Halomonas koreensis sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea

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A moderately halophilic bacterium, strain SS20^T, capable of growing at salinities of 1–20 % (w/v) NaCl was isolated from a solar saltern of the Dangjin area in Korea and was characterized taxonomically. Strain SS20^T was a Gram-negative bacterium comprising motile, short rods. Its major cellular fatty acids were $C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo and $C_{16:0}$. The DNA G+C content was 70 mol% and the predominant ubiquinone was Q-9. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SS20^T belonged to the genus *Halomonas*. The levels of 16S rRNA gene sequence similarity to the type strains of *Halomonas* species were in the range 93·0–97·5 %. The levels of DNA–DNA relatedness between strain SS20^T and the type strains of phylogenetically closely related *Halomonas* species were in the range 5·3–12·3 %. On the basis of physiological and molecular properties, strain SS20^T represents a novel species of the genus *Halomonas*, for which the name *Halomonas koreensis* sp. nov. is proposed. The type strain is SS20^T (=KCTC 12127^T=JCM 12237^T).

The family *Halomonadaceae* of the *γ-Proteobacteria* includes four genera of halophilic bacteria, *Halomonas*, *Chromohalobacter*, *Alcanivorax* and *Cobetia*, and two genera of non-halophilic bacteria, *Zymobacter* and *Carnimonas* (Arahal *et al.*, 2001, 2002a; Dobson & Franzmann, 1996; Yakimov *et al.*, 1998). *Halomonas* was described as comprising Gram-negative, halotolerant, aerobic bacteria capable of growing over a wide range of salt concentrations. Moderately halophilic bacteria are abundant in saline habitats, and a number of *Halomonas* species have been isolated from different terrestrial and aquatic saline environments, mainly salterns, estuarine water, salt lakes, salty foods, sea ice and deep-sea hydrothermal vent environments, in the last few years (Baumann *et al.*, 1983; Mellado *et al.*, 1995; Romanenko *et al.*, 2002; Vreeland *et al.*, 1980; Yoon *et al.*, 2002; Reddy *et al.*, 2003; Kaye *et al.*, 2004). Furthermore, this group of bacteria has great biotechnological potential for the production of compatible solutes or hydrolytic enzymes (Margesin & Schinner, 2001; García *et al.*, 2004).

The features of these moderately halophilic bacteria are too heterogeneous to justify their placement in the single genus *Halomonas*, and the descriptions of some of the species do not match the emended genus description (Romanenko *et al.*, 2002). In addition, the genus *Halomonas* has an unusually wide range of G+C contents (about 52–68 mol%; Arahal *et al.*, 2002b). Therefore some researchers have suggested that it might become possible to split the genus *Halomonas* into two or more groups (Arahal *et al.*, 2002b; Romanenko *et al.*, 2002).

During screening of halophilic micro-organisms, a Gram-negative, moderately halophilic, rod-shaped bacterium (SS20^T) with a DNA G+C content of 70 mol% was isolated from a solar saltern in Korea. The aim of this study was to determine the taxonomic status of this organism: we describe this bacterium, strain SS20^T, as the type strain of a novel species designated *Halomonas koreensis* sp. nov.

Strain SS20^T was isolated from a solar saltern in the Dangjin area of the Yellow Sea in Korea. For isolation, soil samples were diluted serially, spread on marine agar 2216 (MA) (Difco) with the addition of 10 % (w/v) NaCl (final concentration, 11·94 % NaCl, w/v) and incubated for 3 days at 35 °C. Isolate SS20^T was routinely grown aerobically on MA for 3 days at 35 °C except where indicated otherwise. Requirement for, and tolerance of, NaCl were determined

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in nutrient broth (3·0 g beef extract l^{-1}, 5·0 g peptone l^{-1}; Difco) supplemented with modified artificial sea water [ASW: 0–30 % (w/v) NaCl, 5·94 g $MgSO_4.7H_2O$ l^{-}, 4·53 g $MgCl_2.6H_2O$ l^{-}, 0·64 g KCl l^{-} and 1·3 g $CaCl_2$ l^{-}]. Growth was tested at different temperatures (4–55 °C) and pH values (5·0–10·0) in nutrient broth with the addition of ASW containing 7 % (w/v) NaCl. Growth was monitored by measuring the optical density at 600 nm.

