Halobacillus dabanensis sp. nov. and *Halobacillus aidingensis* sp. nov., isolated from salt lakes in Xinjiang, China

W. Y. Liu,^{1,2} J. Zeng,¹ L. Wang,¹ Y. T. Dou¹ and S. S. Yang¹

Correspondence

S. S. Yang

yangssh@cau.edu.cn

¹Department of Microbiology, College of Biological Sciences, China Agricultural University, Key Laboratory for Agro-Microbial Resource and Application, Ministry of Agriculture, Beijing, 100094, P. R. China

²Division of Mineral Resources, Metallurgy and Materials, General Research Institute for Nonferrous Metals, Beijing, 100088, P. R. China

Two moderately halophilic spore-forming bacteria were isolated from salt lakes in the Xinjiang region of China. The two strains, designated AD-6^T and D-8^T, were aerobic, Gram-positive, rod-shaped and motile by means of peritrichous flagella. Strains AD-6^T and D-8^T grew in the presence of 0.5–20% and 0.5–25% (w/v) NaCl in complex medium, respectively. Their cell-wall peptidoglycan was of the L-Orn-D-Asp type. The major menaquinone found in both strains was menaquinone-7 (MK-7). The fatty acid profile contained a large amount of branched fatty acids; the main fatty acids were anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0} and iso-C_{16:0}. The DNA G + C content of strains D-8^T and AD-6^T was 41.4 and 42.2 mol%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains D-8^T and AD-6^T were located in the genus *Halobacillus*. Levels of 16S rRNA gene sequence similarity between the isolated strains and the type strains of *Halobacillus* species were in the range 96.2–99.5%. DNA–DNA relatedness values of 17.0–52.2% were found between the two strains and other *Halobacillus* species. The DNA–DNA relatedness value between D-8^T and AD-6^T was 50.6%. On the basis of phenotypic and chemotaxonomic properties, phylogenetic analysis and genomic distinctiveness, strains D-8^T and AD-6^T should be placed in the genus *Halobacillus* as two novel species, for which the names *Halobacillus dabanensis* sp. nov. (type strain = JCM 12772^T = CGMCC 1.3704^T) and *Halobacillus aidingensis* sp. nov. (type strain = JCM 12771^T = CGMCC 1.3703^T) are proposed, respectively.

Moderately halophilic bacteria constitute a heterogeneous physiological group of micro-organisms consisting of different genera and species growing in saline environments (Ventosa *et al.*, 1998). The spore-forming moderately halophilic bacteria, an important group found mostly in marine or hypersaline environments, were formerly placed in the genus *Bacillus* (Claus & Berkeley, 1986). Based on 16S rRNA gene sequence and chemotaxonomic analysis, several phylogenetically distinct lineages in the genus *Bacillus* have been revealed (Ash *et al.*, 1991; Nielsen *et al.*, 1994; Priest, 1981) and subsequently many spore-forming bacteria have been placed in separate genera. Some moderately halophilic spore-forming bacteria were described as genus *Halobacillus* (Spring *et al.*, 1996). Initially, the genus *Halobacillus* was

proposed with two novel species, *Halobacillus litoralis* DSM 10405^T and *Halobacillus trueperi* DSM 10404^T. In addition, *Halobacillus halophilus* DSM 2266^T was transferred from *Sporosarcina halophila* (Claus *et al.*, 1983). Recently, the moderately halophilic bacterial strains HSL-3^T, MSS-155^T and MA-2^T were proposed as representatives of three novel species: *Halobacillus salinus* JCM 11546^T (Yoon *et al.*, 2003), *Halobacillus locisalis* KCCM 41687^T (Yoon *et al.*, 2004) and *Halobacillus karajensis* DSM 14948^T (Amoozegar *et al.*, 2003).

In order to investigate the moderately halophilic bacteria of China, we isolated some strains from the hypersaline waters and saline deposits of the Daban and Aiding salt lakes, which are located in the arid region of Xinjiang, China. Isolates D-8^T and AD-6^T were considered to be *Halobacillus*-like strains from the results of 16S rRNA gene sequence analysis. The aim of this study was to determine the exact taxonomic position of D-8^T and AD-6^T by using a polyphasic taxonomic approach, including the investigation of phenotypic

Published online ahead of print on 10 June 2005 as DOI 10.1099/ijs.0.63787-0.

