Thalassobacillus cyri sp. nov., a moderately halophilic Gram-positive bacterium from a hypersaline lake

C. Sanchez-Porro,¹ M. A. Amoozegar,² R. Rohban,³ M. Hajighasemi² and A. Ventosa¹

Correspondence

A. Ventosa
ventosa@us.es

¹Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain

²Extremophiles Lab; Department of Microbiology, Faculty of Biology, College of Science, University of Tehran, Tehran, Iran

³Department of Biology, Islamic Azad University, Science and Research Campus, Tehran, Iran

A Gram-positive, moderately halophilic bacterium, designated strain HS286^T, was isolated from water of the hypersaline Lake Howz-Soltan in Iran. Cells were strictly aerobic, rod-shaped, motile and able to produce ellipsoidal endospores at a central-subterminal position in swollen sporangia. Isolate HS286^T grew in a complex medium supplemented with 1–15 % (w/v) NaCl, with optimum growth at 8.0 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain HS286^T was closely related to *Thalassobacillus devorans* G-19.1^T (99.4 % gene sequence similarity). The other closest species were *Halobacillus yeomjeoni* MSS-402^T (96.9 %) and other species of the genus *Halobacillus* (with 96.7–93.5 % similarity). Strain HS286^T had cell-wall peptidoglycan based on *meso*-diaminopimelic acid and MK-7 as the respiratory isoprenoid quinone. The major fatty acids were anteiso-C_{15:0} (43.8 %), iso-C_{16:0} (21.4 %), iso-C_{14:0} (9.4 %), anteiso-C_{17:0} (8.7 %) and iso-C_{15:0} (7.0 %) and the polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, two phospholipids and a glycolipid. The DNA G + C content was 43.0 mol%. All of these features confirmed the placement of isolate HS286^T within the genus *Thalassobacillus*. However DNA–DNA hybridization between strain HS286^T and the only recognized species of the genus *Thalassobacillus*, *T. devorans* G-19.1^T, was 27.3 %, showing unequivocally that the novel isolate constituted a new genospecies. Strain HS286^T could be clearly differentiated from *T. devorans* and other phylogenetic neighbours on the basis of several phenotypic, genotypic and chemotaxonomic features. Therefore, strain HS286^T constitutes a novel species, for which the name *Thalassobacillus cyri* sp. nov. is proposed. The type strain is HS286^T (=CCM 7597^T=JCM 15722^T).

Moderately halophilic bacteria are defined as those that grow optimally in media containing 3–15 % (w/v) NaCl and such bacteria are widely distributed in hypersaline habitats (Ventosa *et al.*, 1998). They are represented by heterogeneous physiological and taxonomic groups including both Gram-negative and Gram-positive micro-organisms (Ventosa *et al.*, 1998; Ventosa, 2006). Among the Gram-positive examples, several aerobic, endospore-forming halophilic genera and species have been reported (Arahal & Ventosa, 2002; Ventosa, 2006). The genus *Thalassobacillus* was proposed by García *et al.* (2005) for Gram-positive, endospore-forming motile rods that are moderately halophilic and have cell-wall peptidoglycan of

type A1γ, with *meso*-diaminopimelic acid as the diagnostic diamino acid. The major fatty acids of members of this genus are anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0}, the predominant menaquinone is MK-7 and the G + C content of the DNA is 42.4 mol%. This genus is currently represented by a single species, *Thalassobacillus devorans*, which is able to degrade phenol and other aromatic compounds and was isolated from a saline soil in South Spain. Based on the comparison of the 16S rRNA gene sequence, this species is phylogenetically related to species of the genus *Halobacillus*; however, in contrast to *T. devorans*, a key characteristic of species of the genus *Halobacillus* is a cell-wall peptidoglycan type based on Orn-D-Asp (Spring *et al.*, 1996). The aim of the present study was to determine the taxonomic position of strain HS286^T, isolated from a hypersaline lake in Iran, by using a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HS286^T is FM864226.

polyphasic approach. The results presented here show that this isolate represents a novel species of the genus *Thalassobacillus*.