Cell morphology was studied using phase-contrast microscopy and transmission electron microscopy. The flagellum type was examined by transmission electron microscopy using cells from the exponential growth phase. Cells were mounted on Formvar-coated copper grids and negatively stained with 1 % potassium phosphotungstate (pH 7·0). Grids were examined in a Phillips 201 transmission electron microscope operated at 80 kV.

For quantitative analysis of whole-cell fatty acids, strain SS20^T was cultivated on either MA or MA with the addition of 5 % (w/v) NaCl for 3 days at 35 °C. Fatty acid methyl esters were analysed by GC/MS according to the instructions of the Microbial Identification System (MIDI; Microbial ID).

Catalase activity was determined by bubble production in 3 % (v/v) aqueous hydrogen peroxide solution. Oxidase activity, nitrate reduction and hydrolysis of aesculin, casein, starch, Tween 80, urea, hypoxanthine, tyrosine, gelatin and xanthine were determined according to methods described previously (Cowan & Steel, 1965; Lanyi, 1987). Other physiological tests were carried out using the API 20 test kits (bioMérieux) according to the manufacturer's instructions, except that the cultured cells were suspended in ASW with 3 % (w/v) NaCl.

Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using HPLC apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; Chromalytic Technology). Methanol/2-propanol (2 : 1, v/v) was used as the mobile phase and quinones were detected at 270 nm. The G+C content (mol%) was determined by reverse-phase HPLC using the method of Tamaoka & Komagata (1984).

DNA–DNA hybridization was carried out fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), except that ribonuclease T1 was used together with ribonuclease A. *Halomonas alimentaria* JCM 10888^T, *Halomonas elongata* DSM 2581^T, *Halomonas maura* DSM 13445^T, *Halomonas pacifica* KCTC 2683^T and *Halomonas salina* DSM 5928^T were used as reference strains for DNA–DNA hybridization. Reference strains were grown in marine broth (Difco) at appropriate temperatures.

The 16S rRNA gene was amplified by PCR using the Eubac 27F and 1492R primers (DeLong, 1992) and PCR products were purified using the QIAquick PCR purification kit (Qiagen). The purified PCR product was sequenced using the ABI PRISM BigDye Terminator cycle sequencing kit and an Applied Biosystems model 310 automatic DNA sequencer. The sequence data were assembled using SeqMan (DNASTAR) and were compared with available 16S rRNA gene sequences from GenBank using the BLAST program (NCBI) to determine the approximate phylogenetic affiliation. The 16S rRNA gene sequence of strain SS20^T was aligned with those of *Halomonas* species and some other related taxa by using the CLUSTAL W software (Thompson *et al.*, 1994). Sequence similarity values were computed using SIMILARITY MATRIX version 1.1 (Ribosomal Database Project II; http://rdp.cme.msu.edu/html/analyses.html). Evolutionary distance matrices were calculated using the algorithm of the Kimura two-parameter model (Kimura, 1980) with the DNADIST program within the PHYLIP software package, version 3.6 (Felsenstein, 2002). Phylogenetic trees were constructed using two different methods, the maximum-likelihood method (Felsenstein, 1981) and the neighbour-joining method (Saitou & Nei, 1987) available in the PHYLIP software package. To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1000 replications) was performed with the SEQBOOT, DNADIST, NEIGHBOR and CONSENSE programs in the PHYLIP package.

Strain SS20^T grew on nutrient agar supplemented with ASW, but not on nutrient agar (Difco) with just NaCl. Strain SS20^T on MA medium formed creamy, smooth, glistening and circular/slightly irregular colonies. Strain

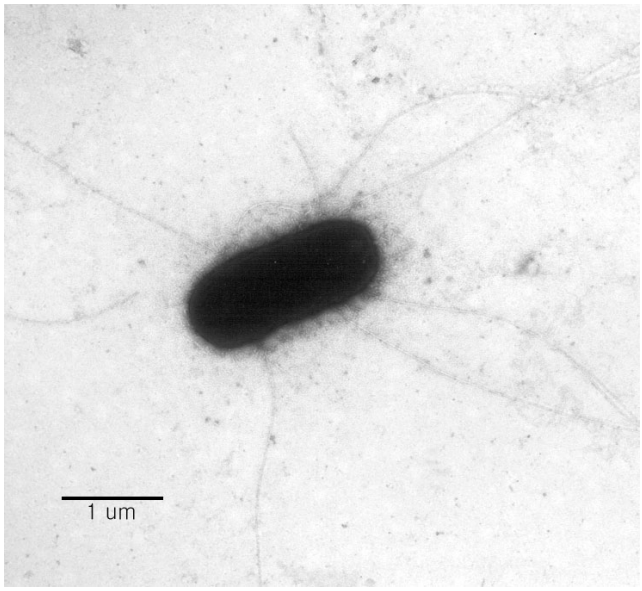

Fig. 1. Transmission electron micrograph showing general morphology of a negatively stained cell of strain SS20^T from an exponentially growing culture. Bar, 1 μm.

