Halobacillus alkaliphilus sp. nov., a halophilic bacterium isolated from a salt lake in Fuente de Piedra, southern Spain

Ida Romano,¹ Ilaria Finore,¹ Giancarlo Nicolaus,² F. Javier Huertas,³ Licia Lama,¹ Barbara Nicolaus¹ and Annarita Poli¹

Correspondence

Annarita Poli
apoli@icmib.na.cnr.it

¹Istituto di Chimica Biomolecolare, Comprensorio ex Olivetti, via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy

²Istituto di Ricerche di Biologia Molecolare, IRBM, Pomezia, Italy

³CSIC, Estacion Experimental del Zaidin, Department of Earth Sciences and Environmental Chemistry, Profesor Albareda 1, 18008 Granada, Spain

A Gram-positive, spore-forming, halophilic bacterial strain, FP5^T, was isolated from a salt lake in southern Spain and subjected to a polyphasic taxonomic study. Strain FP5^T was strictly aerobic. Cells were coccoidal, occurring singly or in clusters. The cell-wall peptidoglycan type of strain FP5^T was A4 β based on L-Orn–D-Asp. Strain FP5^T was characterized chemotaxonomically by having MK-7 as the major menaquinone and anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0} and iso-C_{16:0} as the main fatty acids. The isolate grew optimally at 37 °C and in presence of 10% NaCl; no growth was observed in the absence of NaCl. The DNA G + C content was 43.5 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain FP5^T falls within the evolutionary radiation of species of the genus *Halobacillus*. Levels of 16S rRNA gene sequence similarity between strain FP5^T and the type strains of nine recognized *Halobacillus* species were in the range 97.0–99.0%. Levels of DNA–DNA relatedness indicated that strain FP5^T represents a genomic species that is distinct from recognized *Halobacillus* species. Strain FP5^T could be differentiated from recognized *Halobacillus* species based on several phenotypic characteristics. On the basis of phenotypic, phylogenetic and genomic data, strain FP5^T is considered to represent a novel species of the genus *Halobacillus*, for which the name *Halobacillus alkaliphilus* sp. nov. is proposed. The type strain is FP5^T (=DSM 18525^T =ATCC BAA-1361^T).

The genus *Halobacillus* was first described by Spring *et al.* (1996) to accommodate two novel species, *Halobacillus litoralis* and *Halobacillus trueperi*, and the transfer of *Sporosarcina halophila* (Claus *et al.*, 1983) to *Halobacillus* as *Halobacillus halophilus*. At the time of writing, the genus comprises 13 species with validly published names, with the addition of *Halobacillus salinus* (Yoon *et al.*, 2003), *H. karajensis* (Amoozegar *et al.*, 2003), *H. locisalis* (Yoon *et al.*, 2004), *H. yeomjeoni* (Yoon *et al.*, 2005), *H. dabanensis* and *H. aidingensis* (Liu *et al.*, 2005), *H. profundi* and *H. kuroshimensis* (Hua *et al.*, 2007), *H. campisalis* (Yoon *et al.*, 2007) and *H. faecis* (An *et al.*, 2007).

The genus *Halobacillus* can be differentiated clearly from other related genera based on the cell-wall peptidoglycan type based on L-Orn–D-Asp (Spring *et al.*, 1996; Shida *et al.*, 1997; Yoon *et al.*, 2001), with the exception of that for *H. campisalis*, which is based on *meso*-diaminopimelic acid (Yoon *et al.*, 2007). The aim of the present study was to determine the exact taxonomic status of a halophilic bacterial strain, FP5^T, by using a polyphasic approach, including phenotypic properties, lipid analyses, phylogenetic analysis based on 16S rRNA gene sequences and levels of genotypic relatedness.

Strain FP5^T was isolated from samples collected during summer 2005 from Fuente de Piedra saline lake, Malaga province, southern Spain (37° 6' N 4° 44' W). It was isolated from a saltern crystallizer pond by the dilution-plating technique. Strain FP5^T represented the predominant organism in the enrichment and was the only colony-forming organism at the highest dilutions. The enrichment medium (medium A) contained the following components

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FP5^T is AM295006.

A scanning electron micrograph of cells of strain FP5^T and thin-layer chromatographs of total polar lipids of strain FP5^T are available as supplementary material with the online version of this paper.

