Halomonas saccharevitans sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China

Xue-Wei Xu,[1]† Yue-Hong Wu,[1]† Zhen Zhou,[1] Chun-Sheng Wang,[2] Yu-Guang Zhou,[3] Hui-Bin Zhang,[4] Yong Wang[1] and Min Wu[1]

Correspondence
Min Wu
wumin@zju.edu.cn

[1]College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

[2]Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, People's Republic of China

[3]Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

[4]Altun Mountain National Nature Reserve Administration, Kuerle 841000, People's Republic of China

Three strains of Gram-negative, aerobic, neutrophilic and halophilic bacteria were isolated from samples of a salt lake on the Qinghai–Tibet Plateau and a subterranean saline well in the Si-Chuan Basin of China. These isolates, designated AJ275^T, AJ282^T and ZG16^T, were investigated using a polyphasic approach. Based on 16S rRNA gene sequence analysis, the isolates could be affiliated to the genus *Halomonas*. Genomic DNA G+C contents were 65.9 mol% for AJ275^T, 56.7 mol% for AJ282^T and 57.6 mol% for ZG16^T. The results of DNA–DNA hybridizations, fatty acid analysis and physiological and biochemical tests allowed the isolates to be differentiated genotypically and phenotypically from closely related species. It is proposed that strains AJ275^T (=CGMCC 1.6493^T=JCM 14606^T=LMG 23976^T), AJ282^T (=CGMCC 1.6494^T=JCM 14607^T=LMG 23978^T) and ZG16^T (=CGMCC 1.6495^T=JCM 14608^T=LMG 23977^T) represent the type strains of three novel species in the genus *Halomonas*: *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., respectively.

The genus *Halomonas*, belonging to the family *Halomonadaceae* within the class *Gammaproteobacteria*, was originally proposed by Vreeland *et al.* (1980). During the past two decades, many *Halomonas* species have been isolated from different saline environments, such as saline or soda lakes (Franzmann *et al.*, 1987; James *et al.*, 1990; Mormile *et al.*, 1999; Duckworth *et al.*, 2000; Quillaguamán *et al.*, 2004), solar salterns (Bouchotroch *et al.*, 2001; Lim *et al.*, 2004; Martínez-Checa *et al.*, 2005; Lee *et al.*, 2005), saline sands or soils (Romano *et al.*, 1996; Martínez-Cánovas *et al.*, 2004a, b; García *et al.*, 2004), mineral pools (Romano *et al.*, 2005), marine environments (Yoon *et al.*, 2001; Kaye *et al.*, 2004), animals (Romanenko *et al.*, 2002), seafoods (Yoon *et al.*, 2002), artificial sewage treatments (Berendes *et al.*, 1996) and walls and mural paintings (Heyrman *et al.*, 2002). Additionally, some bacteria that were assigned initially to other genera have been reclassified (Mellado *et al.*, 1995; Dobson & Franzmann, 1996). In total, 35 species of *Halomonas* have been described at the time of writing.

In this study, three novel halophilic bacteria are described using a polyphasic approach. Strains AJ282^T and AJ275^T were isolated from a water sample from Ayakekum salt lake (37° 33′ N 89° 42′ E; 3884 m altitude) located in Altun Mountain on the Qinghai–Tibet Plateau, China. Strain ZG16^T was isolated from subterranean hypersaline waters taken from a saline well located in Zigong (29° 3′ N 105° 7′ E) in the Si-Chuan Basin, China.

The medium (HM) used for isolation and maintenance of the strains was that described by Ventosa *et al.* (1982). The medium (pH 7.5) contained (%, w/v): NaCl, 5.0; KCl, 0.2; $MgSO_4.7H_2O$, 0.1; $CaCl_2.2H_2O$, 0.036; NaBr, 0.023; $NaHCO_3$, 0.006; yeast extract (Difco), 1.0; peptone (Difco), 0.5; glucose, 0.1. Water samples were filtered through

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains AJ282^T, ZG16^T and AJ275^T are EF144147, EF144148 and EF144149, respectively.

