Pontibacillus yanchengensis sp. nov., a moderately halophilic bacterium isolated from salt field soil

Yan Yang, Zhengzhong Zou, Minyan He and Gejiao Wang

Correspondence

Gejiao Wang
gejiaow@yahoo.com.cn or
gejiao@mail.hzau.edu.cn

State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

A Gram-positive-staining, aerobic, moderately halophilic bacterium, designated strain Y32^T, was isolated from subsurface soil of the Sanwei salt field, Yancheng, Jiangsu province, South-east China. The cell-wall peptidoglycan type of strain Y32^T was *meso*-diaminopimelic acid. The menaquinone was menaquinone-7 (MK-7). The major fatty acids were anteiso-C_{15:0} and iso-C_{15:0} and the DNA G + C content of strain Y32^T was 40.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Y32^T was closely related to the type strains of the genus *Pontibacillus*. The 16S rRNA gene sequence similarities of strain Y32^T with the type strains of species of the genus *Pontibacillus* were 97.8 % (*Pontibacillus marinus* KCTC 3917^T), 96.9 % (*Pontibacillus chungwhensis* BH030062^T), 96.8 % (*Pontibacillus litoralis* JSM 072002^T) and 96.0 % (*Pontibacillus halophilus* JSM 076056^T). DNA–DNA relatedness between strain Y32^T and *P. marinus* KCTC 3917^T was 42 %. On the basis of phenotypic, chemotaxonomic and phylogenetic evidences, strain Y32^T was found to be affiliated to the genus *Pontibacillus*, but was clearly differentiated from other members of this genus. Strain Y32^T represents a novel member of the genus, for which the name *Pontibacillus yanchengensis* sp. nov. is proposed. The type strain is Y32^T (=CGMCC 1.10680^T=CCTCC AB209311^T=NRRL B-59408^T).

Moderately halophilic bacteria that grow optimally in media containing 1–15 % (w/v) NaCl constitute a heterogeneous physiological group of micro-organisms which belong to many different genera (Ventosa *et al.*, 1998). Spore-forming moderately halophilic Gram-positive rods isolated from marine or hypersaline environments were formerly placed in the genus *Bacillus*. Through chemotaxonomic and molecular analyses, several phylogenetically distinct lineages in the genus *Bacillus* have been revealed and many spore-forming bacteria have been placed into separate genera (Ash *et al.*, 1991; Nielsen *et al.*, 1994). In 2005, Lim *et al.* (2005a) proposed the genus *Pontibacillus*, members of which were described as Gram-positive, spore-forming, rod-shaped, strictly aerobic and moderately halophilic and containing MK-7 as the major isoprenoid quinone and possessing *Aly*-type *meso*-diaminopimelic acid in the cell wall (Lim *et al.*, 2005a). Recently, Chen *et al.* (2010) proposed the species *Pontibacillus litoralis* and found that it differed from the original genus description as presented by Lim *et al.* (2005a) by the ability to perform facultative anaerobiosis and by having creamy-white pigmentation. Thus, the genus description was emended to remove strictly aerobic growth as a characteristic of the

genus. At the time of writing, there are four recognized members of this genus, *Pontibacillus chungwhensis* (Lim *et al.*, 2005a), *Pontibacillus marinus* (Lim *et al.*, 2005b), *Pontibacillus halophilus* (Chen *et al.*, 2009) and *Pontibacillus litoralis* (Chen *et al.*, 2010). These species were isolated from a solar saltern on the Yellow Sea (*P. chungwhensis* and *P. marinus*), a sea urchin (*P. halophilus*) and a sea anemone (*P. litoralis*). In this study, a Gram-positive-staining, moderately halophilic bacterium, strain Y32^T, was isolated from the soil of a salt field. According to the minimal standards for describing new taxa of aerobic, endospore-forming bacteria (Logan *et al.*, 2009), and on the basis of morphological, physiological, chemotaxonomic and phylogenetic characteristics, it is concluded that strain Y32^T represents a new member of the genus *Pontibacillus*.

Strain Y32^T was isolated from a mixed subsurface soil sample (5–15 cm deep) from the Sanwei salt field, which is located near the Yellow Sea in Yancheng City (32° 23' 43" N, 120° 0' 34" E, with an annual mean temperature of 14 °C), Jiangsu Province, South-east China. This salt field belongs to the Sanwei salt factory, one of the largest salt production factories in China, which produces salt from seawater. The texture of the soil sample was clay; the pH was 7.95; and the Na⁺, K⁺, Mg²⁺ and organic matter contents were 8.13 g kg⁻¹, 1.22 g kg⁻¹, 1.44 mg kg⁻¹ and 0.80 %, respectively. For strain isolation, a 10 g filtered soil sample was added to 90 ml sterilized

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y32^T is EF533969.