(per litre distilled water): 10.0 g yeast extract (Oxoid), 3.0 g trisodium citrate (Applichem), 2.0 g KCl (Applichem), 1.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Carlo Erba), 100 g NaCl (Applichem), 0.36 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (J. T. Baker), 0.05 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Carlo Erba) and 3.0 g Na_2CO_3 (Applichem). Solid media were prepared by the addition of 1.8 % (w/v) agar. The pH of medium A was 9.0.

Growth on single carbon sources was tested on liquid media containing (per litre distilled water) 100 g NaCl (Applichem), 2.0 g KCl, 1.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 16.4 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (Riedel-de Haën), 0.2 g NaHCO_3 (J. T. Baker), 2.29 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (J. T. Baker), 152 mg NH_4Cl (Applichem), 33 mg K_2HPO_4 (Applichem), 0.26 mg $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (J. T. Baker) and 10.0 g of the tested compound.

Reference strains *H. yeomjeoni* DSM 17110^T, *H. karajensis* DSM 14948^T, *H. trueperi* DSM 10404^T and *H. halophilus* DSM 2266^T, obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ), were grown in the media suggested by the culture collection [DSMZ medium no. 514 plus 3 % (w/v) NaCl at 30 °C for *H. yeomjeoni*; DSMZ medium no. 1 plus 10 % (w/v) NaCl at 38 °C for *H. karajensis*; DSMZ medium no. 755 at 30 °C for *H. trueperi*; DSMZ medium no. 123 at 30 °C for *H. halophilus*].

Cell morphology was determined by phase-contrast microscopy (Zeiss) and by scanning electron microscopy. For scanning electron microscopy analysis, the samples were fixed for 24 h in 2.5 % glutaraldehyde. Samples were dehydrated in ethanol, critical-point-dried, sputter-coated with gold (SEM BALTECMED 020) and observed by use of a Philips XL 20 environmental scanning electron microscope. Colony morphology was analysed on solid medium via a Leica M8 stereomicroscope. Tolerance of NaCl and MgSO_4 and growth at various temperatures and pH were tested in medium A. Growth tests were performed at the optimal growth temperature (37 °C) for 2 days. The pH range for growth was determined by adjusting the pH to 4.0–11.0 with HCl and NaOH. The temperature range for growth was determined by incubation in liquid medium at temperatures between 20 and 60 °C. Sensitivity of the strain to antibiotics was tested by using medium A with agar (1.8 %, w/v) and Sensi discs (6 mm; Oxoid) (Romano *et al.*, 1993). Motility was assessed by using test tubes containing medium A with agar (0.5 %, w/v). Phenotypic tests were performed according to the proposed minimal standards for the description of novel taxa within the order *Halobacteriales* (Oren *et al.*, 1997). Gelatin hydrolysis was determined as described by Oren *et al.* (2002). Hydrolysis of casein and activities of oxidase, tyrosinase, aminopeptidase (Bactident-Merck) and catalase were tested in medium A as described by Oren *et al.* (1997). For tests of nitrate reduction, medium A plus 0.1 % (w/v) KNO_3 was employed. Hydrolysis of hippurate was assessed in medium A plus hippurate (1 %, w/v) (Poli *et al.*, 2006). To test for the presence of indole, strain FP5^T was grown at pH 8.0 in

medium A. Gram-staining was performed according to Dussault (1955). The KOH test was performed according to Halebian *et al.* (1981). Tests for the hydrolysis of *N*'-benzoyl-arginine-*p*-nitroanilide stereoisomers were performed according to Oren & Galinski (1994). Cell mass for quinone and lipid analysis was obtained from cultures of test strains grown under their optimal growth conditions for 24 h. Lipid analysis, lipid hydrolysis and identification of core lipids were performed as reported by Nicolaus *et al.* (2001). For quantitative determination of fatty acid composition, fatty acid methyl ester mixtures were prepared following the instructions of the Microbial Identification System (MIDI). Quinones were analysed by LC/MS on a reversed-phase column and by electron ionization/MS and ¹H NMR spectra. Phospholipids and glycolipids were separated by TLC on silica gel plates (10 × 10 cm, 10 × 25 cm, 0.25 mm, F₂₅₄; Merck) and were analysed according to Nicolaus *et al.* (2001). Peptidoglycan analyses were performed by using the methods of Schleifer & Kandler (1972), Schleifer (1985), MacKenzie (1987) and Groth *et al.* (1996).

