Halomonas vilamensis sp. nov., isolated from high-altitude Andean lakes

Rodolfo Javier Menes,[1] Claudia Elizabeth Viera,[1] María Eugenia Farías[2,3] and Manfredo J. Seufferheld[4]

Correspondence
Rodolfo Javier Menes
jmenes@fq.edu.uy

[1]Cátedra de Microbiología, Facultad de Química y Facultad de Ciencias, UDELAR C.C. 1157. Montevideo, Uruguay

[2]CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

[3]PROIMI, Planta Piloto de Procesos Industriales Microbiológicos, Av. Belgrano y Pasaje Caseros-(4000), Tucumán, Argentina

[4]Department of Crop Sciences, 311 Edgar R. Madigan Lab, University of Illinois at Urbana-Champaign, 1201 W. Gregory Drive, Urbana, IL 61801, USA

A Gram-stain-negative, aerobic, rod-shaped, non-spore-forming bacterium (SV325^T) was isolated from the sediment of a hypersaline lake located 4600 m above sea level (Laguna Vilama, Argentina). Strain SV325^T formed cream to pink colonies, was motile and moderately halophilic, and tolerated NaCl concentrations of 1–25 % (w/v) with an optimum of 5–10 % (w/v). Growth occurred at 5–40 °C (optimum around 30 °C) and at pH 5.0–10.0 (optimum 7.0–8.0). The bacterium did not produce exopolysaccharides and stained positively for intracellular polyphosphate granules but not for poly-β-hydroxyalkanoates. It produced catalase and oxidase, reduced nitrate to nitrite, hydrolysed gelatin, did not produce acids from sugars and utilized a limited range of substrates as carbon and energy sources: acetate, caproate, fumarate, DL-β-hydroxybutyrate, malate, maleate, malonate and succinate. The predominant ubiquinones were Q-9 (92.5 %) and Q-8 (7.5 %), the major fatty acids were $C_{19:0}$ cyclo ω8c, $C_{16:0}$, $C_{17:0}$ cyclo and $C_{16:1}\omega$7c/iso-$C_{15:0}$ 2-OH, and the DNA G+C content was 55.0 mol%. Phylogenetic analyses based on the 16S rRNA gene indicated that strain SV325^T belongs to the genus *Halomonas* in the class *Gammaproteobacteria*. Physiological and biochemical tests allowed phenotypic differentiation of strain SV325^T from closely related species with validly published names. We therefore propose a novel species, *Halomonas vilamensis* sp. nov., with type strain SV325^T (=DSM 21020^T =LMG 24332^T).

Halophiles constitute an important group of extremophilic micro-organisms adapted to live and thrive in diverse hypersaline niches such as solar salterns, brines, hypersaline soils and lakes, as well as in salty foods (Horikoshi & Grant, 1998). This group of organisms are a promising valuable resource for biotechnological applications such as the production of compounds of industrial interest (osmoprotectants, extracellular enzymes and polymers among others). Moderately halophilic bacteria constitute a heterogeneous physiological group of micro-organisms which belong to different genera. Besides members of the family *Halomonadaceae*, several other Gram-negative, strictly aerobic or facultatively anaerobic species have been described as moderate halophiles (Ventosa *et al.*, 1998). The family *Halomonadaceae* of the class *Gammaproteobacteria* was originally proposed by Franzmann *et al.* (1988) to accommodate the genera *Deleya* and *Halomonas*. At the time of writing, it comprises ten genera (*Aidingimonas*, *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Halomonas*, *Halotalea*, *Kushneria*, *Modicisalibacter*, *Salinicola* and *Zymobacter*) of which *Halomonas* (Vreeland *et al.*, 1980; Dobson & Franzmann, 1996) is the largest genus, with around 70 species. The most recently described species are *Halomonas andesensis* (Sánchez-Porro *et al.*, 2010) and *Halomonas titanicae* (Guzmán *et al.*, 2010).

The microbial communities that have evolved within the high-altitude aquatic ecosystem of Laguna Vilama (Vilama Lake, Jujuy, Argentina) must tolerate chemical and physical stresses such as hypersalinity, wide fluctuations in daily temperature (−20 to 30 °C), variable pH, high levels of UV radiation, low nutrient availability and high heavy metal

Abbreviation: SW, saltwater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SV325^T is EU557315.