0.45 μm and 0.22 μm filters in sequence. The 0.22 μm membranes were added to HM medium and plated by using a tenfold dilution series method. Plates were incubated aerobically at 25 °C. After 3–7 days incubation, representative colonies were picked and maintained at 30 °C. Strains were purified by repeated restreaking; purity was confirmed by the uniformity of colony morphology. Cell morphology and motility were examined by optical microscopy (Olympus BX40). The optimal conditions for growth were determined in HM medium with 0–30 % (w/v) NaCl. The pH range for growth was determined by adding the buffers MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CHES (pH 9.0–10.0) to HM medium at a concentration of 50 mM. The temperature range for growth was determined by incubating the strains at 4–55 °C.

Phenotypic characteristics, including oxidase and catalase reactions, H_2S production, hydrolysis of aesculin, gelatin, casein, DNA, starch, Tween 20, Tween 80, tyrosine and urea, indole production, gluconate oxidation, phenylalanine deamination, substrate utilization and acid production from sugars, were tested in HM medium according to the methods of Mata *et al.* (2002). Antimicrobial susceptibility tests were performed in liquid HM medium containing 50 μg antimicrobial agent ml^{-1}. Detailed results are given in the species description.

Fatty acid methyl esters were obtained from cells grown in HM medium for 1 day at 30 °C and analysed by using GC/MS (Kuykendall *et al.*, 1988); data are given in Table 1. The 16S rRNA genes were amplified as described previously (Xu

Table 1. Differential phenotypic characteristics of the novel isolates and the type strains of related *Halomonas* species

Strains: 1, AJ282^T; 2, ZG16^T; 3, AJ275^T; 4, *H. sulfidaeris* DSM 15722^T; 5, *H. hydrothermalis* DSM 15725^T; 6, *H. venusta* CGMCC 1.2315^T; 7, *H. ventosae* DSM 15911^T. +, Positive; −, negative; ND, not determined; TR, trace. Data were from our comparative tests. All strains were positive for catalase and gluconate oxidation and negative for indole production and hydrolysis of gelatin and Tween 80.

Characteristic	1	2	3	4	5	6	7
Oxidase	−	−	+	+	+	+	+
H_2S formation	+	+	−	−	ND	−	−
Phenylalanine deamination	+	−	+	+	+	−	−
Hydrolysis of:							
Aesculin	−	−	−	−	−	−	+
Casein	+	+	−	+	−	−	−
Tween 20	+	+	+	+	−	−	+
Tyrosine	−	−	−	−	−	+	+
Urea	−	+	−	−	+	+	−
Utilization of:							
Citrate	+	+	−	+	+	+	+
Ethanol	+	−	−	+	−	+	−
Histidine	−	+	−	−	−	−	−
Isoleucine	−	−	+	+	+	+	ND
Malate	+	+	−	+	+	+	+
Acid production from:							
Galactose	+	+	−	+	ND	+	+
Glucose	+	+	−	+	+	+	+
Inositol	−	+	−	+	+	+	+
Maltose	+	+	−	+	ND	+	+
Sorbitol	+	+	−	−	−	+	+
Sucrose	+	+	−	−	ND	+	+
Susceptibility to:							
Nitrofurantoin	−	+	+	+	+	+	+
Penicillin	−	−	+	+	−	−	−
Major fatty acid methyl esters (%):							
16 : 1ω7c	8.94	3.31	10.37	5.75	3.84	9.84	11.77
16 : 0	17.89	21.30	20.60	18.12	17.79	9.38	21.62
18 : 1ω7c	30.03	29.46	30.65	42.89	36.46	42.36	27.11
18 : 0	11.41	12.42	14.78	TR	14.26	13.92	10.90
19 : 0 cyclo ω8c	9.03	11.57	4.42	7.37	TR	TR	6.65

et al., 2005) with primers 1 (5′-AGAGTTTGATCCTGGCT-CAG-3′; positions 8–27 according to the *Escherichia coli* 16S rRNA gene) and 2 (5′-GGTTACCTTGTTACGACTT-3′; 1510–1492).