The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was isolated by using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). The 16S rRNA gene sequence was determined by direct sequencing of the PCR products. Genomic DNA extraction, amplification of the 16S rRNA gene and purification of the PCR products were carried out as described by Reed *et al.* (2006) with primers 5'-ATGTA-AGCTCCTGGGGTTTAC-3' and 5'-AGAAAGGAGGTGATCCAGCC-3'. Purified PCR products were sequenced at the DSMZ by using the ABI PRISM Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) according to the manufacturer's protocol. Sequence reaction products were electrophoresed by using an Applied Biosystems 373A DNA sequencer. Sequencing of the 16S rRNA gene and phylogenetic analysis were performed as described by Romano *et al.* (2007). DNA-DNA hybridization experiments were performed as described by De Ley *et al.* (1970) with the modifications given by Huß *et al.* (1983) by using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). Hybridizations were carried out between strain FP5^T and the type strains of related species (*H. yeomjeoni* DSM 17110^T, *H. karajensis* DSM 14948^T, *H. trueperi* DSM 10404^T and *H. halophilus* DSM 2266^T).

Cells of strain FP5^T were Gram-positive, spore-forming cocci, occurring singly or in bunches, and measured 1.57 µm in diameter (see Supplementary Fig. S1 in IJSEM Online). The coccoid morphology of the cells remained the same in all phases of growth. After incubation for 2 days, colonies of strain FP5^T were about 1–2 mm in diameter, circular, smooth and pale orange. Strain FP5^T required Na⁺ and Mg²⁺ for growth, but was

also able to grow using K^+ instead of Na^+ . Strain FP5^T grew in media containing 0.5–20% (w/v) NaCl; optimal growth occurred with 10% (w/v) NaCl. Strain FP5^T grew at temperatures of 25–45 °C, with optimum growth at 37 °C. The optimum pH for growth was 9.0. Growth was observed up to pH 10, but no growth occurred below pH 6.0. This optimum alkaline pH for growth was not found for other *Halobacillus* species (Spring *et al.*, 1996; Claus *et al.*, 1983; Yoon *et al.*, 2003, 2004, 2005; Amoozgar *et al.*, 2003; Liu *et al.*, 2005).

Strain FP5^T was aerobic and catalase- and oxidase-positive. Acid was produced from glucose, trehalose, maltose, ribose, sucrose, raffinose, fructose and mannose, but not from galactose, cellobiose or xylose. Tyrosine was hydrolysed, but starch, casein, hippurate, gelatin and urease were not. The strain was negative for nitrate reduction and phenylalanine deaminase. Strain FP5^T was sensitive to (µg per disc) bacitracin (10), tetracycline (30), novobiocin (30), erythromycin (5), ampicillin (25), chloramphenicol

(10), fusidic acid (10), penicillin (10 U), lincomycin (10) and vancomycin (30), but resistant to (µg per disc) streptomycin (25), nystatin (100), kanamycin (30) and neomycin (30). Detailed results of morphological analyses and biochemical tests for strain FP5^T are given in the species description. Differential characteristics between strain FP5^T and recognized species of the genus *Halobacillus* are given in Table 1. Of particular note was that only strain FP5^T was positive for tyrosine hydrolysis and that no growth was observed at <5% (w/v) NaCl for this strain. This appears to be the first report of an alkaliphilic member of the genus *Halobacillus*. Cell-wall analysis revealed that the peptidoglycan type of strain FP5^T was A4β based on L-Orn-D-Asp. The predominant menaquinone was MK-7, as reported for *H. halophilus*, the type species of the genus. The major fatty acids were anteiso-C_{15:0} (40.4%), anteiso-C_{17:0} (31.0%), iso-C_{15:0} (9.6%) and iso-C_{16:0} (8.3%). The polar lipid profile of strain FP5^T was quite similar to the complex lipid patterns reported for recognized *Halobacillus* species, with the

Table 1. Differential characteristics between strain FP5^T and recognized *Halobacillus* species

