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Halomonas gomseomensis sp. nov., *Halomonas janggokensis* sp. nov., *Halomonas salaria* sp. nov. and *Halomonas denitrificans* sp. nov., moderately halophilic bacteria isolated from saline water

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A total of 34 *Halomonas* strains were isolated from saline water in Anmyeondo, Korea. Ten of these strains, considered to belong to novel species, were subjected to a polyphasic taxonomic investigation. The strains were Gram-negative, moderately halophilic, motile and non-spore-forming rods that contained Q-9 as the predominant ubiquinone and $C_{18:1}\omega7c$, $C_{16:0}$ and either summed feature 4 ($C_{16:1}\omega7c$/$C_{15:0}$ iso 2-OH) or $C_{19:0}$ cyclo $\omega8c$ as the major fatty acids. Phylogenetic analysis, based on 16S rRNA gene sequencing, showed that the ten isolates formed four separate lineages in the genus *Halomonas*. Combined phenotypic data and DNA–DNA hybridization data supported the conclusion that they represent four novel species in the genus *Halomonas*, for which the names *Halomonas gomseomensis* sp. nov. (type strain M12^T = KCTC 12662^T = DSM 18042^T), *Halomonas janggokensis* sp. nov. (type strain M24^T = KCTC 12663^T = DSM 18043^T), *Halomonas salaria* sp. nov. (type strain M27^T = KCTC 12664^T = DSM 18044^T) and *Halomonas denitrificans* sp. nov. (type strain M29^T = KCTC 12665^T = DSM 18045^T) are proposed.

The family *Halomonadaceae* of the class *Gammaproteobacteria* comprises five genera, *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Halomonas* and *Zymobacter*, which accommodate halophilic/halotolerant and non-halophilic bacteria (Arahal *et al.*, 2002a, b; Franzmann *et al.*, 1988). Nearly one-third of the species within the family *Halomonadaceae* have undergone taxonomic changes (Arahal *et al.*, 2001, 2002a; Dobson & Franzmann, 1996; Mellado *et al.*, 1995) and, recently, [*Pseudomonas*] *beijerinckii* has been reclassified as *Chromohalobacter beijerinckii* (Peçonek *et al.*, 2006). Nevertheless, the taxonomic status of some taxa, e.g. *Cobetia marina* and *Halomonas marisflavi*, is still in doubt.

The members of genus *Halomonas* are moderately halophilic bacteria, and the genus contains 35 recognized species. The type species is *Halomonas elongata*. Recently, *Halomonas almeriensis* (Martínez-Checa *et al.*, 2005), *Halomonas campaniensis* (Romano *et al.*, 2005) and *Halomonas taeanensis* (Lee *et al.*, 2005) have been described. During the screening of salt-loving bacteria that might be applied to the bioremediation of saline environments, we isolated 34 *Halomonas* strains from saline samples of sea and solar salterns in Anmyeondo, ten strains of which were considered to belong to novel species in the genus *Halomonas* and were subjected to a polyphasic taxonomic investigation.

Strains M12^T and M59 were isolated from saline water of Gomseom solar saltern, strains M24^T and M58 were isolated from saline water of Janggok solar saltern and strains M27^T, M66, M67, M29^T, M69 and M70 were isolated from seawater in Anmyeondo. For most experiments, strains were cultured on marine agar or broth (Difco) containing 10 % NaCl at 28 °C for 48 h. When strains were cultured on other complex media for phenotypic tests, the final NaCl concentration was adjusted to 10 %.

The Gram reaction was performed as described by Gerhardt *et al.* (1994). Cell morphology and motility was observed under a phase-contrast microscope (Nikon Optiphot; ×1000 magnification) with cells grown for 1–7 days, and the presence of flagella was determined by transmission electron microscopy (JEM-10111; JEOL) after negative staining with 2 % (w/v) uranyl acetate. Oxidase activity was tested using 1 % tetramethyl-*p*-phenylenediamine (Tarrand & Groschel, 1982) and catalase activity was tested using 3 % H_2O_2. Growth was investigated at temperatures ranging from 5 to 55 °C at intervals of 5 °C and at pH 4–11 at intervals of 1 pH unit. Requirement for and tolerance of

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *H. gomseomensis* M12^T, *H. janggokensis* M24^T, *H. salaria* M27^T and *H. denitrificans* M29^T are AM229314–AM229317, respectively.