Three supplementary figures are available with the online version of this paper.

HGM liquid medium (Fairley *et al.*, 2002) and incubated at 37 °C for 30 min with shaking at 160 r.p.m. An aliquot of the suspension was diluted with 10 % (w/v) NaCl solution and streaked onto HGM agar plates that were then incubated at 37 °C for one week. About 10^6 c.f.u. g⁻¹ colonies were obtained. After several rounds of restreaking, single colonies were used for DNA isolation and 16S rRNA gene identification. By analysing 50 colonies, six halophilic bacterial strains were obtained that were pre-identified as belonging to the genera *Bacillus* (GenBank accession no. EF533965), *Halobacillus* (EF533963 and EF533970), *Halomonas* (EF533962 and EF533966) and *Pontibacillus* (Y32^T, EF533969). Strain Y32^T was considered to represent a novel species of the genus *Pontibacillus* and was chosen for further study. Strain Y32^T was routinely grown aerobically on marine agar 2216 (MA medium, pH 7.5, Difco) supplemented with 5 % (w/v) NaCl for 2 days at 37 °C, except where mentioned otherwise. Anaerobic growth was determined by incubation in an anaerobic chamber (Mitsubishi Gas Chemical Co. Inc.) at 37 °C on the same medium.

The morphology of cells was observed using light and transmission electron microscopes. Gram staining was determined using the method described by Dussault (1955). Endospores and flagella were stained according to the methods of Schaeffer-Fulton and Leifsson (Smibert & Krieg, 1994), respectively. The temperature for growth was tested at different temperatures (4–55 °C) on MA medium supplemented with 5 % (w/v) NaCl. The pH range for growth was determined in filter-sterilized MA medium supplemented with 5 % NaCl by adding acetic acid/sodium acetate (pH 4.0–4.5), MES (pH 5.0–6.0, 10 mM), PIPES (pH 6.5–7.0, 10 mM), Tris/HCl (pH 7.5–9.0, 10 mM) and adjusted with NaOH or HCl. Requirement of and tolerance to NaCl were determined in nutrient broth supplemented with modified artificial seawater containing 0–30 % (w/v) NaCl (Lim *et al.*, 2005a). Growth was monitored by measuring the optical density at 600 nm. Hydrolysis of casein, gelatin, xanthine, hypoxanthine, urea, starch, Tween 80, L-tyrosine and urea was performed as described by Cowan & Steel (1965). Acid production from carbohydrates was determined as described by Leifson (1963). Nitrate and nitrite reduction were tested by the method described by Lányi (1987). Methyl red and Voges–Proskauer tests, H₂S and indole production and determination of aesculin hydrolysis were performed as recommended by Smibert & Krieg (1994). The oxidation/fermentation of glucose was determined according to the method of Mata *et al.* (2002). Catalase and oxidase activities were determined by the method described by Lim *et al.* (2005a). Other enzyme activities were tested using API ZYM strips (bioMérieux) according to the manufacturer's instructions except for the suspension medium being supplemented with 4 % (w/v) NaCl. The experiments were performed using *P. marinus* KCTC 3917^T, *P. chungwhensis* KCTC 3890^T, *P. litoralis* KCTC 13237^T and *P. halophilus* KCTC 13190^T as reference strains.

The utilization of sole carbon and nitrogen sources was analysed using a traditional method (Ventosa *et al.*, 1982) and API 32 GN strips (bioMérieux).

Genomic DNA was isolated from the novel strain using a previously described procedure (Cashion *et al.*, 1977). The 16S rRNA gene sequence was amplified by PCR with universal primers Uni-27F and Uni-1492R (Wilson *et al.*, 1990). The PCR product was ligated into a pGEM-T vector (Promega). DNA sequencing was performed by Beijing Genomics Institute (China). The resulting 16S rRNA gene sequence was compared with the sequences from the NCBI GenBank database to determine which reference strains were most closely related to strain Y32^T. The similarities were calculated using the NCBI alignment and EzTaxon programs (Chun *et al.*, 2007). Multiple alignments of sequences determined in this study together with reference sequences obtained from the databases and calculations of levels of sequence similarity were carried out using CLUSTAL W (Thompson *et al.*, 1994). Neighbour-joining and maximum-parsimony trees were constructed using MEGA 4.0 (Tamura *et al.*, 2007), and a maximum-likelihood tree was generated using the PHYLIP package (Felsenstein, 2002). The confidence values for the branches of phylogenetic trees were determined using bootstrap analyses based on 1000 replications. DNA–DNA hybridization was performed by the thermal denaturation and renaturation method (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 × SSC at the optimum temperature.