The GenBank/EMBL/DDBJ accession numbers for the almost-complete 16S rRNA gene sequences of strains D-8^T and AD-6^T are AY351395 and AY351389, respectively.

characteristics, chemotaxonomic properties, DNA G + C content, DNA–DNA relatedness and a phylogenetic analysis.

Bacterial strains were isolated from the samples according to the method of Spring *et al.* (1996). The complex medium used for the isolation and maintenance of bacterial strains contained 5 g tryptone, 10 g yeast extract, 5 g Casamino acids, 3 g citrate sodium, 2 g KCl and 20 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ dissolved in 1 l of water (Xu *et al.*, 1995). If required, media were solidified by the addition of agar (15 g l^{-1}). The pH value was adjusted to 7.5. The sea water medium described by Spring *et al.* (1996) was used for the investigation of spore formation. Unless indicated, all tests were carried out in medium with 10% NaCl (w/v), at pH 7.5 and incubated at 35°C . The recommended media and conditions for growth were used to culture the reference strains, including *H. halophilus* DSM 2266^T, *H. litoralis* DSM 10405^T, *H. karajensis* DSM 14948^T, *H. salinus* JCM 11546^T, *H. locisalis* KCCM 41687^T and *H. trueperi* DSM 10404^T (Amoozegar *et al.*, 2003; Spring *et al.*, 1996; Yoon *et al.*, 2003, 2004).

To determine cell morphology, bacterial cultures were grown on complex medium plates for 16 h and then examined by phase-contrast microscopy. Photomicrographs of spores were obtained from cultures grown on sea water medium for 30 h. Transmission electron microscopy was used to observe bacterial flagellation. Motility was analysed by the hanging-drop method with cultures in the early exponential growth phase. The Gram reaction was determined by the KOH lysis method of Gregersen (1978). Colony morphology was examined after 3 days incubation on solid complex medium. The NaCl concentration for growth was determined in liquid media containing 0–30% (w/v) NaCl. The pH range for growth was determined by adjusting the pH to values between pH 4.0 and pH 11.0. The temperature range for growth was determined by incubation in liquid medium at temperatures between 5 and 60°C . Physiological and biochemical studies were carried out according to Quesada *et al.* (1984) and Ventosa *et al.* (1982).

The interpeptide bridge present in the cell-wall peptidoglycan was analysed using the method described by Schleifer & Kandler (1972). The preparation and hydrolysis of the cell wall were carried out according to Schleifer & Kandler (1972) and Schleifer (1985). Cell-wall hydrolysates were separated by one-dimensional chromatography on micro-cellulose thin layers using *H. litoralis* as a reference. Menaquinones were analysed as described previously (Komagata & Suzuki, 1987), using reverse-phase HPLC and employing *H. locisalis* as a reference. For quantitative determination of fatty acid composition, the fatty acid methyl ester mixtures were prepared and identified following the manufacturer's instructions for the Microbial Identification System (MIDI).

Chromosomal DNA was extracted and purified according to standard methods (Mellado *et al.*, 1996; Sambrook *et al.*, 1989). The 16S rRNA gene sequence was amplified as

described by Duckworth *et al.* (1996). PCR products were sequenced with a DNA Sequencer (373A; Applied Biosystems) using the software provided by the manufacturer. The almost-complete 16S rRNA gene sequences of strains D-8^T and AD-6^T were compared with sequences from the GenBank, EMBL, DDBJ and PDB databases using the MEGABLAST program (NCBI). Alignment of sequences was carried out using CLUSTAL W software (Thompson *et al.*, 1994). The phylogenetic tree was inferred by using the TREECON application. Evolutionary distance matrices for the neighbour-joining method were calculated with the algorithm of Kimura (1980) and the stability of the relationships was assessed by 1000 bootstrap analyses.

The DNA G + C content was determined by the thermal denaturation method using a BIO-20 UV spectrophotometer according to De Ley *et al.* (1970) and Huß *et al.* (1983). For the calculation of DNA G + C content, the equation of De Ley *et al.* (1970) was used and corrected using *Escherichia coli* strain K-12 as a reference. Levels of DNA–DNA relatedness were estimated spectrophotometrically (De Ley *et al.*, 1970; Huß *et al.*, 1983). DNA was sheared by sonication to generate fragments of $2\text{--}5 \times 10^5$ Da and renaturation was performed in $2 \times \text{SSC}$ at the optimum temperature.