Strain HS286^T was isolated from water of the hypersaline lake Howz-Soltan, which is located near Qom city in the centre of Iran, with an extent of about 240 and 280 km² in dry and wet seasons, respectively. The depth of the salt layer, which covers almost all the surface of the lake bed (playa), varies between 20–46 m and the pH of the water, saline soil and salt sediments ranges between 6.5 and 8.2. The major chemical composition of the soil, brine, mud and salt consists of NaCl, KCl, MgSO₄, MgCl₂ and Na₂SO₄. At the time of sampling, the temperature of the water was 32 °C and the pH was 7.6. Strain HS286^T was isolated by diluting a water sample in sterile 10 % (w/v) salt solution, plating on 10 % HM medium and incubation at 37 °C aerobically. The 10 % HM medium contained (l⁻¹): 81 g NaCl, 7 g MgCl₂, 9.6 g MgSO₄, 0.36 g CaCl₂, 2 g KCl, 0.06 g NaHCO₃, 0.026 g NaBr, 5 g proteose-peptone no. 3 (Difco); 10 g yeast extract (Difco) and 1 g glucose (Ventosa *et al.*, 1982). The pH of this medium was adjusted to 7.5. The strain was subsequently purified three times by plating on the same medium and was maintained on the same HM medium and at –80 °C on HM medium without agar and supplemented with 30 % (v/v) glycerol.

In order to characterize strain HS286^T phenotypically, standard tests were performed. Cell morphology was examined by light microscopy (model CX31; Olympus) using cells from exponentially growing cultures. Gram staining was performed by the Burke method (Murray *et al.*, 1994) and the result was confirmed by the KOH test (Baron & Finegold, 1990). The presence of endospores was investigated by using the Schaeffer–Fulton staining method (Murray *et al.*, 1994). Motility was analysed by the wet-mount method (Murray *et al.*, 1994). Catalase, oxidase and urease activities, nitrate reduction, hydrolysis of aesculin, production of indole and methyl red and Voges–Proskauer reactions were tested as recommended by Smibert & Krieg (1994). Hydrolysis of Tween 80 was examined as described by Harrigan & McCance (1976). Determination of acid production from carbohydrates, as well as utilization of carbon and nitrogen sources, was performed as recommended by Ventosa *et al.* (1982). Antibiotic susceptibility tests were performed on plates of Mueller–Hinton agar plus 10 % (w/v) sea salts seeded with a bacterial suspension containing 1.5×10^6 c.f.u. ml⁻¹ using discs (HiMedia) impregnated with various antibiotics. The plates were incubated at 37 °C for 48 h and the inhibition zone was interpreted according to the manufacturer's manual. To determine the optimal temperature and pH for growth of the novel strain, broth cultures were incubated at temperatures of 10–50 °C at intervals of 5 °C and at pH 5–11 at intervals of 0.5 pH units. pH values below 6, pH 6–9 and pH values above 9 were obtained using sodium acetate/acetic acid, Tris/HCl and glycine/sodium hydroxide buffers, respectively. Growth at different salt concentrations (0, 1, 2.5, 5, 8, 10, 15, 20 and 25 %, w/v) was

tested on HM medium at pH 7.5. Growth was monitored by turbidity at OD₆₀₀ using a spectrophotometric method (model UV-160 A; Shimadzu). Other physiological and biochemical tests were performed as described previously (Mata *et al.*, 2002; Quesada *et al.*, 1984; Ventosa *et al.*, 1982).

Cells of strain HS286^T were Gram-positive, motile and strictly aerobic. They were able to produce ellipsoidal endospores at a central to subterminal position in swollen sporangia. The new isolate was moderately halophilic, showing optimal growth at 8 % (w/v) NaCl and being able to grow in media with 1–15 % (w/v) NaCl. It was not able to grow in media without NaCl. Other phenotypic features are included in Table 1 and are detailed in the species description.