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

concentrations, especially arsenic. This isolated, undisturbed and biotically extreme aquatic ecosystem is a unique site for the discovery of novel bacterial species as well as for studying the interactions between the environment and the evolution of biodiversity (Seufferheld *et al.*, 2008). The lake is located 4600 m above sea level in the north-western section of the Andean Argentinean Puna desert (22° 35′ S 66° 55′ W). In this paper, we describe a novel species of the genus *Halomonas* for which we propose the name *Halomonas vilamensis* sp. nov.

Strain SV325^T was isolated from lake sediment samples by serial dilutions in 0.9 % NaCl solution, spread-plated on modified growth medium 25 (MGM 25) agar and incubated aerobically for 10 days at 30 °C. MGM has the following composition (l^{-1}): 5.0 g tryptone, 1.0 g yeast extract and 333 ml (for MGM 10) or 830 ml (for MGM 25) saltwater (SW) solution (as described in the Halohandbook; Dyall-Smith, 2008). The saltwater solution contained (l^{-1}) 240 g NaCl, 30 g $MgCl_2.6H_2O$, 35 g $MgSO_4.7H_2O$, 7 g KCl and 5 ml 1M $CaCl_2.2H_2O$. Fifteen grams of agar was added for solid medium. The pH was adjusted to 7.5 ± 0.2 and the medium was autoclaved for 15 min at 121 °C. The strain was routinely grown on MGM 10 agar at 30 °C for 24–48 h and preserved at −70 °C in MGM 10 broth supplemented with 20 % (v/v) glycerol.

The following culture collection strains were obtained from DSMZ (Braunschweig, Germany): *Halomonas gomseomensis* DSM 18042^T, *Halomonas sulfidaeris* DSM 15722^T, *Halomonas neptunia* DSM 15720^T and *Halomonas janggokensis* DSM 18043^T. Strains *Halomonas arcis* LMG 23978^T and *Halomonas subterranea* LMG 23977^T were obtained from BCCM/LMG (Gent, Belgium). The strains were cultured on MGM 10 agar at 30 °C for 48 h.

The characterization of strain SV325^T was performed according to Arahal *et al.* (2007). All physiological tests were incubated at 30 °C for up to 10 days unless otherwise stated.