Strains D-8^T and AD-6^T were Gram-positive and cells occurred singly, in pairs or in short chains. Under optimal conditions, cells of strain D-8^T were rods with round ends and measured $2.2\text{--}4.2 \times 0.6\text{--}0.9 \mu\text{m}$ (Fig. 1). Cells of strain AD-6^T were club-shaped rods, $1.0\text{--}2.8 \times 0.6\text{--}1.2 \mu\text{m}$ in size

Fig. 1. Phase-contrast micrographs of cells from exponentially growing cultures. (a) Strain AD-6^T. (b) Strain D-8^T. Bars, $5 \mu\text{m}$.

(Fig. 1). After incubation on sea water medium plates for 30 h, both strains formed endospores located at a central or subterminal position. Both strains were motile with peritrichous flagella. After incubation for 3 days, colonies of strain D-8^T were about 3–4 mm in diameter, twice as large as those of strain AD-6^T. Colonies of both strains were circular, smooth and slightly raised. Colonies of strain AD-6^T were orange. Colonies of strain D-8^T were initially cream coloured with orange pigment appearing after a few additional days incubation.

Strain D-8^T grew in medium containing 0.5–25 % (w/v) NaCl; optimal growth occurred at 10 % (w/v) NaCl. This strain grew at temperatures ranging from 15 to 50 °C with an optimum of 35 °C. Strain AD-6^T grew in medium containing 0.5–20 % (w/v) NaCl; optimal growth occurred at 10 % (w/v) NaCl. The temperature range for growth of this strain was 15–40 °C with an optimum of 32 °C. The optimal pH for both strains was 7.5. Growth was observed up to pH 10–11 but no growth occurred below pH 5.

Strains D-8^T and AD-6^T were aerobic and showed catalase, oxidase and DNase activities. For both strains, acid was produced from D-fructose, D-glucose, D-trehalose, D-mannitol, maltose and sucrose, but not from D-galactose.

Starch and casein were hydrolysed but no hydrolysis of tyrosine, aesculin or Tween 80 was observed. There were some minor differences between strains D-8^T and AD-6^T: acid was produced from D-xylose by strain D-8^T and gelatin was hydrolysed by strain AD-6^T (Table 1). From the results of the cell-wall analysis, the peptidoglycan type of the strains D-8^T and AD-6^T was determined as L-Orn–D-Asp. The predominant menaquinone found in the two strains was MK-7 as found in the type species of the genus, *H. locisalis*. The major branched fatty acids found in the two isolated strains were anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0} and iso-C_{16:0} (Table 2).

The almost-complete 16S rRNA nucleotide sequences for strains D-8^T and AD-6^T were submitted to GenBank under the accession numbers AY351395 and AY351389, respectively. Similarity searches were conducted within GenBank, EMBL, DDBJ and PDB to infer a possible phylogenetic classification for the isolated strains. The results showed that strains D-8^T and AD-6^T were most closely related to *Halobacillus* species. When the 16S rRNA gene sequences were compared using MEGABLAST (NCBI), strains D-8^T and AD-6^T showed high levels of similarity with *H. halophilus* DSM 2266^T (97.6 %, 96.2 %), *H. litoralis* DSM 10405^T (99.3 %, 98.0 %), *H. karajensis* DSM 14948^T (99.5 %, 98.2 %),

Table 1. Differential characteristics of strains D-8^T and AD-6^T and *Halobacillus* species