The sequence was compared with closely related sequences of reference organisms from the FASTA network service. Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by the neighbour-joining method with the MEGA3 program package (Kumar *et al.*, 2004).The DNA G+C content was determined by thermal denaturation (T_m) (Marmur & Doty, 1962) using *E. coli* K12 DNA as the calibration standard. DNA–DNA hybridizations were performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983), using a Beckman DU 800 spectrophotometer.

16S rRNA gene sequence analysis indicated that strains AJ275^T, AJ282^T and ZG16^T clustered within the genus *Halomonas* (Fig. 1). Strain AJ275^T exhibited the closest phylogenetic affinity and highest sequence similarity to *Halomonas ventosae* DSM 15911^T (97.6 %). 16S rRNA gene sequence similarity values between strain AJ275^T and other *Halomonas* species were below 96.5 %. The DNA G+C content of strain AJ275^T (65.9 mol%) was close to the upper limit of typical values for *Halomonas* species (52–68 mol%; Franzmann *et al.*, 1988), but was notably lower than that of *H. ventosae* DSM 15911^T (73.4 mol%; Martínez-Cánovas *et al.*, 2004a). DNA–DNA hybridization was carried out at 80 °C. The DNA–DNA relatedness level between strain AJ275^T and *H. ventosae* DSM 15911^T was 17 %. Phylogenetic analysis based on 16S rRNA gene sequence comparison showed that strains AJ282^T and ZG16^T could be placed in a parallel branch with *Halomonas sulfidaeris* and *Halomonas hydrothermalis* with high bootstrap values (Fig. 1). The 16S rRNA gene sequence similarities of these two novel isolates were around 97 % to *H. sulfidaeris* DSM 15722^T and *H. hydrothermalis* DSM 15725^T. DNA–DNA relatedness between the novel isolates and *H. sulfidaeris* DSM 15722^T, *H. hydrothermalis* DSM 15725^T and *Halomonas venusta* CGMCC 1.2315^T was less than 50 % (Table 2). In addition, comparison of phenotypic properties (Table 1) also indicated differences between the novel isolates and other *Halomonas* species, such as hydrolysis of substrates, acid production from sugars, sensitivity to antimicrobial agents and fatty acid composition.

Based on 16S rRNA gene sequence analysis, as well as DNA–DNA hybridization data and differential phenotypic properties, it is concluded that strains AJ275^T, AJ282^T and ZG16^T represent three novel species within the genus *Halomonas*, *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., respectively.

Description of *Halomonas saccharevitans* sp. nov.

Halomonas saccharevitans (sac.char.e′vi.tans. L. n. *saccharon -i* a kind of sugar; L. part. adj. *evitans* avoiding; N.L. part. adj. *saccharevitans* sugar avoiding, because it uses very few sugars).

Gram-negative. Aerobic. Oxidase- and catalase-positive. Motile cocci, 0.8–1.2 μm in diameter. Young cultures show ovoid-like cells (1–2 μm wide and 2–4 μm long). Colonies on complex agar medium are 1–2 mm in diameter, smooth, circular, elevated and light yellow after 2 days. Moderately halophilic. NaCl concentration for growth is between 0.5 and 15.0 % (w/v), with optimum growth at 3.0–7.5 %. Grows at pH 6.0–10.0 and 4–48 °C (optimum growth at pH 7.0–8.0 and 30 °C). Tween 20 is hydrolysed. Aesculin, casein, DNA, gelatin, starch, Tween 80 and tyrosine are not hydrolysed. Phenylalanine deamination and gluconate