Transmission electron micrographs of cells of strains M12^T, M24^T, M27^T and M29^T, detailed DNA–DNA hybridization results and results of API ZYM tests are available as supplementary material in IJSEM Online.

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NaCl were determined in nutrient broth (Difco) supplemented with modified artificial seawater [containing (l^{-1}): 0–30 (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 15, 20, 25 and 30) % NaCl, 5.94 g $MgSO_4.7H_2O$, 4.53 g $MgCl_2.6H_2O$, 0.64 g KCl and 1.3 g $CaCl_2$; Lee *et al.*, 2005]. Hydrolysis of casein and starch was tested on casein agar and starch agar (Difco). The H_2S production test was performed on triple-sugar-iron agar (BBL). Carbon source utilization tests, acid production tests and additional physiological tests were performed using API 20NE, API 32GN, API 50CH and API ZYM galleries according to the instructions of the manufacturer (bioMérieux).

For the analysis of fatty acids, strains were cultured on tryptic soy agar (TSA; Difco) containing 10 % NaCl at 28 °C for 48 h. *Halomonas anticariensis* LMG 22089^T, *Halomonas cupida* DSM 4740^T, *Halomonas desiderata* DSM 9502^T, *Halomonas hydrothermalis* DSM 15725^T, *Halomonas maura* DSM 13445^T, *Halomonas sulfidaeris* DSM 15722^T, *Halomonas ventosae* DSM 15911^T, *Halomonas venusta* DSM 4743^T and *Chromohalobacter marismortui* DSM 6770^T were used as reference strains under the same conditions.

Fatty acid methyl esters were prepared and analysed as described previously (Klatte *et al.*, 1994) using the standard Microbial Identification System (MIDI Inc.) for automated gas chromatographic analysis (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones were extracted and purified as described previously (Tindall, 1990); dried preparations were dissolved in 200 µl 2-propanol and 1–10 µl samples were separated by HPLC without further purification.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA genes and sequencing of purified PCR products were carried out according to Rainey *et al.* (1996). The 16S rRNA gene sequences were aligned with published sequences retrieved from EMBL using CLUSTAL X (Thompson *et al.*, 1997) and edited using BioEdit (Hall, 1999). Phylogenetic trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods; distances were estimated by the method of Jukes & Cantor (1969) using MEGA version 2.1 (Kumar *et al.*, 2001). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. DNA G+C contents were determined by HPLC after hydrolysis as described by Tamaoka & Komagata (1984) and non-methylated λ DNA (Sigma) was used as a standard. DNA–DNA hybridization to determine genomic relatedness was performed fluorometrically by the method of Ezaki *et al.* (1989) using DNA probes labelled with photobiotin (Sigma; A1935) and microdilution wells (Greiner Bio-one; 96-well microplate).

The novel *Halomonas* strains formed visible colonies (0.5–1.5 mm diameter) on marine agar with 10 % NaCl at 28 °C within 48 h. Good growth occurred at temperatures ranging from 15 to 40 °C. The colonies were convex, translucent and circular with entire edges. Cells were aerobic, Gram-negative, catalase-positive, non-spore-forming rods and were motile with peritrichous or lateral/polar flagella. Transmission electron micrographs of cells of strains M12^T, M24^T, M27^T and M29^T are available as Supplementary Fig. S1 in IJSEM Online. Detailed physiological and biochemical characteristics are summarized in Table 1 and in the species descriptions.

The predominant quinone was ubiquinone Q-9; a small amount of Q-8 was also present. The fatty acid profiles of strains M12^T, M24^T and M29^T were similar to those of related taxa; the fatty acids $C_{18:1}\omega7c$, $C_{16:0}$ and summed feature 4 ($C_{16:1}\omega7c$/$C_{15:0}$ iso 2-OH) were predominant. Strain M27^T showed a slightly different profile; $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{19:0}$ cyclo $\omega8c$ were predominant. Detailed fatty acid compositions are shown in Table 2.