Genomic DNA from strain HS286^T was obtained using the method described by Marmur (1961). The 16S rRNA gene of the strain was amplified by PCR using the forward primer 16F27 and the reverse primer 16R1488 (Mellado *et al.*, 1995). Direct sequence determination of the PCR-amplified DNA was carried out by using an automated DNA sequencer (model ABI 3130XL; Applied Biosystems). The 16S rRNA gene sequence analysis was performed with the ARB software package (Ludwig *et al.*, 2004). The 16S rRNA gene sequence was aligned with those of phylogenetically closely related organisms (as determined by BLAST and EzTaxon analyses) (Maidak *et al.*, 1996; Chun *et al.*, 2007) and the alignment was confirmed and checked against both primary and secondary structures of the 16S rRNA molecule using the alignment tool of the ARB software package. The phylogenetic trees were constructed using three different methods: maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms integrated in the ARB software for phylogenetic inference. The 16S rRNA gene sequences used for phylogenetic comparisons were obtained from the GenBank database and the strain designations and their accession numbers are shown in Fig. 1.

The almost-complete 16S rRNA gene sequence of strain HS286^T (1453 nucleotides) was obtained and used for initial BLAST and EzTaxon searches in GenBank and phylogenetic analysis. Comparative 16S rRNA gene sequence analysis revealed that strain HS286^T was most closely related to the genus *Thalassobacillus*. In the phylogenetic tree based on the neighbour-joining algorithm (Fig. 1), strain HS286^T formed a separate cluster with *Thalassobacillus devorans* G-19.1^T, the sole species of the genus *Thalassobacillus* so far described. This separate branch was phylogenetically related to a cluster that included species of the genera *Halobacillus* and *Salimicrobium*. The highest degree of 16S rRNA gene sequence similarity was found with *T. devorans* (99.4 %), followed by *Halobacillus yeomjeoni* (96.9 %), *Halobacillus alkaliphilus* (96.7 %) and *Halobacillus campisalis* (96.5 %). The similarity values between strain HS286^T and the type

Table 1. Characteristics that distinguish strain HS286^T from species of the genus *Thalassobacillus* and closely related species of the genus *Halobacillus*

Strains: 1, *Thalassobacillus cyri* sp. nov. HS286^T; 2, *Thalassobacillus devorans* G-19.1^T (data from García *et al.*, 2005); 3, *Halobacillus yeomjeoni* MSS-402^T (Yoon *et al.*, 2005); 4, *Halobacillus alkaliphilus* FP5^T (Romano *et al.*, 2008); 5, *Halobacillus campisalis* ASL-17^T (Yoon *et al.*, 2007). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5
Cell size (µm)	0.6–0.8 × 1.3–8.5	1.0–1.2 × 2.0–4.0	0.4–0.6 × 2.0–10.0	1.57 (diameter)	0.7–1.4 × 1.0–1.6
Oxidase	+	–	+	+	+
NaCl (% w/v)					
Range for growth	1–15	0.5–20	0.5–21	0.5–20	0–22
Optimum (% w/v)	8	7.5–10	3–5	10	8
Temperature (°C)					
Range	5–45	15–45	15–48	25–45	4–41
Optimum	40	37	37	37	37
pH					
Range	7.0–9.5	6.0–10.0	5.5–8.0	6.0–10.0	5.5–ND
Optimum	7.5	7.0	7.0–8.0	9.0	7.0–8.0
Acid production from melibiose	+	–	–	ND	+
Methyl red test	+	–	ND	ND	ND
Hydrolysis of:					
Aesculin	+	–	–	ND	+
Casein	+	–	+	–	+
Gelatin	–	+	+	–	–
Starch	+	–	–	–	+
Diamino acid in peptidoglycan	<i>m</i> -Dpm*	<i>m</i> -Dpm	Orn-D-Asp	Orn-D-Asp	<i>m</i> -Dpm
DNA G+C content (mol%)	43.0	42.4	42.9	43.5	42.1

**m*-Dpm, meso-diaminopimelic acid.

Fig. 1. Neighbour-joining phylogenetic tree, based on the 16S rRNA gene sequence comparison, showing the relationship of strain HS286^T with related species. The accession numbers of the sequences used in this study are shown in parentheses after the strain designation. *Paenibacillus polymyxa* NCDO 1774^T (X60632) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

strains of other species of the genus *Halobacillus* were 96.3–93.5 %. The novel strain showed 94.0–91.8 % gene sequence similarity with the three species of the genus *Salimicrobium*. Similar tree topologies were obtained when other treeing methods (maximum-parsimony and maximum-likelihood) were used (results not shown).