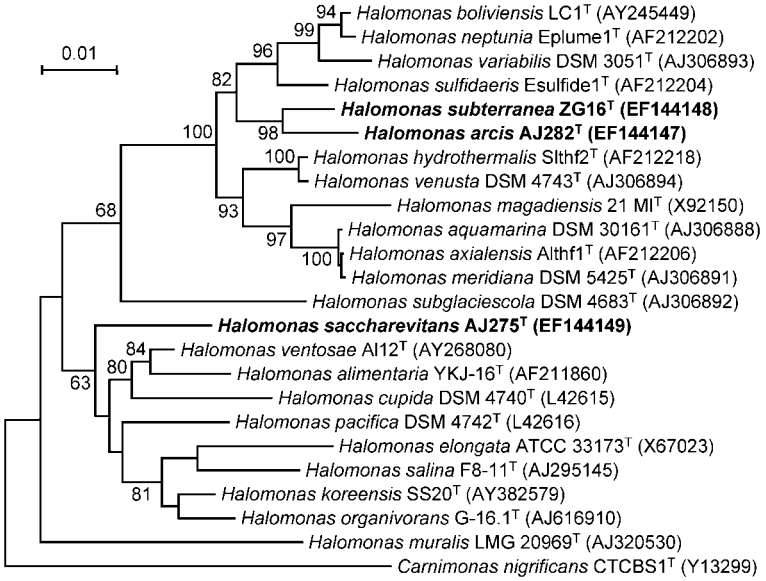

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolates and related taxa. Bootstrap values are based on 1000 replicates; only values >60 % are show. Bar, 0.01 substitutions per nucleotide position.

Table 2. DNA G+C content (T_m) and DNA–DNA hybridization between the novel strains and related species of the genus *Halomonas*

Strain	DNA G+C content (mol%)	DNA–DNA hybridization with: (%)	
		AJ282^T	ZG16^T
AJ282^T	56.7±0.3	100	46
ZG16^T	57.6±1.1	46	100
H. sulfidaeris DSM 15722^T	56.0*	48	49
H. hydrothermalis DSM 15725^T	56.3*	25	7
H. venusta CGMCC 1.2315^T	ND	25	ND

ND, Not determined.
*Data from Kaye *et al.* (2004).

oxidation are positive. Negative for production of indole and urease. H_2S is not produced from thiosulfate. Chemo-organotrophic. Casamino acids are required for growth. The following substrates are utilized for growth: glycerol, fumarate, alanine, aspartate, glutamate, isoleucine, serine and valine. No growth is observed on arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, melezitose, rhamnose, ribose, sorbose, sucrose, trehalose, xylose, adonitol, ethanol, inositol, mannitol, sorbitol, salicin, acetate, citrate, formate, gluconate, malate, malonate, propionate, succinate, arginine, glycine, histidine, leucine, lysine, methionine or ornithine. Susceptible to ampicillin, carbenicillin, cefotaxime, chloramphenicol, erythromycin, nalidixic acid, nitrofurantoin, penicillin, polymyxin B and treptomycin, but not to kanamycin, neomycin, nystain, rifampicin or streptomycin. Principal fatty acids (greater than 5 %) are 18 : 1ω7*c*, 16 : 0, 18 : 0 and 16 : 1ω7*c*.

The type strain is AJ275^T (=CGMCC 1.6493^T=JCM 14606^T=LMG 23976^T), isolated from a water sample taken from a salt lake on the Qinghai–Tibet Plateau, China. The DNA G+C content of strain AJ275^T is 65.9± 0.3 mol% (T_m).

Description of *Halomonas arcis* sp. nov.

Halomonas arcis (ar′cis. L. gen. n. *arcis* of a height, summit or peak, referring to the isolation of the organism from a salt lake on the Qinghai–Tibet Plateau).

Gram-negative and motile. Young cultures show rod-like cells (0.5–1.0×2.0–4.0 μm). Colonies on complex agar medium are smooth, circular, elevated and cream. Halotolerant. NaCl concentration for growth is between 0 and 20 % (w/v), with optimum growth at 1–5 % (w/v). Grows at pH 6.0–10.0 and 4–48 °C (optimum growth at pH 7.0–8.0 and 30 °C). Catalase is produced, but not oxidase. Tween 20 and casein are hydrolysed. H_2S is produced from thiosulfate. Aesculin, DNA, gelatin, starch, Tween 80 and tyrosine are not hydrolysed. Phenylalanine deamination and gluconate oxidation are positive. Indole and urease production are negative. Chemo-organotrophic. Casamino acids are required for growth. Acid is produced from galactose and glucose and, to a lesser extent, from arabinose, fructose, maltose, mannitol, melezitose, sorbitol, sucrose and trehalose. No growth is observed on cellobiose, lactose, mannose, rhamnose, ribose or xylose. The following substrates are utilized for growth: xylose, ethanol, glycerol, acetate, citrate, fumarate, gluconate, malate, malonate, propionate, succinate, alanine, arginine, aspartate, glutamate, lysine, ornithine and valine. Susceptible to chloramphenicol, erythromycin, nalidixic acid, polymyxin B and treptomycin, but not to ampicillin, kanamycin, neomycin, nitrofurantoin, nystain, penicillin, rifampicin or streptomycin. Principal fatty acids (greater than 5 %) are 18 : 1ω7*c*, 16 : 0, 18 : 0, 19 : 0 cyclo ω8*c* and 16 : 1ω7*c*.

