, *T. halophilus* JCM 21760^T and *T. goriensis* DSM 18252^T were grown according to conditions described by An *et al.* (2007) and Kim *et al.* (2007), respectively.

To characterize the isolates phenotypically, standard tests were performed according to the proposed minimal standards for the description of aerobic, endospore-forming bacteria (Logan *et al.*, 2009). Cell morphology and motility were examined after cultivation for 16 h. Endospores were observed after 48 h using phase-contrast microscopy (Eclipse 50i; Nikon). Bacterial flagellation was observed using transmission electron microscopy (JEM-1230; JEOL). Gram reaction was determined according to the methods described by Doetsch (1981) and Gregersen (1978). Colony morphology was examined after 3 days at 35 °C on modified isolation medium (6 % NaCl, pH 7.0). Conditions for growth were determined in liquid isolation medium by monitoring the increase in optical density at 600 nm. Growth was tested with 0, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 21, 22, 23, 25 or 30 % (w/v) NaCl, at pH 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11, achieved using the appropriate biological buffers (pH 8.0, Na₂HPO₄/NaH₂PO₄; pH 9.0, Na₂CO₃/NaHCO₃; and pH 11.0, Na₂HPO₄/NaOH), as described by Gomori (1955), and at 4, 10, 15, 20, 25, 28, 30, 33, 35, 37, 40, 45, 50, 55 and 60 °C. The API 20E and API 50CHB systems (bioMérieux) were used to determine the physiological and biochemical characteristics, such as the Voges–Proskauer test, fermentation of 49 carbohydrates and hydrolysis of gelatin. All suspension media were supplemented with 5 % NaCl (w/v). Tests for catalase, urease and oxidase, hydrolysis of casein, starch, tyrosine, Tween 80 and aesculin, utilization of citrate, reduction of nitrate, Voges–Proskauer reaction, production of indole and H₂S and arginine dihydrolase, tryptophan deaminase, phenylalanine deaminase, lysine decarboxylase and ornithine decarboxylase activities were performed for the isolates and the reference strains according to the

conventional methods described by Dong & Cai (2001). Cells of strains YI7-61^T, IA7 and DB2 were motile, Gram-positive and rod-shaped. Non-swollen ellipsoidal endospores were formed. Catalase and β-galactosidase were negative. Urease and oxidase were positive. Starch could be hydrolysed. H₂S and indole were not produced. Nitrate was not reduced to nitrite. Gelatin was liquefied. The Voges–Proskauer test was negative. The results for the reference strains in this study were the same as those reported before except for total salt concentration for growth (Table 1). The morphological, physiological and biochemical characteristics are given in detail in Table 1, Supplementary Fig. S1, available in IJSEM Online, and the species description.

Table 1. Differential characteristics of *Terribacillus aidingensis* sp. nov. and its closest phylogenetic neighbours

Taxa: 1, *Terribacillus aidingensis* sp. nov. (*n*=3); 2, *T. saccharophilus* JCM 21759^T; 3, *T. halophilus* JCM 21760^T; 4, *T. goriensis* DSM 18252^T. Data were taken from this study unless otherwise indicated. C, Central; E, ellipsoidal; S, swollen; ST, subterminal; T, terminal; +, positive; –, negative; ND, no data available.

Characteristic	1	2	3	4
Motility	+	–	–	+*
Spore shape	E	E, S	E, S	E, S*
Spore position	C, ST	ST, T	ST, T	C, ST*
Voges–Proskauer test	–	+	+	–
Urease	+	–	–	–
Oxidase	+	–	–	–
Catalase	–	+	+	+
Growth with (% NaCl):				
Range	0.5–21	0–16	0–19	0–14*
Optimum	3–7	2–7	2–7	0–2*
Growth at (°C):				
Range	4–48	5–45	5–45	15–43*
Optimum	30–37	28–33	30–33	30–35*
Growth at (pH):				
Range	5–9	6–10	5–10	5.5–9*
Optimum	6–7	7–8	7–8	7.5*
Acid production from:				
Amygdalin	+	–	–	+
Fructose	+	+	+	–
Galactose	+	+	–	+
Glucose	+	+	–	+
Sucrose	+	+	–	+
Trehalose	+	+	–	+
Xylose	–	–	–	+
Hydrolysis of:				
Casein	+	+	–	+
Starch	+	–	–	–
Diamino acid in murein	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	ND*
DNA G+C content (mol%)	44.6–45.0	44.0	45.8	43.0*

*Data were taken from Kim *et al.* (2007) and Krishnamurthi & Chakrabarti (2008).