Gram staining was performed as described by Smibert & Krieg (1994). Poly-β-hydroxyalkanoate and polyphosphate staining was carried out using 15-day-old cultures grown in MGM 10 broth, according to Murray *et al.* (1994). Colony morphology, production of diffusible or non-diffusible pigments and exopolysaccharide production were observed on MGM 10 agar after cultivation of the strain for 3 days. Motility and shape were observed by phase-contrast microscopy after cultivation of the strains in MGM 10 broth for 2 days (Zeiss Axioplan microscope). Oxidase reaction was performed with a 1 % aqueous solution of *N*, *N*-dimethyl-*p*-phenylenediamine, and catalase activity was determined by adding a 1 % H_2O_2 solution to a culture grown on MGM 10 agar for 24 h. To determine the range of substrates used as carbon and energy sources or as carbon, nitrogen and energy sources, we utilized a basal medium, BBSW5 (pH 7.2 ± 0.2), with the following composition (l^{-1}): 0.05 g yeast extract (Difco), 0.1 g NH_4Cl, 0.08 g KH_2PO_4, 10 ml trace element solution (Menes & Muxí, 2002) and 167 ml SW solution (final concentration of total salts 5.0 %). After sterilization, 1 ml filter-sterilized vitamin solution was added (Menes & Muxí, 2002). Each substrate was added aseptically from a filter-sterilized stock solution to a final concentration of 1 g l^{-1}, except for carbohydrates, which were added to 2 g l^{-1}. The basal medium was prepared without NH_4Cl for amino acid substrates. To study different electron acceptors, the basal medium was prepared anaerobically in Hungate tubes (Menes & Muxí, 2002). Electron acceptors and substrates were added from anaerobic stock solutions to final concentrations of 10 mM and 1 g l^{-1}, respectively. Koser medium, which is recommended by Arahal *et al.* (2007) for determining the range of substrates, did not support the growth of strain SV325^T even after vitamin supplementation. Oxidation and fermentation of glucose were determined in OF medium (Hugh & Leifson, 1953) with 7.5 % (w/v) sea salts (Rodríguez-Valera *et al.*, 1981). Tests for acid production from carbohydrates and alcohols (L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melezitose, raffinose, L-rhamnose, D-salicin, sorbose, trehalose, D-xylose, sucrose, adonitol, ethanol, glycerol, *myo*-inositol, D-mannitol, propanol, D-sorbitol); hydrogen sulfide production; aerobic nitrate reduction; hydrolysis of gelatin, casein, starch, Tween 20, Tween 80, tyrosine, aesculin, urea and DNA; phosphatase production; haemolysis; pigment production from tyrosine; phenylalanine deamination; selenite reduction; gluconate oxidation; ONPG activity; indole production; lysine and ornithine decarboxylase activity; and lecithovitellin, methyl red and Voges–Proskauer tests were performed according to Mata *et al.*, (2002). Anaerobic growth was checked in MH medium with 7.5 % (w/v) sea salts (Rodríguez-Valera *et al.*, 1981) using the Generbag Anaer system (bioMérieux). Tributyrin hydrolysis was assessed in MH medium with 7.5 % (w/v) sea salts according to Smibert & Krieg (1994). Tests for growth on MacConkey and cetrimide agar were supplemented with 7.5 % (w/v) sea salts. Respiration on fumarate (10 mM), nitrate (10 mM) and nitrite (5 mM) was assessed in anaerobically prepared MH medium with 7.5 % (w/v) sea salts, dispensed in Balch tubes and gassed with oxygen-free nitrogen. API 20E and API 20NE kits (bioMérieux) were inoculated according to the manufacturer's instructions except that cell suspensions were prepared in diluted SW (1 : 6 in water) and carbon assimilation tests were prepared using 5 ml of AUX medium supplemented with 1 ml of SW. Acid production from carbohydrates was tested with the API 50 CH kit (bioMérieux) with the cell suspension prepared using 10 ml API 50 CHL supplemented with 2 ml SW. For carbon source utilization determination, the kits were inoculated with a cell suspension prepared in the BBSW5 medium described above. Both kits were incubated for up to 10 days. Growth at different temperatures (0, 5 and 20–45 °C at intervals of about 5 °C) and pH (4.0–11.0 at intervals of 1.0 pH unit) was tested in MGM 10 broth.

Growth at different NaCl concentrations [0–30 % NaCl (w/v), equivalent to 2.4–32.4 % total salts] was tested in NaCl-free MGM 10 broth with increasing concentrations of NaCl (0, 1, 2, 5, 10, 15, 20, 25 and 30 %). Growth was monitored by measuring optical density at a wavelength of 600 nm over time and plotting the slope. The mean of triplicate runs at each temperature, pH and salt concentration was used to determine optimal growth rates for each condition. Requirement for sodium, magnesium and potassium salts was assayed in MGM 10 broth supplemented with SW solution prepared without NaCl (for sodium requirement assay), without magnesium salts (for magnesium requirement assay) or without KCl (for potassium requirement assay).

Susceptibility to antimicrobial compounds was assayed in MH medium with 7.5 % (w/v) sea salts by the Kirby-Bauer method (Bauer *et al.,* 1996). Results of the characterization are given in the species description.

Flagella of cells grown in MGM 10 broth for 48 h were stained with 2 % (w/v) uranyl acetate and observed with a JEOL JEM-1010 transmission electron microscope. Cells of strain SV325^T were rod-shaped, occurred singly or in pairs, and had polar and laterally inserted flagella (Fig. 1).