The DNA G+C content was determined by HPLC according to the method of Tamaoka & Komagata (1984). Analysis of menaquinones was performed by HPLC as described by Xie & Yokota (2003). Quinones were extracted with chloroform/methanol (2:1, v/v), evaporated in a vacuum and separated by TLC on silica-gel F₂₅₄ plates with hexane/ethyl ether (85:15) as the solvent system. Quinone components were eluted with the mobile phase of methanol and isopropyl ether (4:1, v/v) by HPLC. For whole-cell fatty acid analysis, strain Y32^T and the four reference strains were grown at 35 °C for 48 h on MA supplemented with 3 % (w/v) NaCl and analysed by GC (6890; Hewlett Packard) according to the instructions of the Sherlock Microbial Identification System (MIDI). Cell walls were purified and analysed using the TLC protocol (Lechevalier & Lechevalier, 1980).

The detailed morphological, physiological and biochemical characteristics of strain Y32^T are given in the species description. A transmission electron micrograph showing the morphology of cells of strain Y32^T and a light micrograph showing endospores are available as supplementary figures (see Supplementary Figs S1 and S2, available in IJSEM Online). The phenotypic characteristics of strain Y32^T that showed that it was closely related to other species of the genus *Pontibacillus* and enabled it to be differentiated from phylogenetically related strains are presented in Table 1. The growth of strain Y32^T in media

Table 1. Differential characteristics of strain Y32^T and other type strains of recognized species of the genus *Pontibacillus*

Strains: 1, Y32^T; 2, *P. marinus* KCTC 3917^T; 3, *P. chungwhensis* KCTC 3890^T; 4, *P. litoralis* KCTC 13237^T; 5, *P. halophilus* KCTC 13190^T. Taxa 2, 3, 4 and 5 were the deposited strains as reported by Lim *et al.* (2005b), Lim *et al.* (2005a), Chen *et al.* (2010) and Chen *et al.* (2009), respectively. The experiments of all five strains were performed under the same laboratory conditions and the data are from this study. Cells of all strains are rod-shaped. +, Positive; –, negative; w, weakly positive; s, spherical, e, ellipsoidal; t, terminal; st, subterminal.

Characteristic	1	2	3	4	5
Pigmentation	Yellow	Cream	Yellow	Creamy white	Yellow
Spore shape	s	s	s	e	e
Spore position	t	t	t	st	st
NaCl range (% w/v)	3–20	1–9	1–16*	0.5–15	1–25
NaCl optimum (% w/v)	6–8	2–5	2–5	2–5	4–8*
Oxidase	+	+	–	+	+
Nitrate reduction to nitrite	+	+	–	+	–
Hydrolysis of:					
Aesculin	+	+	–	+	+
Casein	–	–	+	–	–
Gelatin	–	–	–	+	+
Starch	+	–	+	–	+
Tween 80	–	+	+	–	–
Acid production from:					
D-Fructose	+	+	–	+	–
D-Glucose	+	–	+	+	–
Glycerol	–	–	+	–	–
Maltose	+	+	+	+	–
D-Mannitol	+	–	–	–	–
D-Mannose	–	–	–	–	+
Raffinose	–	+	–	–	–
D-Ribose	–	+	+	–	+
Sucrose	–	+	+†	+	–
Trehalose	+	+	+	+	–
Activity of:					
Acid phosphatase	+	–†	–†	–	w
Esterase (C4)	+	+†	+†	+	–
α-Chymotrypsin	w	+†	+†	–	–
β-Galactosidase	–	–†	+†	–	–
α-Glucosidase	+	+†	+†	–	+
β-Glucosidase	–	–†	–†	–	w
β-Glucuronidase	–	–†	–†	–	+
Leucine arylamidase	+	w†	+†	+	–
DNA G + C content (mol%)	40.3	42.0	41.0	41.3	45.5

*Data that were different from the results obtained in previous studies.