The almost-complete 16S rRNA gene sequences (approx. 1480 nt) of the ten strains were determined and compared with those of existing species within the family *Halomonadaceae*. Strains M12^T and M59, which shared 99.4 % similarity, showed the highest similarity to strain M24^T, *H. hydrothermalis* ATCC BAA-800^T and *H. venusta* DSM 4743^T (97.4, 97.2 and 97.1 %, respectively). Strains M24^T and M58, which shared 99.8 % similarity, showed the highest similarity to strain M12^T and *H. sulfidaeris* ATCC BAA-803^T (97.4 and 97.3 %, respectively). Strains M27^T, M66 and M67, which shared 100 % similarity, showed the highest similarity to *H. anticariensis* LMG 22089^T (94.5 %). Strains M29^T, M69 and M70, which shared 99.8–100 % similarity, showed the highest similarity to *H. ventosae* CECT 5797^T, *H. maura* CECT 5298^T and *H. cupida* DSM 4740^T (97.7, 97.4 and 97.0 %, respectively). The ten strains formed four separate lineages in the genus *Halomonas* in the phylogenetic trees (Fig. 1).

DNA–DNA hybridization tests were performed among the novel *Halomonas* strains and the type strains of closely related *Halomonas* species to confirm the taxonomic status of the novel strains. According to the DNA–DNA hybridization levels, strains M12^T and M59, which shared 90 % relatedness, showed 21–41 % hybridization with strain M24^T, *H. hydrothermalis* DSM 15725^T and *H. venusta* DSM 4743^T. Strains M24^T and M58, which shared 93 % relatedness, showed 31–37 % hybridization with strain M12^T and *H. sulfidaeris* DSM 15722^T. Strains M27^T, M66 and M67 shared 99–100 % hybridization with each other. Strains M29^T, M69 and M70, which shared 99–100 % relatedness, showed 15–39 % hybridization with *H. ventosae* DSM 15911^T, *H. maura* DSM 13445^T and *H. cupida* DSM 4740^T. Consequently, the DNA–DNA hybridization results (Supplementary Table S1 in IJSEM Online) supported the proposal of four novel *Halomonas* species.

Combined phenotypic and genotypic data support that the ten novel strains represent four novel species in the genus *Halomonas*, for which the names *Halomonas gomseomensis* sp. nov., *Halomonas janggokensis* sp. nov., *Halomonas salaria* sp. nov. and *Halomonas denitrificans* sp. nov. are proposed.

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Table 1. Differential phenotypic characteristics of the novel *Halomonas* species, closely related *Halomonas* species and *Chromohalobacter marismortui*