The G + C content of the genomic DNA was determined from the midpoint value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962) by using the equation of Owen & Hill (1979). Cell biomass for analyses of cell-wall peptidoglycan, isoprenoid quinones, polar lipids and fatty acids was obtained by cultivation on HM medium at 37 °C. Cell-wall peptidoglycan type, polar lipids and the menaquinone type of strain HS286^T were analysed by the Identification Service of the DSMZ (Braunschweig, Germany). The whole-cell fatty acid composition was determined by GC using the Microbial Identification System (MIDI, Microbial ID). This analysis was performed by the Identification, Characterization and Molecular Typing Service of the BCCM/LMG bacteria collection (Gent, Belgium).

The DNA G + C content of strain HS286^T was 43.0 mol%, which was close to the value previously described for *T. devorans* (42.4 mol%) (García *et al.*, 2005). Strain HS286^T had cell-wall peptidoglycan based on *meso*-diaminopimelic acid as the diagnostic diamino acid, similar to that described for *T. devorans* (García *et al.*, 2005). However, this feature distinguished strain HS286^T from the majority of the species included in the genus *Halobacillus*, which have been described as having a cell-wall peptidoglycan type based on L-Orn–D-Asp (Amoozgar *et al.*, 2003; An *et al.*, 2007; Hua *et al.*, 2007; Liu *et al.*, 2005; Spring *et al.*, 1996; Yoon *et al.*, 2003, 2004, 2005). *H. campisalis* is the sole species belonging to the genus *Halobacillus* with a cell-wall peptidoglycan type based on *meso*-diaminopimelic acid (Yoon *et al.*, 2007); however, this species is distantly related to our novel isolate (96.5 % gene sequence similarity). The isoprenoid quinone detected in strain HS286^T was menaquinone-7 (MK-7). With respect to the polar lipid composition, strain HS286^T contained diphosphatidylglycerol, phosphatidylglycerol, two phospholipids and a glycolipid. These chemotaxonomic features of the new isolate are similar to those of *T. devorans* DSM 16966^T (García *et al.*, 2005). The major fatty acids of strain HS286^T were anteiso-C_{15:0} (43.8 %), iso-C_{16:0} (21.4 %), iso-C_{14:0} (9.4 %), anteiso-C_{17:0} (8.7 %) and iso-C_{15:0} (7.0 %) (Table 2). Only some minor differences in the proportions of the fatty acids were observed with respect to *T. devorans* (Table 2).

DNA–DNA hybridization studies were performed by the competition procedure of the membrane filter method (Johnson, 1994). The hybridization temperature was 47.3 °C, which is within the limit of validity for the filter method (De Ley & Tijtgat, 1970) and the percentage of hybridization was calculated according to Johnson (1994). The experiments were performed in triplicate. The

Table 2. Cellular fatty acid content (%) of strain HS286^T and the most closely related species

Strains: 1, *Thalassobacillus cyri* sp. nov. HS286^T; 2, *T. devorans* G-19.1^T (García *et al.*, 2005; M. T. García, personal communication).

Fatty acid	1	2
iso-C _{14:0}	9.4	4.8
Summed feature 2*	2.1	—
iso-C _{15:0}	7.0	10.2
anteiso-C _{15:0}	43.8	52.3
iso-C _{16:0}	21.4	10.5
C _{16:1} ω17c	1.4	0.45
C _{16:0}	0.4	0.64
iso-C _{17:0}	1.8	1.4
anteiso-C _{17:0}	8.7	8.3
anteiso-C _{17:1}	2.8	5.1

*Summed feature 2: C_{12:0} alde, iso-C_{16:1}/C_{14:0} 3-OH.

percentage of DNA–DNA hybridization between strain HS286^T and *T. devorans* G-19.1^T was 27.3 %. These levels of DNA–DNA hybridization are low enough to classify strain HS286^T as a genotypically distinct species within the genus *Thalassobacillus* (Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002).