SS20^T grew at salt concentrations in the range 1–20 % (w/v) NaCl. Growth of the strain was consistent at various salinities ranging from 1 to 12 % (w/v) NaCl. Growth occurred from pH 5·5 to 10 (optimum, pH 7·0–8·0) in nutrient broth containing 7 % (w/v) salts. Growth was observed at temperatures between 10 and 47 °C, having an optimum growth temperature of 35 °C. Strain SS20^T was a Gram-negative, non-spore-forming, short rod 0·8–1·0 μm wide and 1·8–2·2 μm long. Cells were motile, each cell having several flagella (Fig. 1). Strain SS20^T showed oxidase- and catalase-positive reactions. It hydrolysed hypoxanthine, urea and L-tyrosine, but hydrolysis of aesculin, casein, gelatin, starch, Tween 80 and xanthine was not observed. The strain reduced nitrate to nitrite. It produced acids from D-glucose and glycerol, but not from L-arabinose, D-fructose, D-mannose, arbutin, D-salicin, maltose, α-D-lactose, D-melibiose, sucrose, D-trehalose, adonitol, D-xylose, D-galactose, D-mannitol or D-ribose. The phenotypic characteristics of strain SS20^T are summarized and compared with those of the type strains of closely related *Halomonas* species in Table 1.

The major isoprenoid quinone of strain SS20^T was Q-9. The fatty acid profile of strain SS20^T was characterized as containing saturated and unsaturated straight-fatty acids such as $C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo and $C_{16:0}$ (Table 2). The fatty acid composition on MA was slightly different from that on MA supplemented with 5 % NaCl (w/v), as reported previously (Arahal *et al.*, 2001; Bouchotroch *et al.*, 2001; Franzmann & Tindall, 1990; Valderrama *et al.*, 1998; Yoon *et al.*, 2001, 2002). However, the major fatty acid profile of strain SS20^T was similar to those of other members of the genus *Halomonas*, but was distinguishable from that of the genus *Zymobacter*, the closest phylogenetic neighbour

Table 1. Differential phenotypic characteristics of strain SS20^T and other related type strains of *Halomonas* species

Species/strain: 1, strain SS20^T; 2, *H. pacifica*; 3, *H. salina*; 4, *H. alimentaria*; 5, *H. maura*; 6, *H. elongata*; 7, *H. halophila*; 8, *H. eurihalina*. Data are from Arahal *et al.* (2002a), Baumann *et al.* (1983), Bouchotroch *et al.* (2001), Mata *et al.* (2002), Quesada *et al.* (1990), Valderrama *et al.* (1991), Ventosa *et al.* (1998), Yoon *et al.* (2002) and this study. Symbols: +, positive; −, negative; ND, not determined. All of the micro-organisms were negative for the hydrolysis of starch and casein.

Characteristic	1	2	3	4	5	6	7	8
Cell morphology	Short rod	Rod	Short rod	Short rod	Long rod	Rod	Rod	Short rod
Size (μm)	0·9–2·0	ND	0·7–0·8 × 2·0–2·5	0·8–1·2 × 1·3–1·9	0·6–0·7 × 6·0–9·0	2·0–4·0	0·5–0·7 × 1·5–2·0	0·8–1·0 × 2·0–2·5
Pigmentation	Cream	Cream	Cream–yellow	Cream–yellow	Cream	White	Cream	Cream
Flagellation	Peritrichous	Peritrichous	−	−	−	Peritrichous	Peritrichous	−
Motility	+	+	−	−	−	+	+	−
NaCl range (%, w/v)	1–20	0–20	2–20	0·5–23·0	1–15	0–20	2–30	0·5–25
NaCl optimum (%, w/v)	1–12	0·5–3·0	5·0	1–13	7·5–10·0	3·5–8·0	7·5	7·5
pH range	5·5–10·0	5·0–10·0	5·0–10·0	ND	6·0–9·0	5·0–9·0	5·0–10·0	5·0–10·0
Temperature range (°C)	10–47	4–45	4–45	4–45	10–40	15–45	10–45	4–45
Nitrate reduction	+	−	+	+	+	+	+	+
Catalase	+	ND	+	+	+	+	+	+
Oxidase	+	+	+	+	+	−	+	−
Hydrolysis of:								
Aesculin	−	−	−	−	−	+	+	+
Xanthine	−	−	ND	−	ND	ND	ND	ND
L-Tyrosine	+	−	+	−	−	−	−	+
Gelatin	−	−	−	−	−	+	−	+
Tween 80	−	−	−	−	−	−	−	+
Urea	+	+	+	+	−	+	+	+
Acid production from:								
L-Arabinose	−	−	−	ND	−	+	+	−
Sucrose	−	−	−	ND	−	+	−	−
α-D-Lactose	−	−	−	ND	−	+	−	−
D-Mannitol	−	+	−	ND	−	+	−	−
D-Glucose	+	+	−	−	−	+	+	−
D-Fructose	−	−	−	ND	−	−	+	−
D-Galactose	−	−	−	ND	−	−	+	−
D-Trehalose	−	−	−	ND	−	−	+	−
DNA G+C content (mol%)	70	67–68	60·7–64·2	63	62·2–64·1	60·5	66·7	65·7