Species: 1, *H. halophilus* (data from Claus *et al.*, 1983); 2, *H. litoralis* (Spring *et al.*, 1996); 3, *H. trueperi* (Spring *et al.*, 1996); 4, *H. salinus* (Yoon *et al.*, 2003); 5, *H. karajensis* (Amoozegar *et al.*, 2003); 6, *H. locisalis* (Yoon *et al.*, 2004); 7, strain D-8^T; 8, strain AD-6^T. The species are peritrichously flagellated except for *H. locisalis* with a polar flagellum. All the strains are positive for catalase and oxidase and negative for urease and nitrate reduction. +, Positive reaction; –, negative reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Morphology	Coccoid	Rods	Rods	Rods	Rods	Rods	Rods	Clubs
Motility	+	+	+	+	–	+	+	+
Orange pigmentation	+	+	+	+	–	+	+	+
NaCl concentration for growth (% w/v):								
Range	2–20	0.5–25	0.5–30	0–23	1–24	>0–23	0.5–25	0.5–20
Optimum	10	10	10	2–10	10	10	10	10
Temperature range for growth (°C)	15–40	10–43	10–44	10–45	10–49	10–42	15–50	15–40
pH range for growth	7.0–9.0	6.0–9.5	6.0–9.5	ND	6.0–9.6	5.0–9.5	5.0–11.0	6.0–10.0
Acid production from:								
D-Fructose	–	+	+	+	+	+	+	+
D-Glucose	–	+	+	+	+	+	+	+
D-Galactose	–	–	+	+	–	–	–	–
D-Mannitol	–	+	+	+	+	–	+	+
Maltose	–	+	–	+	+	–	+	+
Sucrose	–	+	+	+	–	+	+	+
D-Trehalose	–	+	+	+	ND	+	+	+
D-Xylose	–	+	–	–	–	–	+	–
Hydrolysis of:								
Casein	+	–	–	+	+	–	+	+
Gelatin	+	+	+	+	+	–	–	+
Starch	+	–	–	+	+	+	+	+
DNA G+C content (mol%)	40.1–40.9	42.0	43.0	45.0	41.3	44	41.4	42.2

Table 2. Cellular fatty acid profiles of strains D-8^T and AD-6^T and *Halobacillus* species

Species: 1, *H. halophilus* (data from Yoon *et al.*, 2003); 2, *H. litoralis* (Yoon *et al.*, 2003); 3, *H. trueperi* (Yoon *et al.*, 2003); 4, *H. salinus* (Yoon *et al.*, 2003); 5, *H. karajensis* (Amoozegar *et al.*, 2003); 6, *H. locisalis* (Yoon *et al.*, 2004); 7, strain D-8^T; 8, strain AD-6^T. Strains D-8^T and AD-6^T were grown on complex medium for maintenance with 10% (w/v) NaCl at 35 °C for 2 days. Values are percentages of total fatty acids. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
C _{15:0}	1.5	1.1	ND	1.6	0.3	1.1	2.93	0.26
C _{16:0}	0.9	0.6	0.9	1.0	1.1	0.9	2.03	2.28
anteiso-C _{13:0}	0.4	0.4	ND	ND	ND	0.5	0.07	0.08
anteiso-C _{15:0}	47.3	57.0	25.3	31.7	42.4	42.0	49.7	42.04
anteiso-C _{17:0}	11.9	8.2	6.5	6.2	16.0	13.0	18.63	15.68
iso-C _{14:0}	12.2	6.4	21.7	9.4	2.0	11.2	1.10	2.34
iso-C _{15:0}	7.5	15.8	7.7	26.3	11.3	8.4	7.45	13.47
iso-C _{16:0}	15.2	5.4	31.5	15.7	6.9	15.9	8.15	7.47
iso-C _{17:0}	1.2	1.5	2.1	4.2	5.0	1.4	2.22	4.36
C _{16:1} ω7c alcohol	1.8	2.0	3.8	4.0	9.7	5.7	4.11	3.01
iso-C _{17:1} ω10c	ND	0.2	ND	ND	1.1	ND	0.35	1.42
C _{16:1} ω11c	ND	ND	ND	ND	0.9	ND	0.66	0.67

H. salinus JCM 11546^T (97.9%, 96.9%), *H. locisalis* KCCM 41687^T (98.6%, 97.6%) and *H. trueperi* DSM 10404^T (99.5%, 98.6%), respectively. Levels of 16S rRNA gene sequence similarity between the isolated strains and the type strains used in the construction of the phylogenetic tree were less than 95%. The phylogenetic tree was obtained using TREECON software. The phylogenetic analysis revealed that strains D-8^T and AD-6^T were members of the branch containing the genus *Halobacillus* (Fig. 2).