Biomass for chemotaxonomic analysis was harvested from cultures after incubation on complex medium at 35 °C for 18 h. The analysis of the cell-wall peptidoglycan was carried out using the method described by Schleifer & Kandler (1972) and Schleifer (1985). Cell-wall hydrolysates were separated by one-dimensional chromatography on micro-cellulose thin layers, using *T. halophilus* JCM 21760^T as a reference. Menaquinones were analysed as described by Collins (1985) using reversed-phase HPLC (HP-1050), using *T. halophilus* JCM 21760^T and *Arthrobacter nicotinovorans* CGMCC 1.1933^T as references. The fatty acid compositions of strain YI7-61^T, *T. halophilus* JCM 21760^T and *T. saccharophilus* JCM 21759^T were determined under the same laboratory conditions for cells grown in complex medium supplemented with 5% (w/v) NaCl (pH 7.0) on a rotary shaker (200 r.p.m.) at 35 °C for 18 h, according to the manufacturer's instructions for the Microbial Identification System (MIDI). The diamino acid in the murein and the menaquinone of strain YI7-61^T were *meso*-diaminopimelic acid (*m*-DAP) and MK-7, respectively, which were the same as those of *T. halophilus* JCM 21760^T. The major cellular fatty acids of strains YI7-61^T, IA7 and DB2 were anteiso-C_{15:0} (67.9, 58.8 and 48.6%, respectively) and anteiso-C_{17:0} (19.4, 21.4 and 34.8%, respectively). Detailed cellular fatty acid compositions of strain YI7-61^T, *T. halophilus* JCM 21760^T, *T. saccharophilus* JCM 21759^T and *T. goriensis* DSM 18252^T are given in Supplementary Table S1.

Chromosomal DNA was extracted and purified according to standard methods (Marmur, 1961). The determination of DNA G + C content was carried out using the thermal denaturation method according to Marmur & Doty, (1962)

with a BIO-20 UV spectrophotometer. For the calculation of DNA G + C content, the equation of Owen & Hill (1979) was used. The DNA G + C contents of strains YI7-61^T, IA7 and DB2 were 45.0, 44.6 and 44.9 mol%, respectively. These results are within the emended G + C content range (43–46 mol%) for the genus *Terribacillus* Krishnamurthi & Chakrabarti (2008).

The 16S rRNA gene sequence was amplified as described by Duckworth *et al.* (1996). PCR products were sequenced with a DNA sequencer (ABI 373A; Applied Biosystems) and the software provided by the manufacturer. The almost-complete 16S rRNA gene sequences of strains YI7-61^T, IA7 and DB2 (1472 bp, 1464 bp and 1485 bp) were compared with sequences from public databases using the BLAST program via the National Center for Biotechnology Information. Pairwise sequence similarities were calculated using the BioEdit software package (Hall, 1999). Phylogenetic trees were reconstructed using MEGA version 3.1 (Kumar *et al.*, 2004). Distances were calculated by using distance options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining, maximum-parsimony and minimum-evolution methods. The stability of the relationships was assessed by 1000 bootstrap analyses. A neighbour-joining phylogenetic tree is shown in Fig. 1. The topologies of the trees generated with the minimum-evolution and maximum-parsimony methods were very similar to the neighbour-joining tree (data not shown). The phylogenetic analysis revealed that the three isolates belonged to the same phylogenetic lineage within the genus *Terribacillus*. 16S rRNA gene sequence similarities among the isolates

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between *Terribacillus aidingensis* sp. nov. and members of the genus *Terribacillus* and other closely related genera. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Bar, 0.01 substitutions per site.

were 98.5 % (YI7-61^T and IA7), 98.7 % (YI7-61^T and DB2) and 98.7 % (IA7 and DB2). Strains YI7-61^T, IA7 and DB2 showed 16S rRNA gene sequence similarities of 97.6, 96.8 and 96.9 % with *T. halophilus* 002-051^T, 97.2, 96.4 and 96.7 % with *T. saccharophilus* RB589 and 95.5, 95.4 and 95.4 % with *T. goriensis* CL-GR16^T, respectively. On the basis of 16S rRNA gene sequence analysis, it was evident that the novel isolates should be assigned to the genus *Terribacillus*.

To determine levels of DNA–DNA relatedness, extracted DNA was sheared by sonication (Braun Melsungen) at 50 W for three periods of 10 s, rehybridized in 2 × SSC at 70 °C and analysed spectrophotometrically (De Ley *et al.*, 1970; Huß *et al.*, 1983). DNA–DNA relatedness among the isolates was 88 % (YI7-61^T and IA7), 90 % (YI7-61^T and DB2) and 92 % (IA7 and DB2). Strain YI7-61^T showed DNA–DNA relatedness of 24, 18 and 18 % with *T. halophilus* JCM 21760^T, *T. saccharophilus* JCM 21759^T and *T. goriensis* DSM 18252^T, respectively. Since <70 % DNA–DNA relatedness is a key marker for the delineation of a novel species (Wayne *et al.*, 1987), these results showed that the three isolates belonged to the same species, which was different from the previously recognized members of the genus *Terribacillus*.