16S rRNA gene sequence analysis revealed that strain HS286^T is closely related to *T. devorans* G-19.1^T, constituting a separate branch with respect to the other closely related species, members of the genera *Halobacillus*, *Salimicrobium* and other Firmicutes. Strain HS286^T shared some phenotypic features and similar chemotaxonomic characteristics, such as the cell-wall peptidoglycan type, respiratory lipoquinone, polar lipid and fatty acid composition, with the genus *Thalassobacillus*. However, there are several phenotypic features that differentiate the novel strain from *T. devorans* (shown in Table 1), as well as different reactions for the utilization of D-glucose, D-galactose, glycerol, D-ribose, melibiose, D-alanine and L-asparagine as sole source of carbon and energy. In addition, the DNA–DNA hybridization data clearly support the suggestion that strain HS286^T constitutes a separate species from *T. devorans*. Based on the data presented, we propose to place the new isolate as a novel species with the name *Thalassobacillus cyri* sp. nov.

Description of *Thalassobacillus cyri* sp. nov.

Thalassobacillus cyri (cy'ri. L. masc. gen. n. *cyri* of Cyrus, named after Cyrus, the first king of Persia, where the type strain was isolated).

Cells are Gram-positive, motile, endospore-forming rods, 0.6–0.8 × 1.3–8.5 µm in size. Ellipsoidal endospores are produced at a central-subterminal position in swollen sporangia. Colonies are circular, entire, smooth, cream-beige and are 2 mm in diameter on 10 % HM agar medium

after 48 h incubation at 37 °C. Strictly aerobic. Moderately halophilic, growing at 1–15 % (w/v) NaCl, with optimal growth at 8 % (w/v) NaCl. No growth occurs in the absence of NaCl. Grows at 5–45 °C (optimally at 40 °C) and at pH 7.0–9.5 (optimally at pH 7.5). Catalase- and oxidase-positive. Casein, starch, DNA, aesculin and Tween 80 are hydrolysed but gelatin is not hydrolysed. Nitrate is reduced to nitrite but nitrite is not reduced. Acid is produced from D-fructose, D-glucose, galactose, D-mannose, maltose, melibiose and trehalose but not from *myo*-inositol, lactose or D-xylose. Methyl red test is positive and lysine decarboxylase and arginine dihydrolase activities are positive. Indole or H₂S are not produced. Voges–Proskauer, urease, β -galactosidase, ornithine decarboxylase and phenylalanine deaminase tests are negative. The following compounds are utilized as sole source of carbon and energy: D-fructose, galactose, lactose, maltose, D-mannose, melibiose, D-ribose, sucrose, glycerol, alanine, arginine, asparagine, cysteine, leucine and proline. The following compounds are not utilized as sole source of carbon and energy: D-glucose, *myo*-inositol, glycine, lysine, methionine and valine. Sensitive to amoxicillin (30 µg), carbenicillin (100 µg), gentamicin (30 µg), nitrofurantoin (300 µg), tetracycline (30 µg), rifampicin (5 µg) and polymyxin B (100 U). Resistant to tobramycin (10 µg) and kanamycin (30 µg). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, two phospholipids and a glycolipid. The isoprenoid quinone is MK-7. The cell-wall peptidoglycan type is A1 γ , with *meso*-diaminopimelic acid as the diagnostic diamino acid. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{14:0}, anteiso-C_{17:0} and iso-C_{15:0}.

The type strain, HS286^T (=CCM 7597^T=JCM 15722^T), was isolated from the Howz-Soltan hypersaline lake in Iran. The DNA G+C content of the type strain is 43.0 mol% (*T*_m).

Acknowledgements

We are grateful to J. Euzéby for his help and valuable suggestions regarding the etymology. This study was supported by grants from the Iranian Environment Department (3/42274), the Spanish Ministerio de Educación y Ciencia (BIO2006-06927) and Junta de Andalucía (P06-CVI-01829).