Taxa: 1, strain FP5^T; 2, *H. halophilus* (data from the present study and from Claus *et al.*, 1983; Spring *et al.*, 1996); 3, *H. trueperi* (Spring *et al.*, 1996); 4, *H. litoralis* (Spring *et al.*, 1996); 5, *H. salinus* (Yoon *et al.*, 2003); 6, *H. karajensis* (Amoozgar *et al.*, 2003); 7, *H. locisalis* (Yoon *et al.*, 2004); 8, *H. dabanensis* (Liu *et al.*, 2005); 9, *H. aidingensis* (Liu *et al.*, 2005); 10, *H. yeomjeoni* (Yoon *et al.*, 2005); 11, *H. profundus* (Hua *et al.*, 2007); 12, *H. kuroshimensis* (Hua *et al.*, 2007). +, Positive; –, negative; w, weakly positive; v, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell morphology	Coccoid	Coccoid	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods/long filamentous rods	Rods	Rods
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Spore shape*	s	s	E or S	E or S	E	E or S	E	E	E	E	E or S	E or S
Colony colour†	POW	O	O	O	POW	CW	LOW	O	O	LY	PY	YO
Motility	–	+	+	+	+	–	+	+	+	+	–	–
Maximum temperature for growth (°C)	50	37	44	43	45	49	42	50	40	48	47	48
pH optimum	9.0	7.2	7.5	7.5	7.0	7.5–8.5	7–8	7.5	7.5	7–8	7.5–8.0	7.5–8.0
NaCl concentration for growth (% w/v)												
Range	5–25	2–20	0.5–30	0.5–25	0–23	1–24	>0.5–24	0.5–25	0.5–20	0.5–21	0.5–30.0	0.5–25.0
Optimum	10	10	10	10	2–10	10	10	10	10	3–5	10	6
Hydrolysis of:												
Casein	–	+	–	–	+	+	–	+	+	+	+	+
Gelatin	–	+	+	+	+	+	–	–	+	+	–	+
Starch	–	+	–	–	–	+	+	+	+	–	+	+
Tyrosine	+	–	–	–	–	ND	–	–	–	–	ND	ND
Acid production from:												
D-Fructose	+	–	+	+	+	+	+	+	+	–	+	+
D-Galactose	–	–	+	–	w	–	–	–	–	–	–	–
Maltose	+	–	+	+	+	+	–	+	+	+	+	+
Sucrose	+	–	+	+	+	–	+	+	+	+	+	w
D-Xylose	+	–	–	+	–	–	–	+	–	–	+	–
D-Glucose	+	–	+	+	+	+	+	+	+	+	+	+
Trehalose	+	–	+	+	+	ND	+	+	+	+	+	–
DNA G + C content (mol%)	43.5	40.1–40.9	43	42	45	41.3	44	41.4	42.2	42.9	43.3	42.1

*E, Ellipsoidal; s, spherical.

†CW, Cream or white; LOW, light orange–yellow; LY, light yellow; O, orange; POW, pale orange–yellow; PY, pale yellow; YO, yellow–orange.

presence of two phospholipids, phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid as major lipids. By using two-dimensional TLC, eluted with chloroform/methanol/water (65:25:4 by volume) for the first dimension and with chloroform/methanol/acetic acid/water (85:15:12:4 by volume) for the second dimension, the lipid profile of strain FP5^T revealed the presence of two minor phospholipids and one minor glycolipid (Supplementary Fig. S2).

The complete 16S rRNA gene sequence of strain FP5^T determined in this study comprised 1484 nt. Comparative 16S rRNA gene sequence analyses showed that strain FP5^T was phylogenetically most closely affiliated to members of the genus *Halobacillus* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain FP5^T fell within the radiation of the cluster comprising *Halobacillus* species (Fig. 1). The 16S rRNA gene sequence of strain FP5^T showed similarity levels of 97.0–99.0 % with respect to sequences of the type strains of recognized *Halobacillus* species (Fig. 1).

The above results indicated that strain FP5^T was a member of the genus *Halobacillus*. However, it could be distinguished from recognized species of the genus *Halobacillus* on the basis of several phenotypic characteristics (Table 1).

The DNA G+C content of strain FP5^T was 43 mol%. Mean levels of DNA–DNA relatedness between strain FP5^T and the type strains of recognized *Halobacillus* species were in the range 4.5–35 % (*H. trueperi*, 4.5 %; *H. salinus*, 10.5 %; *H. karajensis*, 18.2 %; *H. yeomjeoni*, 22.5 %; *H. dabanensis*, 30.3 %; *H. halophilus*, 35.0 %). Therefore, on the basis of the data presented, strain FP5^T should be

placed in the genus *Halobacillus* as a member of a novel species, for which the name *Halobacillus alkaliphilus* sp. nov. is proposed.

Description of *Halobacillus alkaliphilus* sp. nov.

Halobacillus alkaliphilus (al.ka.li.phi'lus. N.L. n. *alkali* alkali; Gr. adj. *philos* loving; N.L. masc. adj. *alkaliphilus* loving alkaline conditions).