On the basis of morphological, physiological and chemotaxonomic characteristics, 16S rRNA gene sequence comparison and DNA–DNA relatedness, strains YI7-61^T, IA7 and DB2 should be treated as representatives of a novel species within the genus *Terribacillus*, for which the name *Terribacillus aidingensis* sp. nov. is proposed.

Description of *Terribacillus aidingensis* sp. nov.

Terribacillus aidingensis (ai.ding.en'sis. N.L. masc. adj. *aidingensis* from Aiding salt lake, where the type strain was isolated).

Cells are rod-shaped, 0.3–0.7 × 1.2–3.5 µm, Gram-positive, occurring singly, in pairs or in short chains, and motile by means of peritrichous flagella. Non-swollen ellipsoidal endospores are located mainly in a central position; subterminal endospores observed occasionally. Colonies on solid complex medium are circular, smooth, entire, slightly raised, pale yellow and 1–3 mm in diameter after 3 days. Growth occurs with 0.5–21 % (w/v) NaCl (optimum 3–7 % total salts), at 4–48 °C (optimum 30–37 °C) and at pH 5–9 (optimum pH 6–7). With API 20E and conventional methods, urease and oxidase are produced, but catalase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not. Tween 80, gelatin and casein are hydrolysed, but tyrosine is not. Nitrate is not reduced to nitrite. Citrate is not utilized. H₂S and indole are not produced. Voges–Proskauer reaction (acetoin) is negative. With API 50CHB, acid is produced from *N*-acetylglucosamine, aesculin, amygdalin, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, lactose, D-mannitol, melibiose, raffinose, trehalose, and sucrose,

but not from DL-arabinose, DL-fucose, D-lyxose, maltose, D-mannose, melezitose, L-rhamnose, D-ribose, L-sorbose, starch, D-tagatose, turanose, DL-xylose, gluconate, glycogen, inulin, salicin, D-adonitol, dulcitol, DL-arabitol, erythritol, glycerol, inositol, D-sorbitol, xylitol, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, 2-ketogluconate or 5-ketogluconate. The diamino acid in the murein is *m*-DAP and the menaquinone is MK-7. The major cellular fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. The DNA G+C content is 44.6–45.0 mol% and the DNA G+C content of the type strain is 45.0 mol%.

The type strain is YI7-61^T (=CGMCC 1.8913^T =NBRC 105790^T), isolated from the Aiding salt lake in Xinjiang, China.

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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.
- Arahal, D. R., Márquez, M. C., Volcani, B. E., Schleifer, K. H. & Ventosa, A. (1999). *Bacillus marismortui* sp. nov., a new moderately halophilic species from the Dead Sea. *Int J Syst Bacteriol* **49**, 521–530.
- Carrasco, I. J., Márquez, M. C., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2007a). *Salsuginibacillus kocurii* gen. nov., sp. nov., a moderately halophilic bacterium from soda-lake sediment. *Int J Syst Evol Microbiol* **57**, 2381–2386.
- Carrasco, I. J., Márquez, M. C., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2007b). *Bacillus chagannorensis* sp. nov., a moderate halophile from a soda lake in Inner Mongolia, China. *Int J Syst Evol Microbiol* **57**, 2084–2088.
- Carrasco, I. J., Márquez, M. C., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2008). *Sediminibacillus halophilus* gen. nov., sp. nov., a moderately halophilic, Gram-positive bacterium from a hypersaline lake. *Int J Syst Evol Microbiol* **58**, 1961–1967.
- Chen, Y.-G., Zhang, Y.-Q., Liu, Z.-X., Zhuang, D.-C., Klenk, H.-P., Tang, S.-K., Cui, X.-L. & Li, W.-J. (2009a). *Halobacillus salsuginis* sp. nov., a moderately halophilic bacterium from a subterranean brine. *Int J Syst Evol Microbiol* **59**, 2505–2509.
- Chen, Y.-G., Cui, X.-L., Wang, Y.-X., Zhang, Y.-Q., Tang, S.-K., Li, W.-J., Liu, Z.-X., Wen, M.-L. & Peng, Q. (2009b). *Virgibacillus sediminis* sp. nov., a moderately halophilic bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* **59**, 2058–2063.
- Chen, Y.-G., Zhang, Y.-Q., Xiao, H.-D., Liu, Z.-X., Yi, L.-B., Shi, J.-X., Zhi, X.-Y., Cui, X.-L. & Li, W.-J. (2009c). *Pontibacillus halophilus* sp. nov., a moderately halophilic bacterium isolated from a sea urchin. *Int J Syst Evol Microbiol* **59**, 1635–1639.
- Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