Extraction of genomic DNA was carried out with the QIAamp DNA Mini kit (Qiagen) and amplification of nearly full-length 16S rRNA gene fragments was performed using primers 27F (5′-AGAGTTTGATC(A/C)TGGCTCAG-3′) and 1492R (5′-ACGG(C/T)TACCTTGTTACGACTT-3′) as described previously (Menes & Muxí, 2002). The resulting PCR products were purified with the QIAquick PCR purification kit (Qiagen) and sequenced using an Applied Biosystems automatic sequencer ABI 3730XL at Macrogen, Seoul, Korea. The 16S rRNA gene sequence (1430 bp) was obtained and compared with other sequences in GenBank using a BLAST search (National Center for Biotechnology Information) and in the Ribosomal Database Project II (release 9.47) using the SIMILARITY_RANK (RDP) algorithm (Cole *et al.*, 2007, 2009). Phylogenetic analysis was performed with MEGA version 2.1 (Kumar *et al.*, 2001) using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms with Jukes & Cantor distance corrections. Bootstrap analyses (1000 replicates) were performed to evaluate the phylogenetic trees. Phylogenetic analysis (Fig. 2 and Supplementary Figs S1 and S2, available in IJSEM Online) clearly placed strain SV325^T in the genus *Halomonas*, located within group 2 of species of the genus *Halomonas* (Arahal *et al.*, 2002; de la Haba *et al.*, 2010). The 16S rRNA gene sequence of strain SV325^T had highest similarities to *Halomonas gomseomensis*, *Halomonas arcis*, *Halomonas subterranea* and *Halomonas janggokensis* (96.7 % sequence similarity) and *Halomonas sulfidaeris* (96.6 %). Lower sequence similarities (<96.3 %) were found with all other described species of the genus *Halomonas*. The 16S RNA gene fragment analysed contained the 18 signature nucleotides defined for the family *Halomonadaceae* (Ben Ali Gam *et al.*, 2007; Dobson & Franzmann, 1996). Extended phylogenetic trees are available as Supplementary Figs S1 and S2.

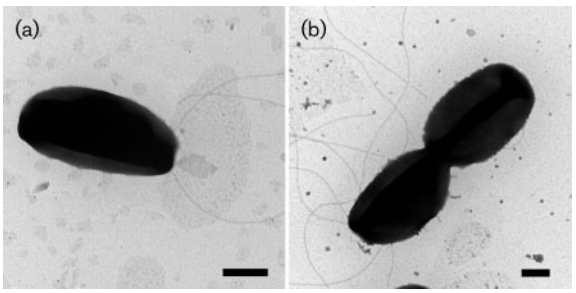

Fig. 1. Transmission electron micrograph of negatively stained cells of strain SV325^T. (a) Flagellar arrangement and (b) dividing cells. Bars, 500 nm.

Whole-cell fatty acid composition, genomic DNA G+C content and respiratory quinone analysis (from cells grown on MGM 10 agar for 3 days at 30 °C) were carried out by the Identification Service of DSMZ (Braunschweig, Germany). The major cellular fatty acids (95.23 %) of strain SV325^T were: $C_{19:0}$ cyclo $\omega 8c$ (30.79 %), $C_{16:0}$ (22.68 %), $C_{17:0}$ cyclo (18.13 %), $C_{16:1}\omega 7c$/iso-$C_{15:0}$ 2-OH (11.40 %), $C_{12:0}$ 3-OH (6.75 %), $C_{18:1}\omega 7c$ (3.11 %) and $C_{10:0}$ (2.37 %). Traces of $C_{10:0}$ 3-OH, $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{18:0}$, 11-methyl $C_{18:1}\omega 7c$, iso-$C_{19:0}$ and $C_{20:2}\omega 6,9c$ were also detected (each <1 %). The DNA G+C content of strain SV325^T was 55.0 mol%. The predominant quinones were Q-9 (92.5 %) and Q-8 (7.5 %).

Several morphological and taxonomical features were compared with those of highly phylogenetically related species (Table 1). Strain SV325^T differed from *Halomonas gomseomensis*, *Halomonas arcis*, *Halomonas subterranea* and *Halomonas janggokensis* in that it did not grow on L-arabinose, D-fructose, D-glucose, maltose, sucrose, D-xylose, glycerol, citrate or gluconate, was gelatinase- and oxidase-positive and did not produce H_2S from cysteine. Strain SV325^T differed from *Halomonas sulfidaeris* in that it did not grow on L-arabinose, D-fructose, sucrose, D-xylose, ethanol, D-mannitol, gluconate or tartrate but grew on acetate, succinate, L-alanine, L-proline and L-valine, was gelatinase-positive and did not hydrolyse casein or Tween 20.