Cells are Gram-positive, spore-forming cocci (1.57 µm in diameter). Colonies on agar medium are circular (1–2 mm in diameter), smooth and pale orange. Growth occurs at NaCl concentrations of 0.5–20 % (w/v), with optimal growth at 10 % (w/v), at temperatures of 25–45 °C, with optimum growth at 37 °C, and at pH 6.0–10.0, with optimum growth at pH 9.0. Aerobic. Catalase- and oxidase-positive. Acid is produced from xylose, glucose, trehalose, maltose, ribose, sucrose, raffinose, fructose and mannose, but not from galactose or cellobiose. Hydrolyses tyrosine, but not starch, casein, hippurate, gelatin or urease. Negative for nitrate reduction and phenylalanine deaminase. The peptidoglycan type is A4β based on L-Orn-D-Asp. The major menaquinone is MK-7. The major fatty acids are anteiso-C_{15:0} (40.4 %), anteiso-C_{17:0} (31.0 %), iso-C_{15:0} (9.6 %) and iso-C_{16:0} (8.3 %). Major lipids are two phospholipids (phosphatidylglycerol and diphosphatidylglycerol) and one unidentified glycolipid. The DNA G+C content of the type strain is 43.5 mol% (*T_m*).

The type strain, FP5^T (=DSM 18525^T =ATCC BAA-1361^T), was isolated from Fuente de Piedra salt lake, southern Spain.

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic positions of strain FP5^T, recognized *Halobacillus* species and some other related taxa. Bootstrap values (%) are based on 1000 replicates and are shown at each node. Bar, 0.01 expected changes per site.

Acknowledgements

This work was partially supported by Regione Campania. We thank Valeria Calandrelli and Eduardo Pagnotta for technical assistance, Vincenzo Mirra and Dominique Melck for NMR imaging and Raffaele Turco for artwork. We are grateful to Dr Salvatore Pelliccione and Dr Manuel Rendon (Director-Curator of Fuente de Piedra Natural Reserve) for their assistance during sampling and to Dr J. P. Euzéby for help with etymology.

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.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- 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 Microbiol* **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.
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Halebian, S., Harris, B., Finegold, S. M. & Rolfe, R. (1981). Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. *J Clin Microbiol* **13**, 444–448.
- 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.
- Huß, 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.
- 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.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Nicolaus, B., Manca, M. C., Lama, L., Esposito, E. & Gambacorta, A. (2001). Lipid modulation by environmental stresses in two models of extremophiles isolated from Antarctica. *Polar Biol* **24**, 1–8.
- Oren, A. & Galinski, E. A. (1994). Hydrolysis of *N*'-benzoyl-arginine-*p*-nitroanilide stereoisomers as a phenotypic test: a study of Gram-positive halotolerant bacteria. *Syst Appl Microbiol* **17**, 7–10.
- Oren, A., Ventosa, A. & Grant, W. D. (1997). Proposal of minimal standards for the description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* **47**, 233–238.
- Oren, A., Elevi, R., Watanabe, S., Ihara, K. & Corcelli, A. (2002). *Halomicrobium mukohataei* gen. nov., comb. nov., and emended description of *Halomicrobium mukohataei*. *Int J Syst Evol Microbiol* **52**, 1831–1835.
- Poli, A., Esposito, E., Lama, L., Orlando, P., Nicolaus, G., de Apollonia, F., Gambacorta, A. & Nicolaus, B. (2006). *Anoxybacillus amylolyticus* sp. nov., a thermophilic amylase producing bacterium isolated from Mount Rittmann (Antarctica). *Syst Appl Microbiol* **29**, 300–307.
- Reed, A. J., Lutz, R. A. & Vetriani, C. (2006). Vertical distribution and diversity of bacteria and archaea in sulphide and methane-rich cold seep sediments located at the base of the Florid Escarpment. *Extremophiles* **10**, 199–211.
- Romano, I., Manca, M. C., Lama, L., Nicolaus, B. & Gambacorta, A. (1993). A method for antibiotic assay on *Sulfolobales*. *Biotechnol Tech* **7**, 439–440.
- Romano, I., Poli, A., Finore, I., Huertas, F. J., Gambacorta, A., Pelliccione, S., Nicolaus, G., Lama, L. & Nicolaus, B. (2007). *Haloterigena hispanica* sp. nov., an extremely halophilic archaeon from Fuente de Piedra, southern Spain. *Int J Syst Evol Microbiol* **57**, 1499–1503.
- 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.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.
- 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.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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* Rüter 1983 as *Marinibacillus marinus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 2087–2093.
- 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, S.-J., 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, S.-J., 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.