- Collins, M. D. (1985).** Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970).** The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Doetsch, R. N. (1981).** Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. H. Phillips. Washington, DC: American Society for Microbiology.
- Dong, X.-Z. & Cai, M.-Y. (editors) (2001).** Determination of biochemical properties. In *Manual for the Systematic Identification of General Bacteria*, pp. 370–398. Beijing: Science Press (in Chinese).
- Duckworth, A. W., Grant, W. D., Jones, B. E. & van Steenberg, R. (1996).** Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiol Ecol* **19**, 181–191.
- Garabito, M. J., Arahall, D. R., Mellado, E., Márquez, M. C. & Ventosa, A. (1997).** *Bacillus salexigens* sp. nov., a new moderately halophilic *Bacillus* species. *Int J Syst Bacteriol* **47**, 735–741.
- García, M. T., Gallego, V., Ventosa, A. & 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.
- Gomori, G. (1955).** Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.
- Gregersen, T. (1978).** Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.
- Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, C. & Logan, N. A. (1998).** *Virgibacillus*: a new genus to accommodate *Bacillus pantothenicus* (Proom and Knight 1950). Emended description of *Virgibacillus pantothenicus*. *Int J Syst Bacteriol* **48**, 99–106.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983).** Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- 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., Itaiya, 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.
- 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.
- Krishnamurthi, S. & Chakrabarti, T. (2008).** Proposal for transfer of *Pelagibacillus goriensis* Kim et al. 2007 to the genus *Terribacillus* as *Terribacillus goriensis* comb. nov. *Int J Syst Evol Microbiol* **58**, 2287–2291.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2004).** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- 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.
- Liu, W. Y., Zeng, J., Wang, L., Dou, Y. T. & Yang, S. S. (2005).** *Halobacillus dabanensis* sp. nov. and *Halobacillus aidingensis* sp. nov., isolated from salt lakes in Xinjiang, China. *Int J Syst Evol Microbiol* **55**, 1991–1996.
- Logan, N. A., Berge, O., Bishop, A. H., Busse, H.-J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Ventosa, A. & other authors (2009).** Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* **59**, 2114–2121.
- Lu, J., Nogi, Y. & Takami, H. (2001).** *Oceanobacillus ihayensis* 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.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Márquez, M. C., Carrasco, I. J., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2008).** *Aquisalibacillus elongatus* gen. nov., sp. nov., a moderately halophilic bacterium of the family *Bacillaceae* isolated from a saline lake. *Int J Syst Evol Microbiol* **58**, 1922–1926.
- Namwong, S., Tanasupawat, S., Lee, K. C. & Lee, J. S. (2009).** *Oceanobacillus kapialis* sp. nov., from fermented shrimp paste in Thailand. *Int J Syst Evol Microbiol* **59**, 2254–2259.
- Niederberger, T. D., Steven, B., Charvet, S., Barbier, B. & Whyte, L. G. (2009).** *Virgibacillus arcticus* sp. nov., a moderately halophilic, endospore-forming bacterium from permafrost in the Canadian high Arctic. *Int J Syst Evol Microbiol* **59**, 2219–2225.
- Owen, R. J. & Hill, L. R. (1979).** The estimation of base compositions, base pairing and genome size of bacterial deoxyribonucleic acids. In *Identification Methods for Microbiologists*, 2nd edn, pp. 217–296. Edited by F. A. Skinner & D. W. Lovelock. London: Academic Press.
- 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.
- Schleifer, K. H. (1985).** Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schlesner, H., Lawson, P. A., Collins, M. D., Weiss, N., Wehmeyer, U., Volker, 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.
- 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.

- 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.
- Tang, S.-K., Wang, Y., Lou, K., Mao, P.-H., Jin, X., Jiang, C.-L., Xu, L.-H. & Li, W.-J. (2009).** *Gracilibacillus saliphilus* sp. nov., a moderately halophilic bacterium isolated from a salt lake. *Int J Syst Evol Microbiol* **59**, 1620–1624.
- 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.
- 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.
- Xue, Y., Ventosa, A., Wang, X., Ren, P., Zhou, P. & Ma, Y. (2008).** *Bacillus aidingensis* sp. nov., a moderately halophilic bacterium isolated from Ai-Ding salt lake in China. *Int J Syst Evol Microbiol* **58**, 2828–2832.
- 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* Ruger 1983 as *Marinibacillus marinus* gen. nov., comb. nov. *Int J Syst Bacteriol* **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.