16S rRNA gene-based phylogenetic analysis revealed that strain SV325^T exhibits less than 97 % sequence similarity to known species of the genus *Halomonas*. This similarity value (Stackebrandt & Goebel, 1994), as well as its phenotypic characteristics, demonstrates that strain SV325^T represents a distinct species belonging to the genus *Halomonas*, for which we propose the name *Halomonas vilamensis* sp. nov.

Description of *Halomonas vilamensis* sp. nov.

Halomonas vilamensis (vi.la.men′sis. N.L. fem. adj. *vilamensis* pertaining to Laguna Vilama, Jujuy, Argentina).

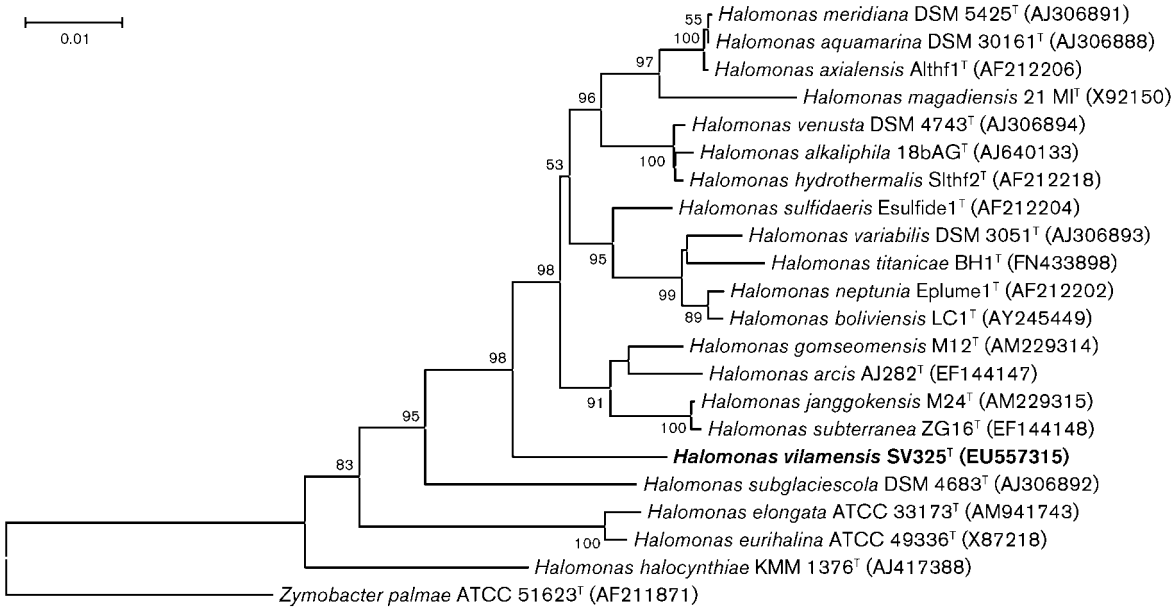

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences of strain SV325^T and related species using the neighbour-joining method. Bootstrap values above 50 % are shown at branch points (1000 resamplings). *Zymobacter palmae* ATCC 51623^T was used as the outgroup. An extended version of this tree is available as Supplementary Fig. S1. Bar, 1 % estimated sequence divergence.