The DNA G + C content of strains D-8^T and AD-6^T was 41.4 and 42.2 mol%, respectively. DNA–DNA hybridization

tests were performed to determine the genotypic relatedness between the two novel strains and the type strains of *Halobacillus* species. Low levels of DNA–DNA relatedness were found between strains D-8^T and AD-6^T and *H. halophilus* DSM 2266^T (20.6%, 28%), *H. litoralis* DSM 10405^T (37%, 29.8%), *H. karajensis* DSM 14948^T (52.2%, 37.1%), *H. salinus* JCM 11546^T (21.1%, 17%), *H. locisalis* KCCM 41687^T (47%, 35.6%) and *H. trueperi* DSM 10404^T (34.9%, 45.4%), respectively. The DNA–DNA relatedness value between strains D-8^T and AD-6^T was 50.6%.

In conclusion, strains D-8^T and AD-6^T showed high levels of 16S rRNA gene sequence similarity to the type strains of *Halobacillus* species. Phylogenetic analysis revealed that these strains fall within the branch of the genus *Halobacillus*. Chemotaxonomic analyses indicated that the two strains have the same properties as other *Halobacillus* species (Amoozegar *et al.*, 2003; Spring *et al.*, 1996; Yoon *et al.*, 2003), although there are a few differences in cellular fatty acid content and some phenotypic properties. Thus, it is evident that the newly isolated strains are members of the genus *Halobacillus*.

Strains D-8^T and AD-6^T are similar to *Halobacillus* species in their morphological characteristics and in most of the physiological characteristics. However, there are some minor differences between the two strains and other *Halobacillus* species, including the range of temperatures and pH values for growth, tolerance of NaCl, the ability to hydrolyse some substrates and in acid production (Table 1). The fatty acid profiles of the two strains also differ from those of the reference strains (Table 2). DNA–DNA hybridization is deemed to be a key marker for the identification of a novel species (Wayne *et al.*, 1987). In the present study, levels of DNA–DNA hybridization were low enough to classify strains D-8^T and AD-6^T as separate species in the genus *Halobacillus*. On the basis of phenotypic, chemotaxonomic and genetic characteristics, it is proposed that strains D-8^T

Fig. 2. Neighbour-joining tree showing the phylogenetic position of strains D-8^T and AD-6^T, *Halobacillus* species and reference strains based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

and AD-6^T should be placed in the genus *Halobacillus* as novel species, with the names *Halobacillus dabanensis* sp. nov. and *Halobacillus aidingensis* sp. nov., respectively.

Description of *Halobacillus dabanensis* sp. nov.

Halobacillus dabanensis (da.ban.en'sis. N.L. masc. adj. *dabanensis* from Daban salt lake, where the type strain was isolated).

Cells are Gram-positive rods with round ends, 2.2–4.2 × 0.6–0.9 µm, occurring singly, in pairs or in short chains (Fig. 1). Cells are motile by means of peritrichous flagella. Ellipsoidal endospores are located at a central or subterminal position. Colonies are circular, smooth, entire, slightly raised and 3–4 mm in diameter after incubation for 3 days. Colonies are initially cream coloured but orange pigment is produced after a few additional days incubation. Growth occurs in 0.5–25 % (w/v) NaCl with 10 % (w/v) optimal for growth. The temperature range for growth is 15–50 °C; optimum is 35 °C. The pH range for growth is pH 5.0–11.0; optimum pH value is 7.5. Catalase, oxidase and DNase are produced, but urease is negative. Acid is produced from D-fructose, D-glucose, D-xylose, D-trehalose, D-mannitol, maltose and sucrose but not from D-galactose. Starch and casein are hydrolysed; tyrosine, aesculin, gelatin and Tween 80 are not hydrolysed. Voges–Proskauer test is negative. Nitrate is not reduced to nitrite. The cell-wall peptidoglycan is of the L-Orn–D-Asp type. The predominant menaquinone is MK-7. The major cellular fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0} and iso-C_{16:0}. The DNA G + C content is 41.4 mol%.

The type strain, D-8^T (=JCM 12772^T=CGMCC 1.3704^T), was isolated from the Daban salt lake in Xinjiang region, China.

Description of *Halobacillus aidingensis* sp. nov.