References

- Amoozegar, M. A., Malekzadeh, F., Malik, K. A., Schumann, P. & Spröer, C. (2003). *Halobacillus karajensis* sp. nov., a novel moderate halophile. *Int J Syst Evol Microbiol* **53**, 1059–1063.
- An, S.-Y., Kanoh, K., Kasai, H., Goto, K. & Yokota, A. (2007). *Halobacillus faecis* sp. nov., a spore-forming bacterium isolated from a mangrove area on Ishigaki Island, Japan. *Int J Syst Evol Microbiol* **57**, 2476–2479.
- Arahal, D. R. & Ventosa, A. (2002). Moderately halophilic and halotolerant species of *Bacillus* and related genera. In *Applications and Systematics of Bacillus and Relatives*, pp. 83–99. Edited by R. Berkeley, M. Heyndrickx, N. Logan & P. De Vos. Oxford: Blackwell.

- Baron, E. J. & Finegold, S. M. (1990). *Bailey and Scott's Diagnostic Microbiology*, 8th edn. St Louis: Mosby.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- De Ley, J. & Tijtgat, R. (1970). Evaluation of membrane filter methods for DNA-DNA hybridization. *Antonie van Leeuwenhoek* **36**, 461–474.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- 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.
- Harrigan, W. F. & McCance, M. E. (1976). *Laboratory Methods in Food and Dairy Microbiology*. London: Academic Press.
- Hua, N.-P., Kanekiyo, A., Fujikura, K., Yasuda, H. & Naganuma, T. (2007). *Halobacillus profundus* sp. nov., and *Halobacillus kuroshimensis* sp. nov., moderately halophilic bacteria isolated from a deep-sea methane cold seep. *Int J Syst Evol Microbiol* **57**, 1243–1249.
- Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*, pp. 655–681. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- 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.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The Ribosomal Database Project (RDP). *Nucleic Acids Res* **24**, 82–85.
- 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.
- Mata, J. A., Martínez-Canovas, J., Quesada, E. & Bejar, V. (2002). A detailed phenotypic characterization of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.
- Mellado, E., Moore, E. R. B., Nieto, J. J. & Ventosa, A. (1995). Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina* and *Deleya salina*, and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. *Int J Syst Bacteriol* **45**, 712–716.
- Murray, R. G. E., Doetsch, R. N. & Robinow, F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Owen, R. J. & Hill, L. R. (1979). The estimation of base compositions, base pairing and genome sizes of bacterial deoxyribonucleic acids. In *Identification Methods for Microbiologists* (Society for Applied Bacteriology Technical Series no. 14), 2nd edn, pp. 277–296. Edited by F. A. Skinner & D. W. Lovelock. 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.
- Romano, I., Finore, I., Nicolaus, G., Huertas, F. J., Lama, L., Nicolaus, B. & Poli, A. (2008).** *Halobacillus alkaliphilus* sp. nov., a halophilic bacterium isolated from a salt lake in Fuente de Piedra, southern Spain. *Int J Syst Evol Microbiol* **58**, 886–890.
- Saitou, N. & Nei, M. (1987).** The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Spring, S., Ludwig, W., Marquez, M. C., Ventosa, A. & Schleifer, K. H. (1996).** *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int J Syst Bacteriol* **46**, 492–496.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Fredericksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rossello-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.
- Ventosa, A. (2006).** Unusual micro-organisms from unusual habitats: hypersaline environments. In *Prokaryotic diversity: Mechanisms and Significance*, pp. 223–253. Edited by N. A. Logan, H. M. Lappin-Scott & P. C. F. Oyston. Cambridge: Cambridge University Press.
- 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.
- 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.
- Yoon, J.-H., Kang, K. H., Lee, C.-H., Oh, H.-W. & Oh, T.-K. (2005).** *Halobacillus yeomjeoni* sp. nov., isolated from a marine solar saltern in Korea. *Int J Syst Evol Microbiol* **55**, 2413–2417.
- Yoon, J.-H., Kang, J. K., Jung, Y.-T. & Oh, T.-K. (2007).** *Halobacillus campisalis* sp. nov., containing meso-diaminopimelic acid in the cell-wall peptidoglycan, and emended description of the genus *Halobacillus*. *Int J Syst Evol Microbiol* **57**, 2021–2025.