Cells are Gram-stain-negative, rod-shaped (2.0–4.0 × 1.0–1.6 μm) and occur singly and in pairs. Cells are motile by means of polar and laterally inserted flagella, non-spore-forming and aerobic. Does not produce exopolysaccharides or diffusible pigments and stains positively for intracellular polyphosphate granules but not for poly-β-hydroxyalkanoates. Colonies are cream to pale pink, circular, smooth, convex and 1 to 2 mm in diameter after 3 days of incubation on MGM 10 agar or MH medium with 7.5 % (w/v) sea salts. Grows at temperatures ranging from 5 to 40 °C (optimum growth around 30 °C) and at pH 5.0–10.0 (optimum growth at pH 7.0–8.0) (Supplementary Fig. S3). Moderately halophilic, capable of growing at NaCl concentrations from 1 to 25 % (w/v) with optimum growth at 5–10 % (w/v) in media with 2.4 % (w/v) magnesium and potassium salts. Requires NaCl, potassium salts and magnesium salts for growth. No growth on MacConkey agar or cetrimide agar supplemented with 7.5 % (w/v) sea salts. Catalase- and oxidase-positive. No oxidation or fermentation of glucose in OF Hugh and Leifson medium (after 15 days). Does not produce acids from carbohydrates and alcohols. Phenylalanine deaminase-positive. Blood is haemolysed. Gelatin is hydrolysed but not aesculin, casein, DNA, Tween 20, Tween 80, tributyrin, tyrosine, starch or urea. Nitrate but not nitrite is reduced aerobically. Selenite is reduced. The following tests are negative: anaerobic growth, gluconate oxidation, respiration on nitrite and fumarate, H_2S from L-cysteine, pigment from tyrosine, phosphatase, ONPG, lecitovitellin, lysine decarboxylase, ornithine decarboxylase, indole, methyl red and Voges–Proskauer. Respiration on nitrate is positive. Arsenate, fumarate, molybdate, nitrite, selenate, selenite and tungstate are not reduced with tryptone, malate or acetate as electron donors. The following compounds are utilized as sole carbon and energy sources: acetate, caproate, fumarate, DL-β-hydroxybutyrate, malate, maleate, malonate and succinate. L-Alanine, L-aspartate, L-glutamate, glycine, L-lysine, L-proline and L-valine are utilized as sole carbon, energy and nitrogen sources but not L-arginine, L-cysteine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-ornithine, L-phenylalanine, L-serine or L-threonine. No growth is observed on the following compounds as sole carbon and energy sources: aesculin, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melezitose, raffinose, L-rhamnose, D-salicin, sorbose, trehalose, D-xylose, sucrose, benzoate, butyrate, caprylate, citrate, formate, gluconate, lactate, propionate, pyruvate, tartrate, adonitol, ethanol, glycerol, *myo*-inositol, D-mannitol, propanol, D-sorbitol or creatine. In API 20 NE, malic acid is utilized, gelatinase is produced and nitrate is reduced to nitrite, but tests for D-glucose, L-arabinose, gluconate, adipic acid, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, capric acid, trisodium citrate, phenylacetic acid, aesculin, arginine dihydrolase and indole are negative. Does not produce acid or utilize any of the carbohydrates in API 50 CH: *N*-acetylglucosamine, D-adonitol, aesculin, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, D-glucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, maltose, α-methyl D-glucoside, α-methyl

Table 1. Differential characteristics of strain SV325^T and its closest phylogenetic relatives

Strains: 1, SV325^T; 2, *Halomonas gomseomensis* M12^T (DSM 18042^T); 3, *Halomonas arcis* AJ282^T (LMG 23978^T); 4, *Halomonas subterranea* ZG16^T (LMG 23977^T); 5, *Halomonas janggokensis* M24^T (DSM 18043^T); 6, *Halomonas sulfidaeris* Esulfide1^T (DSM 15722^T); 7, *Halomonas neptunia* Eplume1^T (DSM 15720^T). +, Positive; −, negative; W, weakly positive reaction; ND, no data available; L-P, lateral–polar; Pe, peritrichous. Data for temperature range, pH range, flagellar arrangement and DNA G+C content for taxa 2 and 5 from Kim *et al.* (2007), for taxa 3 and 4 from Xu *et al.* (2007), for taxa 6 and 7 from Kaye *et al.* (2004); all other data are from this study. All strains were positive for motility, catalase activity, haemolysis, selenite reduction, and growth on fumarate, malate, malonate, L-aspartate and L-glutamate. All strains were negative for anaerobic growth, aerobic nitrite reduction, cetrimide agar growth, starch and Tween 80 hydrolysis, respiration on nitrite and fumarate, growth on L-serine and the lecitovitellin test.