The type strain is AJ282^T (=CGMCC 1.6494^T=JCM 14607^T=LMG 23978^T), isolated from a water sample taken from a salt lake located in Altun Mountain on the Qinghai–Tibet Plateau, China. The DNA G+C content of strain AJ282^T is 56.7±0.3 mol% (T_m).

Description of *Halomonas subterranea* sp. nov.

Halomonas subterranea (sub.ter.ra′ne.a. L. fem. adj. *subterranea* underground, subterranean, referring to the isolation of the organism from the subterranean brines).

Gram-negative and motile. Young cultures show rod-like cells (0.5–1.0×3.0–5 μm). Colonies on complex agar medium are smooth, circular, elevated and cream. Halotolerant. NaCl concentration for growth is between 0 and 15 % (w/v), with optimum growth at 1–5 % (w/v). Grows at pH 6.0–10.0 and 4–48 °C (optimum growth at pH 7.0–8.0 and 30 °C). Catalase is produced, but not oxidase. Tween 20, casein and urea are hydrolysed. H_2S is produced from thiosulfate. Aesculin, DNA, gelatin, starch, Tween 80 and tyrosine are not hydrolysed. Gluconate oxidation is positive. Indole production and phenylalanine deamination are negative. Chemo-organotrophic. Casamino acids are required for growth. Acid is produced from arabinose, galactose and glucose and, to a lesser extent, from fructose, inositol, maltose, mannitol, melezitose, sorbitol, sucrose and trehalose. No growth is observed on cellobiose, lactose,

mannose, rhamnose, ribose, sorbose or xylose. The following substrates are utilized for growth: xylose, glycerol, acetate, citrate, fumarate, gluconate, malate, succinate, alanine, arginine, aspartate, glutamate, histidine and lysine. Susceptible to chloramphenicol, erythromycin, nalidixic acid, nitrofurantoin, polymyxin B and treptomycin, but not to ampicillin, kanamycin, neomycin, nystain, penicillin, rifampicin or streptomycin. Principal fatty acids (greater than 5 %) are 18 : 1ω7*c*, 16 : 0, 18 : 0 and 19 : 0 cyclo ω8*c*.

The type strain is ZG16^T (=CGMCC 1.6495^T=JCM 14608^T=LMG 23977^T), isolated from hypersaline waters taken from a subterranean saline well on the Si-Chuan Basin, China. The DNA G+C content of strain ZG16^T is 57.6$\pm$1.1 mol% (T_m).

Acknowledgements

We thank Jean Euzéby for his help with the specific etymology and nomenclature. This work was supported by grants from the Major State Basic Research Development Program of China (973 Program) (grant no. 2004CB719604-3) and the National Natural Science Foundation of China (grant no. 30670048).

References

Berendes, F., Gottschalk, G., Heine-Dobbernack, E., Moore, E. R. B. & Tindall, B. J. (1996). *Halomonas desiderata* sp. nov., a new alkaliphilic, halotolerant and denitrifying bacterium isolated from a municipal sewage works. *Syst Appl Microbiol* **19**, 158–167.

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.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

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.

Duckworth, A. W., Grant, W. D., Jones, B. E., Meijer, D., Márquez, M. C. & Ventosa, A. (2000). *Halomonas magadii* sp. nov., a new member of the genus *Halomonas*, isolated from a soda lake of the East African Rift Valley. *Extremophiles* **4**, 53–60.

Franzmann, P. D., Burton, H. R. & McMeekin, T. A. (1987). *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from Antarctica. *Int J Syst Bacteriol* **37**, 27–34.