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

Cells are club-shaped rods, 1.0–2.8 × 0.6–1.2 µm. Gram-positive, occurring singly or in pairs (Fig. 1) and motile by means of peritrichous flagella. Ellipsoidal endospores are located at a central or subterminal position. Colonies are round, smooth, orange in colour, entire and 1–2 mm in diameter after incubation for 3 days. Growth occurs in 0.5–20 % (w/v) NaCl with an optimum concentration of 10 % (w/v) NaCl. The temperature range for growth is 15–40 °C; optimum temperature is 32 °C. Growth occurs at pH 6–10; optimum pH is 7.5. Acid is produced from D-fructose, D-glucose, D-trehalose, D-mannitol, maltose and sucrose but not from D-galactose and D-xylose. Catalase, oxidase and DNase are produced, but urease is negative. Starch, gelatin and casein are hydrolysed but tyrosine, aesculin and Tween 80 are not hydrolysed. Phosphatase, lecithinase, phenylalanine deaminase and arginine dihydrolase are not produced. The Voges–Proskauer test is

negative. Nitrate is not reduced to nitrite. The cell-wall peptidoglycan type is L-Orn–D-Asp. The predominant menaquinone is MK-7. The major cellular fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0} and iso-C_{16:0}. The DNA G + C content is 42.2 mol%.

The type strain, AD-6^T (=JCM 12771^T=CGMCC 1.3703^T), was isolated from Aiding salt lake in Xinjiang region, China.

Acknowledgements

We are grateful to Dr Carmen Marquez and Dr Jose Enrique Ruiz Sainz of University of Sevilla, Spain, for critical reading of the manuscript. This work was supported by the Chinese National Program for High Technology Research and Development (2003AA241150).

References

- Amoozegar, M. A., Malekzadeh, F., Malik, K. A. & Spröer, C. (2003). *Halobacillus karajensis* sp. nov., a novel moderate halophile. *Int J Syst Evol Microbiol* **53**, 1059–1063.
- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of genus *Bacillus* as revealed by comparative analysis of small-subunit-ribosomal RNA sequence. *Lett Appl Microbiol* **13**, 202–206.
- 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.
- Claus, D., Fahmy, F., Rolf, H. J. & Tosunoglu, N. (1983). *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. *Syst Appl Bacteriol* **4**, 496–506.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- 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.
- Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.
- HuB, V. A. R., Festal, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- 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). Lipids and cell wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Mellado, E., Moore, E. R. B., Nieto, J. J. & Ventosa, A. (1996). Analysis of 16S rRNA gene sequences of *Vibrio costicola* strains: description of *Salinivibrio costicola* gen. nov., comb. nov. *Int J Syst Bacteriol* **46**, 817–821.
- 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.
- Priest, F. G. (1981). DNA homology in genus *Bacillus*. In *The Aerobic Endospore-Forming Bacteria*, pp. 33–57. Edited by R. C. Berkeley & M. Goodfellow. London: Academic Press.

- Quesada, E., Ventosa, A., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1984).** *Deleya halophila*, a new species of moderately halophilic bacteria. *Int J Syst Bacteriol* **34**, 287–292.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- 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.
- 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.
- 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., Quesada, E., Rodriguez-Valera, F., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1982).** Numerical taxonomy of moderately halophilic Gram-negative rods. *J Gen Microbiol* **128**, 1959–1968.
- Ventosa, A., Nieto, J. J. & Oren, A. (1998).** Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.
- Xu, D. Q., Huang, J. J., Zhang, J. Z., Fan, Q. & Liu, D. L. (1995).** *Halomonas huanghaiensis* sp. nov., a new species of genus *Halomonas*. *Acta Microbiol Sin* **35**, 315–321 (in Chinese).
- Yoon, J.-H., Kang, K.-H. & Park, Y.-H. (2003).** *Halobacillus salinus* sp. nov., isolated from a salt lake on the coast of the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 687–693.
- Yoon, J.-H., Kang, K.-H., Oh, T. K. & Park, Y.-H. (2004).** *Halobacillus locisalis* sp. nov., a halophilic bacterium isolated from a marine solar saltern of the Yellow Sea in Korea. *Extremophiles* **8**, 23–28.