Characteristic	1	2	3	4	5	6	7
Pigmentation	Cream–pink	Cream–beige	Cream	Cream	White	Cream	Cream
Temperature range (°C)	5–40	5–45	4–48	4–48	5–45	−1–35	−1–35
pH range	5–10	6–10	6–10	6–10	6–10	5–10	5–12
Oxidase	+	−	−	−	−	+	+
Flagellar arrangement	L-P	Pe	ND	ND	Pe	Pe	ND
Aerobic nitrate reduction	+	−	+†	−†	−	+	+
Growth on MacConkey agar	−	+†	+†	−†	+†	−†	+†
Growth on*:							
L-Arabinose	−	+	+	+†	+	+	+
Cellobiose	−	+†	−	−	−†	−	+
D-Fructose	−	+	+†	+†	+	+	+
D-Galactose	−	+†	+†	+†	+†	−	+
D-Glucose	−	+	+†	+†	+	−	+
Lactose	−	+†	−	−	−†	−	+
Maltose	−	+	+†	+†	+	−	+
D-Mannose	−	−	−	−	−	−	+
Sucrose	−	+	+†	+	+	+	+
D-Xylose	−	+†	+	+	+†	+	+
Ethanol	−	+†	+	−	−†	+	+
Glycerol	−	+	+	+	+	−	+
D-Mannitol	−	−	+†	+†	+	+	+
D-Sorbitol	−	−	+†	+†	+	−	+
Acetate	+	+	+	+	+	−	+
Citrate	−	+	+	+	+	−	+
Gluconate	−	+	+	+	+	+†	+†
DL-Lactate	−	+	−†	−†	+	−	−
Propionate	−	+	+	−†	+	−	−
Succinate	+	−†	+	+	+†	−	+
Tartrate	−	−†	−†	+†	−	+	+
L-Arginine	−	+†	+	−‡	+†	−‡	+
L-Alanine	+	+	+	+	+	−	+
L-Histidine	−	+	−	+	−	−	−
L-Lysine	+	−†	−‡	+	−†	+	+
L-Proline	+	+	+†	+‡	+†	−	+
L-Valine	+	+†	+	+‡	+†	−	−
Hydrolysis of:							
Aesculin	−	+	−	−	−	−†	+†
Casein	−	−	W	W	−	+	−
DNA	−	+	−	−	+	−	−
Gelatin	+	−	−	−	−	−	−
Tween 20	−	−†	−‡	−‡	−†	+†	−†
Tyrosine	−	−†	−	−	−†	−†	+†
Phosphatase	−	W†	W†	−†	−†	−†	+
Tyrosine pigment	−	−†	−†	−†	−†	−†	+
Phenylalanine deaminase	+	+†	+	+‡	+†	+‡	−
H_2S production from cysteine	−	+†	+†	+†	+†	−	+‡
DNA G+C content (mol%)	55.0	62.0–63.6	56.7	57.6	60.2–61.0	56.0	57.3

*Substrates as sole carbon and energy source, or as sole carbon, energy and nitrogen source.
†Characteristics not reported in the original description.
‡Different results were reported in the original description.

D-mannoside, β-methyl D-xyloside, D-mannitol, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, sucrose, D-tagatose, trehalose, turanose, D-xylose, L-xylose and xylitol. Susceptible to ampicillin (10 μg), azithromycin (15 μg), bacitracin (10 μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), clindamycin (2 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), levofloxacin (5 μg), nalidixic acid (30 μg), neomycin (30 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), oxacillin (1 μg), polymyxin B (300 IU), rifampicin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg) and vancomycin (30 μg). Resistant to amikacin (30 μg), penicillin (10 IU), streptomycin (10 μg), sulfisoxazole (0.25 mg) and tobramycin (10 μg). The DNA G+C content of the type strain is 55.0 mol% (by HPLC). The predominant quinones are Q-9 and Q-8. The major cellular fatty acids (up to 90 %) are $C_{19:0}$ cyclo *ω8c*, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH and $C_{12:0}$ 3-OH.

The type strain, SV325^T (=DSM 21020^T =LMG 24332^T), was isolated from the sediment of a hypersaline lake located 4600 m above sea level (Laguna Vilama, Argentina).