†Additional data from this study that have not been published in previous studies.

with all single sole carbon and nitrogen sources was negative. The length of 16S rRNA gene sequence of strain Y32^T obtained was 1485 bp. The NCBI alignments indicated that strain Y32^T was closely related to *P. marinus* KCTC 3917^T (97.8 % gene sequence similarity), *P. chungwhensis* BH030062^T (96.9 %), *P. litoralis* JSM 072002^T (96.8 %) and *P. halophilus* JSM 076056^T (96.0 %). Using the EzTaxon program, all the other gene sequence similarities, except for *P. marinus*, were below 97 %. Phylogenetic trees based on 16S rRNA gene sequences revealed that strain Y32^T fell within the branch of the genus *Pontibacillus*. The members of the genus *Pontibacillus*

constituted an independent lineage in the phylogenetic trees using all three alignment methods (Fig. 1 and Supplementary Fig. S3a, b). In the neighbour-joining tree, strain Y32^T clustered with the clade comprising *P. marinus* and *P. chungwhensis* and the relationship between this cluster and *P. litoralis* was supported by bootstrap analysis at a confidence level of 75. The maximum-parsimony tree and maximum-likelihood tree showed that strain Y32^T and *P. litoralis* comprised a clade.

DNA–DNA relatedness between strain Y32^T and *P. marinus* KCTC 3917^T was 42 % (the range of values

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain Y32^T and some related bacterial species. Numbers at branching nodes are bootstrap percentages from 1000 replications. Bar, 1 % nucleotide substitution.

obtained in replicate assays was 41–42 %). The DNA G + C content of strain Y32^T was 40.3 mol%. The novel strain possessed *meso*-diaminopimelic acid as the diagnostic diamino acid, which was in agreement with the description of genus *Pontibacillus* and with the properties of the majority of endospore-forming Gram-positive bacilli. Menaquinone 7 (MK-7) was the respiratory quinone. No minor quinone was detected. The major cellular fatty acids on MA plus 3 % (w/v) NaCl were anteiso-*C*_{15:0}, iso-*C*_{15:0}, iso-*C*_{16:0}, iso-*C*_{14:0}, anteiso-*C*_{17:0} and *C*_{16:1} ω 7*c* alcohol (see details in Table 2), which was in agreement with those of the type strains of the other recognized species of the genus *Pontibacillus*.

On the basis of distinctive physiological, morphological and other key traits of strain Y32^T, it is concluded that the strain represents a novel species, for which the name *Pontibacillus yanchengensis* sp. nov. is proposed.

Description of *Pontibacillus yanchengensis* sp. nov.

Pontibacillus yanchengensis (yan.chen.gen'sis. N.L. masc. adj. *yanchengensis* of or belonging to Yancheng, as the type strain was isolated from a salt field in Yancheng).

Cells are Gram-positive-staining, rod-shaped (0.5–0.9 × 1.9–2.5 µm), motile with flagella and are unable to grow in anaerobic conditions. Spherical endospores are

formed at the terminal position in swollen sporangia. Colonies are yellow, smooth and have circular/slightly irregular margins after incubation on MA plus 5 % NaCl (w/v) at 37 °C for 48 h. Grows in media containing 3–20 % (w/v) NaCl; optimum growth occurs on media containing 6–8 % (w/v) NaCl. No growth in the absence of NaCl. Growth is observed at pH 6.0–9.5 and optimum growth occurs at pH 7.0–8.0. The temperature range for growth is 15–45 °C (optimum, 35–40 °C). Oxidase- and catalase-positive. Reduces nitrate to nitrite. Hydrolyses aesculin and starch, but not casein, L-tyrosine, Tween 80, hypoxanthine, xanthine, gelatin or urea. Negative result in tests for the production of H₂S or indole, for the fermentation of glucose and for nitrite reduction. Negative result in methyl red and Voges-Proskauer tests. Produces acid from D-glucose, D-fructose, D-mannitol, maltose, trehalose and starch, but not from D-ribose, D-xylose, D-mannose, L-arabinose, L-rhamnose, α -lactose, D-galactose, raffinose, D-salicin, melibiose, adonitol or sucrose. Enzyme activities are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, α -glucosidase, naphthol-AS-BI-phosphohydrolase and leucine arylamidase. Weak or negative activity for α -chymotrypsin. Negative for lipase (C14), cystine arylamidase, β -glucuronidase, α -fucosidase, α -galactosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase, lysine decarboxylase, α -mannosidase, trypsin and valine arylamidase. Major cellular fatty acids on MA plus 3 % (w/v) NaCl are anteiso-*C*_{15:0}, iso-*C*_{15:0}, iso-*C*_{16:0},

Table 2. Whole-cell fatty acids of strain Y32^T and type strains of the genus *Pontibacillus*