Table 2. Cellular fatty acid composition of strain SS20^T on MA and MA supplemented with 5 % (w/v) NaCl

Data are expressed as percentages of total fatty acids. −, Not detected. Fatty acids representing less than 0·5 % are not included.

Fatty acid	MA	MA + 5 % NaCl
$C_{10:0}$	2·76	2·86
$C_{12:0}$ 3-OH	6·18	6·52
$C_{16:0}$	24·95	30·06
$C_{17:0}$ cyclo	1·63	4·12
$C_{18:1}\omega7c$	32·02	17·26
$C_{19:0}\omega8c$ cyclo	12·93	27·88
Summed feature 3*	17·37	8·76
Summed feature 7*	0·62	−

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso $C_{15:0}$ 2-OH; summed feature 7 comprised an unknown fatty acid with an equivalent chain length of 18·846, $C_{19:1}\omega6c$ and/or $C_{19:0}\omega10c$ cyclo.

(Arahal *et al.*, 2001; Bouchotroch *et al.*, 2001; Franzmann & Tindall, 1990; Okamoto *et al.*, 1993; Yoon *et al.*, 2002).

The DNA G+C content of strain SS20^T was 70 mol%, which is slightly higher than the level of previously reported *Halomonas* species (52–68 mol%; Arahal *et al.*, 2002b). However, in agreement with the fatty acid profiles, phylogenetic analysis based on the nearly complete 16S rRNA gene sequence (1399 nt) showed that strain SS20^T was positioned within the radiation of *Halomonas* species and formed a distinct phyletic line within a diffuse subclade of the genus in the neighbour-joining analysis (Fig. 2) as well as according to the maximum-likelihood method (data not shown). In addition, strain SS20^T displayed some phenotypic properties that were different from those of related *Halomonas* species (Table 1).

Strain SS20^T joined a clade including the type strains of *Halomonas elongata*, *Halomonas eurihalina*, *Halomonas halmophila*, *Halomonas halophila*, *H. salina*, *H. maura* and *H. pacifica* (Fig. 2). The levels of 16S rRNA gene sequence similarity between strain SS20^T and *H. pacifica* DSM 4742^T, *H. salina* ATCC 49509^T, *H. alimentaria* KCCM 41042^T, *H. maura* CECT 5298^T, *H. elongata* ATCC 33173^T, *H. halophila* DSM 4770^T, *H. eurihalina* ATCC 49336^T and *H. halmophila* ATCC 19717^T were 97·5, 97·2, 97·2, 97·1, 96·8, 96·5, 96·0 and 95·9 %, respectively. DNA–DNA relatedness between strain SS20^T and the type strains of closely related *Halomonas* species with high 16S rRNA gene sequence similarities was assessed. The values obtained were 10·8, 8·5, 11·2, 12·3, 5·5, 5·3, 7·5 and 7·8 % with *H. pacifica*, *H. salina*, *H. alimentaria*, *H. maura*, *H. elongata*, *H. eurihalina*, *H. halmophila* and *H. halophila*, respectively; these values are too low to allocate strain SS20^T to any species within the genus *Halomonas* (Wayne, 1994). Because some researchers have suggested that the genus *Halomonas* could be split into several genera, strain SS20^T may require reclassification in the future. On the basis of the phenotypic and molecular properties that have been reported to