Acknowledgements

We thank Professor Jean Euzéby and Professor Dr Hans G. Trüper for their help with the naming of the organism. We also thank Mylene Rodríguez for helping with the susceptibility testing. M. J. S. acknowledges support from the Cooperative State Research Education and Extension Service, US Department of Agriculture, project no. ILLU-875-389.

References

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

Arahal, D. R., Vreeland, R. H., Litchfield, C. D., Mormile, M. R., Tindall, B. J., Oren, A., Béjar, V., Quesada, E. & Ventosa, A. (2007). Recommended minimal standards for describing new taxa of the family *Halomonadaceae*. *Int J Syst Evol Microbiol* **57**, 2436–2446.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.

Ben Ali Gam, Z., Abdelkafi, S., Casalot, L., Tholozan, J. L., Oueslati, R. & Labat, M. (2007). *Modicisalibacter tunisiensis* gen. nov., sp. nov., an aerobic, moderately halophilic bacterium isolated from an oilfield-water injection sample, and emended description of the family *Halomonadaceae* Franzmann *et al.* 1989 emend Dobson and Franzmann 1996 emend. Ntougias *et al.* 2007. *Int J Syst Evol Microbiol* **57**, 2307–2313.

Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & Tiedje, J. M. (2007). The ribosomal database project (RDP-II): introducing *myRDP* space and quality controlled public data. *Nucleic Acids Res* **35** (Database issue), D169–D172.

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T. & other authors (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37** (Database issue), D141–D145.

de la Haba, R. R., Arahal, D. R., Márquez, M. C. & Ventosa, A. (2010). Phylogenetic relationships within the family *Halomonadaceae* based on comparative 23S and 16S rRNA gene sequence analysis. *Int J Syst Evol Microbiol* **60**, 737–748.

Dobson, S. J. & Franzmann, P. D. (1996). Unification of the genera *Deleya* (Bauman *et al.* 1993), *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.

Dyall-Smith, M. (2008). The Halohandbook. Protocols for Haloarchaeal Genetics, version 7.0. http://haloarchaea.com/resources/halohandbook/index.html

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

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.

Guzmán, D., Quillaguamán, J., Muñoz, M. & Hatti-Kaul, R. (2010). *Halomonas andesensis* sp. nov., a moderate halophile isolated from the saline lake Laguna Colorada in Bolivia. *Int J Syst Evol Microbiol* **60**, 749–753.

Horikoshi, K. & Grant, W. D. (editors) (1998). *Extremophiles: Microbial Life in Extreme Environments*. New York: Wiley.

Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J Bacteriol* **66**, 24–26.

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.

Kim, K. K., Jin, L., Yang, H. C. & Lee, S.-T. (2007). *Halomonas gomseomensis* sp. nov., *Halomonas janggokensis* sp. nov., *Halomonas salaria* sp. nov. and *Halomonas denitrificans* sp. nov., moderately halophilic bacteria isolated from saline water. *Int J Syst Evol Microbiol* **57**, 675–681.

Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

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.

Menes, R. J. & Muxí, L. (2002). *Anaerobaculum mobile* sp. nov., a novel anaerobic, moderately thermophilic, peptide-fermenting bacterium that uses crotonate as an electron acceptor, and emended description of the genus *Anaerobaculum*. *Int J Syst Evol Microbiol* **52**, 157–164.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, D.C.: American Society for Microbiology.

Rodríguez-Valera, F., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1981). Characteristics of the heterotrophic bacterial populations in hypersaline environments of different salt concentrations. *Microb Ecol* **7**, 235–243.

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

Sánchez-Porro, C., Kaur, B., Mann, H. & Ventosa, A. (2010). *Halomonas titanicae* sp. nov., a halophilic bacterium isolated from the RMS Titanic. *Int J Syst Evol Microbiol* **60**, 2768–2774.

Seufferheld, M. J., Alvarez, H. M. & Farias, M. E. (2008). Role of polyphosphates in microbial adaptation to extreme environments. *Appl Environ Microbiol* **74**, 5867–5874.

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, D.C.: American Society for Microbiology.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

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.

Xu, X.-W., Wu, Y.-H., Zhou, Z., Wang, C.-S., Zhou, Y.-G., Zhang, H.-B., Wang, Y. & Wu, M. (2007). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619–1624.