Strains: 1, Y32^T; 2, *P. chungwhensis* KCTC 3890^T; 3, *P. marinus* KCTC 3917^T; 4, *P. litoralis* KCTC 13237^T; 5, *P. halophilus* KCTC 13190^T. All data are from this study. Cells of all strains used in the experiment were grown at 35 °C for 2 days on MA supplemented with 3 % (w/v) NaCl. –, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain fatty acids:					
C _{15:0}	1.0	0.7	0.5	–	–
C _{16:0}	1.0	2.5	1.0	1.0	0.8
Branched fatty acids:					
iso-C _{14:0}	18.5	10.3	5.2	1.7	17.8
iso-C _{15:0}	25.2	23.9	68.9	63.8	48.5
anteiso-C _{15:0}	35.2	38.6	11.1	13.8	10.5
iso-C _{16:0}	5.1	6.9	3.1	2.5	7.6
iso-C _{17:0}	1.1	0.8	2.1	4.1	2.3
anteiso-C _{17:0}	4.1	5.7	1.8	0.7	2.1
iso-C _{17:1} ω10c	0.3	–	0.6	6.8	0.8
Unsaturated fatty acids:					
C _{16:1} ω7c alcohol	6.2	8.3	3.3	1.8	6.8
C _{16:1} ω11c	0.8	0.4	0.7	0.9	0.7
Summed feature 4*	0.6	0.2	0.6	1.2	0.7

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

iso-C_{14:0}, anteiso-C_{17:0} and C_{16:1}ω7c alcohol. The cell-wall peptidoglycan type is *meso*-diaminopimelic acid. The menaquinone is menaquinone-7 (MK-7).

The type strain, Y32^T (=CGMCC 1.10680^T=CCTCC AB209311^T=NRRL B-59408^T), was isolated from subsurface soil of Sanwei salt field, Yancheng, Jiangsu province, South-east China. The DNA G+C content of the type strain is 40.3 mol% (HPLC).

Acknowledgements

We would like to thank Dr Jin-Woo Bae (Kyung Hee University) for purchasing *P. marinus* KCTC 3917^T from the KCTC, the KCTC for providing the other three type strains, and Dr Chengxiang Fang (CCTCC, Wuhan University) for fatty acid analysis. This work was supported by the National Natural Science Foundation of China (30671140) and the Returning Overseas Scientist Fund of the Ministry of Education, P. R. China.

References

- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* as revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.
- 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.

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. (2009). *Pontibacillus halophilus* sp. nov., a moderately halophilic bacterium isolated from a sea urchin. *Int J Syst Evol Microbiol* **59**, 1635–1639.

Chen, Y.-G., Zhang, Y.-Q., Yi, L.-B., Li, Z.-Y., Wang, Y.-X., Xiao, H.-D., Chen, Q.-H., Cui, X.-L. & Li, W.-J. (2010). *Pontibacillus litoralis* sp. nov., a facultatively anaerobic bacterium isolated from a sea anemone, and emended description of the genus *Pontibacillus*. *Int J Syst Evol Microbiol* **60**, 560–565.

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.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.

Fairley, D. J., Boyd, D. R., Sharma, N. D., Allen, C. C. R., Morgan, P. & Larkin, M. J. (2002). Aerobic metabolism of 4-hydroxybenzoic acid in Archaea via an unusual pathway involving an intramolecular migration (NIH shift). *Appl Environ Microbiol* **68**, 6246–6255.

Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

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.

Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy* (Special Publication no. 6), pp. 227–291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

Lim, J.-M., Jeon, C. O., Song, S. M. & Kim, C.-J. (2005a). *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.

Lim, J.-M., Jeon, C. O., Park, D.-J., Kim, H.-R., Yoon, B.-J. & Kim, C.-J. (2005b). *Pontibacillus marinus* sp. nov., a moderately halophilic bacterium from a solar saltern, and emended description of the genus *Pontibacillus*. *Int J Syst Evol Microbiol* **55**, 1027–1031.

Logan, N. A., Berge, O., Bishop, A. H., Busse, H.-J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Rabinovitch, L. & other authors (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* **59**, 2114–2121.

Mata, J. A., Martínez-Cánovas, J., Quesada, E. & Béjar, V. (2002). A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.

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.

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.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- 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., Nieto, J. J. & Oren, A. (1998).** Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.
- 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.
- Wilson, K. H., Blitchington, R. B. & Greene, R. C. (1990).** Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol* **28**, 1942–1946.
- Xie, C. H. & Yokota, A. (2003).** Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.