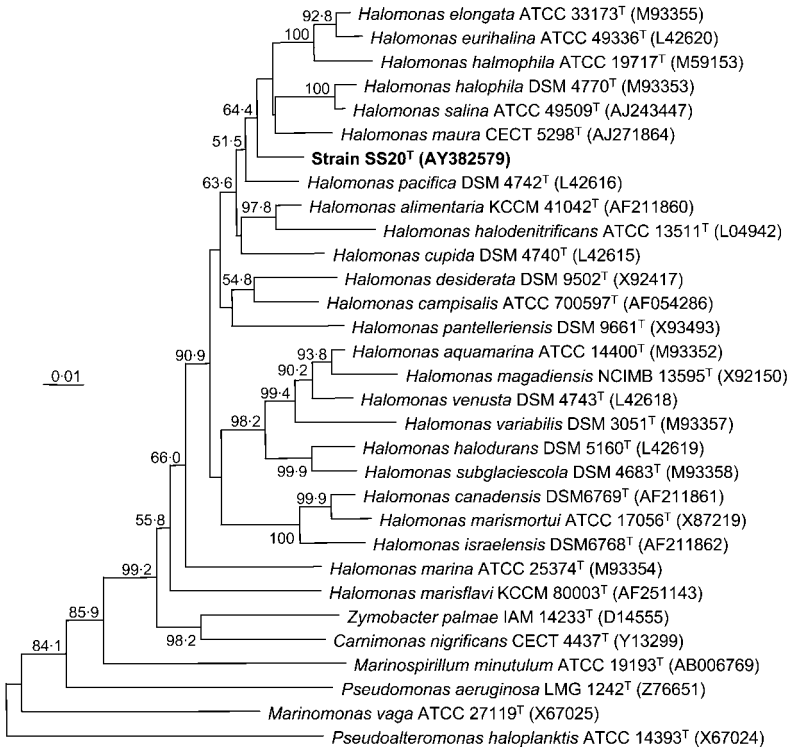

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain SS20^T and other related taxa. Numbers at branching nodes are bootstrap values (percentages of 1000 replications); only values greater than 50 % are indicated. Bar, 0·01 substitutions per nucleotide position.

date, we propose that strain SS20^T should be assigned to a novel species of the genus *Halomonas*, *Halomonas koreensis* sp. nov.

Description of *Halomonas koreensis* sp. nov.

Halomonas koreensis (ko.re.en′sis. N.L. fem. adj. *koreensis* pertaining to Korea).

Cells are Gram-negative, non-spore-forming, short rods measuring 0·8–1·0 μm in width and 1·8–2·2 μm in length. Oxidase- and catalase-positive. Cells are motile, each cell having several flagella. Colonies are creamy, smooth, glistening and circular/slightly irregular. Grows at salinities in the range 1–20 % (w/v) NaCl. Good growth at 1–12 % (w/v) NaCl. No growth occurs in the absence of salts. Grows between 10 and 47 °C (optimum, 35 °C) and from pH 5·5 to 10 (optimum, pH 7·0–8·0). Hypoxanthine, urea and L-tyrosine are hydrolysed. Aesculin, casein, gelatin, starch, Tween 80 and xanthine are not hydrolysed. Nitrate is reduced to nitrite. Acid is produced from D-glucose and glycerol, but not from L-arabinose, D-fructose, D-mannose, arbutin, D-salicin, maltose, α-D-lactose, D-melibiose, sucrose, D-trehalose, adonitol, D-xylose, D-galactose, D-mannitol or D-ribose. The predominant isoprenoid quinone is Q-9. The major fatty acids are $C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo and $C_{16:0}$. The DNA G+C content is 70 mol%.

The type strain is SS20^T (=KCTC 12127^T=JCM 12237^T), isolated from a solar saltern at Sungumi in Korea.

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References

Arahal, D. R., García, M. T., Vargas, C., Cánovas, D., Nieto, J. J. & Ventosa, A. (2001). *Chromohalobacter salexigens* sp. nov., a moderately halophilic species that includes *Halomonas elongata* DSM 3043 and ATCC 33174. *Int J Syst Evol Microbiol* **51**, 1457–1462.

Arahal, D. R., Castillo, A. M., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2002a). Proposal of *Cobetia marina* gen. nov., comb. nov., within the family *Halomonadaceae*, to include the species *Halomonas marina*. *Syst Appl Microbiol* **25**, 207–211.