Franzmann, P. D., Wehmeyer, U. & Stackebrandt, E. (1988). *Halomonadaceae* fam. nov., a new family of the class *Proteobacteria* to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* **11**, 16–19.

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.

Heyrman, J., Balcaen, A., De Vos, P. & Swings, J. (2002). *Halomonas muralis* sp. nov., isolated from microbial biofilms colonizing the walls and murals of the Saint-Catherine chapel (Castle Herberstein, Austria). *Int J Syst Evol Microbiol* **52**, 2049–2054.

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.

James, S. R., Dobson, S. J., Franzmann, P. D. & McMeekin, T. A. (1990). *Halomonas meridiana*, a new species of extremely halotolerant bacteria isolated from Antarctic saline lakes. *Syst Appl Microbiol* **13**, 270–278.

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.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lee, J.-C., Jeon, C.-O., Lim, J.-M., Lee, S.-M., Lee, J.-M., Song, S.-M., Park, D.-J., Li, W.-J. & Kim, C.-J. (2005). *Halomonas taeanensis* sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea. *Int J Syst Evol Microbiol* **55**, 2027–2032.

Lim, J.-M., Yoon, J.-H., Lee, J.-C., Jeon, C.-O., Park, D.-J., Sung, C. & Kim, C.-J. (2004). *Halomonas koreensis* sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea. *Int J Syst Evol Microbiol* **54**, 2037–2042.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Martínez-Cánovas, M. J., Quesada, E., Llamas, I. & Béjar, V. (2004a). *Halomonas ventosae* sp. nov., a moderately halophilic, denitrifying, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **54**, 733–737.

Martínez-Cánovas, M. J., Béjar, V., Martínez-Checa, F. & Quesada, E. (2004b). *Halomonas anticariensis* sp. nov., from Fuente de Piedra, a saline-wetland wildfowl reserve in Málaga, southern Spain. *Int J Syst Evol Microbiol* **54**, 1329–1332.

Martínez-Checa, F., Béjar, V., Martínez-Cánovas, M. J., Llamas, I. & Quesada, E. (2005). *Halomonas almeriensis* sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium from Cabo de Gata, Almería, south-east Spain. *Int J Syst Evol Microbiol* **55**, 2007–2011.

Mata, J. A., Martinez-Canovas, J., Quesada, E. & Bejar, V. (2002). A detailed phenotypic characterisation 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.

Mormile, M. R., Romine, M. F., Garcia, M. T., Ventosa, A., Bailey, T. J. & Peyton, B. M. (1999). *Halomonas campisalis* sp. nov., a denitrifying, moderately haloalkaliphilic bacterium. *Syst Appl Microbiol* **22**, 551–558.

Quillaguamán, J., Hatti-Kaul, R., Mattiasson, B., Alvarez, M. T. & Delgado, O. (2004). *Halomonas boliviensis* sp. nov., an alkalitolerant, moderate halophile isolated from soil around a Bolivian hypersaline lake. *Int J Syst Evol Microbiol* **54**, 721–725.

Romanenko, L. A., Schumann, P., Rohde, 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.

Romano, I., Nicolaus, B., Lama, L., Manca, M. C. & Gambacorta, A. (1996). Characterization of a haloalkalophilic strictly aerobic bacterium, isolated from Pantelleria Island. *Syst Appl Microbiol* **19**, 326–333.

Romano, I., Gioradano, A., Lama, L., Nicolaus, B. & Gambacorta, A. (2005). *Halomonas campaniensis* sp. nov., a haloalkaliphilic bacterium isolated from a mineral pool of Campania Region, Italy. *Syst Appl Microbiol* **28**, 610–618.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Ventosa, A., Quesada, E., Rodriguez-Valera, F., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1982). Numerical taxonomy of moderately halophilic Gram-negative rods. *J Gen Microbiol* **128**, 1959–1968.

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.

Xu, X.-W., Wu, M. & Huang, W.-D. (2005). Isolation and characterization of a novel strain of *Natrinema* containing a *bop* gene. *J Zhejiang Univ Sci B* **6**, 142–146.

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.