Arahal, D. R., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2002b). Phylogeny of the family *Halomonadaceae* based on 23S and 16S rDNA sequence analyses. *Int J Syst Evol Microbiol* **52**, 241–249.

Baumann, L., Bowditch, R. D. & Baumann, P. (1983). Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. *Int J Syst Bacteriol* **33**, 793–802.

Bouchotroch, S., Quesada, E., Moral, A. D., Llamas, I. & Béjar, V. (2001). *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **51**, 1625–1632.

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

DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.

Dobson, S. J. & Franzmann, P. D. (1996). Unification of the genera *Deleya* (Baumann *et al.* 1983), *Halomonas* (Vreeland *et al.* 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family *Halomonadaceae*. *Int J Syst Bacteriol* **46**, 550–558.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Bacteriol* **39**, 224–229.

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

Franzmann, P. D. & Tindall, B. J. (1990). A chemotaxonomic study of members of the family *Halomonadaceae*. *Syst Appl Microbiol* **13**, 142–147.

García, M. T., Mellado, E., Ostos, J. C. & Ventosa, A. (2004). *Halomonas organivorans* sp. nov., a moderate halophile able to degrade aromatic compounds. *Int J Syst Evol Microbiol* **54**, 1723–1728.

Kaye, J. Z., Márquez, M. C., Ventosa, A. & Baross, J. A. (2004). *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *Int J Syst Evol Microbiol* **54**, 499–511.

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). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–208.

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

Margesin, R. & Schinner, F. (2001). Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles* **5**, 73–83.

Mata, J. A., Martínez-Cánovas, M. J., Quesada, E. & Béjar, 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.

Okamoto, T., Taguchi, H., Nakamura, K., Ikenaga, H., Kuraishi, H. & Yamasato, K. (1993). *Zymobacter palmae* gen. nov., sp. nov., a new ethanol-fermenting peritrichous bacterium isolated from palm sap. *Arch Microbiol* **160**, 333–337.

Quesada, E., Valderrama, M. J., Bejar, V., Ventosa, A., Gutiérrez, M. C., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1990). *Volcaniella eurihalina* gen. nov., sp. nov., a moderately halophilic nonmotile gram-negative rod. *Int J Syst Bacteriol* **40**, 261–267.

Reddy, G. S. N., Raghavan, P. U. M., Sarita, N. B., Prakash, J. S. S., Nagesh, N., Delille, D. & Shivaji, S. (2003). *Halomonas glaciei* sp. nov. isolated from fast ice of Adelie Land, Antarctica. *Extremophiles* **7**, 55–61.

Romanenko, L. A., Schumann, P., Roche, M., Mikhailov, V. V. & Stackebrandt, E. (2002). *Halomonas halocynthiae* sp. nov., isolated from the marine ascidian *Halocynthia aurantium*. *Int J Syst Evol Microbiol* **52**, 1767–1772.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

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.

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

Valderrama, M. J., Quesada, E., Bejar, V., Ventosa, A., Gutierrez, M. C., Berraquero, F. R. & Cormenzana, A. R. (1991). *Deleya salina* sp. nov., a moderately halophilic Gram-negative bacterium. *Int J Syst Bacteriol* **41**, 377–384.

Valderrama, M. J., Monteoliva-Sanchez, M., Quesada, E. & Ramos-Cormenzana, A. (1998). Influence of salt concentration on the cellular fatty acid composition of the moderately halophilic bacterium *Halomonas salina*. *Res Microbiol* **149**, 675–679.

Ventosa, A., Nieto, J. J. & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.

Vreeland, R. H., Litchfield, C. D., Martin, E. L. & Elliot, E. (1980). *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. *Int J Syst Bacteriol* **30**, 485–495.

Wayne, L. G. (1994). Actions of the Judicial Commission of the International Committee on Systematic Bacteriology on requests for opinions published between January 1985 and July 1993. *Int J Syst Bacteriol* **44**, 177–178.

Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R., Abraham, W. R., Lunsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.

Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.

Yoon, J.-H., Choi, S. H., Lee, K.-C., Kho, Y. H., Kang, K. H. & Park, Y.-H. (2001). *Halomonas marisflavae* sp. nov., a halophilic bacterium isolated from the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **51**, 1171–1177.

Yoon, J.-H., Lee, K.-C., Kho, Y.-H., Kang, K.-H., Kim, C.-J. & Park, Y.-H. (2002). *Halomonas alimentaria* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **52**, 123–130.