Species: 1, *H. gomseomensis* sp. nov.; 2, *H. janggokensis* sp. nov.; 3, *H. salaria* sp. nov.; 4, *H. denitrificans* sp. nov.; 5, *H. anticariensis*; 6, *H. cupida*; 7, *H. desiderata*; 8, *H. hydrothermalis*; 9, *H. maura*; 10, *H. sulfidaeris*; 11, *H. ventosae*; 12, *H. venusta*; 13, *Chromohalobacter marismortui*. +, Positive; −, negative; ND, not reported. Data were taken from Arahal *et al.* (2002b), Bouchotroch *et al.* (2001), Kaye *et al.* (2004), Martínez-Cánovas *et al.* (2004a, b) and this study.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Morphology	Rods	Rods	Short rods	Short rods	Rods	Rods	Rods	Rods	Long rods	Rods	Short rods	Rods	Rods
Cell length (μm)	1.8–2.4	1.6–2.0	1.3–1.7	1.2–1.6	3.0–3.5	1.5–3.0	1.0–2.6	2.0–3.0	6.0–9.0	2.0–3.0	1.2–1.4	2.0–3.5	1.5–4.0
Cell width (μm)	0.6–0.8	0.5–0.7	0.8–0.9	0.6–0.8	0.8–1.0	0.6–0.8	0.4–0.6	1.0–1.5	0.6–0.7	0.8–1.0	0.7–0.8	1.0–1.3	0.6–1.0
Motility	+	+	+	+	+	+	+	+	−	+	+	+	+
Flagellar arrangement*	Pe	Pe	L–P	Pe	Pe	Pe	Pe	Pe	−	Pe	ND	Pe	Pe
Pigmentation†	C–BG	W	Y	BR–Y	C	W	C	C	C	Y–C	C	Y	BR–Y
Salt range (% NaCl)	1–20	1–20	0–25	2–20	0.5–15	0–20	0–18	0.5–22	1–20	0.5–24	3–15	0–20	2–30
Temperature range (°C)	5–45	5–45	10–45	5–50	20–45	5–40	10–45	2–40	10–45	−1 to 35	15–50	4–40	5–45
pH range	6–10	6–10	5–10	7–10	6–9	5–11	7–11	5–12	5–10	5–10	6–10	5–10	5–10
Hydrolysis of:													
Aesculin	+	−	−	−	−	+	−	−	−	−	−	−	−
Casein	−	−	+	−	−	+	−	−	−	+	−	−	−
DNA	+	+	+	−	−	+	+	+	−	−	−	+	−
Nitrate reduction	−	−	+	+	+	+	+	+	+	+	+	+	+
Nitrite reduction	−	−	−	+	−	−	+	+	+	−	+	−	−
Oxidase	−	−	+	+	+	−	+	+	+	+	+	+	−
H_2S production	−	−	−	−	−	−	+	−	+	−	+	−	−
Urease	−	−	−	−	+	−	−	+	−	−	−	+	−
Growth on:													
Citrate	+	+	+	+	+	+	+	+	+	−	+	+	−
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	−
Glucose	+	+	+	−	+	+	+	+	+	−	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	−	+	+	ND
Mannose	−	+	+	−	+	+	+	+	+	−	−	−	+
Sucrose	+	+	−	−	ND	+	+	+	+	+	−	+	+
Acid production from:													
Fructose	+	+	+	+	−	+	−	−	−	−	−	−	ND
Glucose	+	+	+	−	−	+	−	+	−	+	−	−	+
Mannitol	−	+	+	−	ND	+	−	−	−	+	+	−	+
Mannose	+	−	+	−	−	+	−	−	−	+	−	−	+
Salicin	−	−	−	−	ND	+	−	−	−	−	−	−	−
Trehalose	+	+	+	−	−	+	−	−	−	−	−	−	+
Arabinose	+	+	+	−	−	+	−	−	−	−	−	−	+
Maltose	+	+	+	−	−	+	−	−	−	+	−	−	+
Lactose	−	−	+	−	−	+	−	−	−	+	−	−	+
G+C content (mol%)	62.0–63.6	60.2–61.0	58.8–60.1	53.8–55.2	60–61.4	60–63	66	56.3	62.2–64	56	72.6–74.3	52–55	62–65

*L–P, Lateral/polar; Pe, peritrichous.

†BG, Beige; BR, brown; C, cream; W, white; Y, yellow.

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Table 2. Cellular fatty acids of the type strains of novel *Halomonas* species and related taxa

Strains: 1, *H. gomseomensis* sp. nov. M12^T; 2, *H. janggokensis* sp. nov. M24^T; 3, *H. salaria* sp. nov. M27^T; 4, *H. denitrificans* sp. nov. M29^T; 5, *H. anticariensis* LMG 22089^T; 6, *H. cupida* DSM 4740^T; 7, *H. desiderata* DSM 9502^T; 8, *H. hydrothermalis* DSM 15725^T; 9, *H. maura* DSM 13445^T; 10, *H. sulfidaeris* DSM 15722^T; 11, *H. ventosae* DSM 15911^T; 12, *H. venusta* DSM 4743^T; 13, *Chromohalobacter marismortui* DSM 6770^T. Fatty acids are listed using standard abbreviations (number of carbon atoms : number of double bonds). Fatty acids that account for less than 1.0 % of the total fatty acids in all strains studied are not shown. Therefore, the percentages do not add up to 100 %. tr, Trace (<1.0 %); ND, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
$C_{10:0}$	2.0	2.1	2.2	2.4	2.5	3.2	1.5	1.1	2.8	1.2	2.5	2.2	3.6
$C_{12:0}$	tr	ND	3.0	ND	3.7	4.1	ND	tr	3.3	tr	ND	1.2	4.3
$C_{12:0}$ 3-OH	10.0	8.3	8.3	7.4	5.5	7.5	3.7	6.1	6.5	8.1	6.9	6.6	11.2
$C_{14:0}$	ND	ND	ND	ND	ND	ND	2.6	3.2	tr	ND	ND	2.6	ND
Summed feature 4*	9.7	7.6	10.0	21.2	13.2	14.2	3.8	11.7	19.5	8.4	18.3	11.5	9.2
$C_{16:0}$	16.5	12.4	20.4	21.1	25.6	21.5	15.9	14.8	20.5	8.7	20.3	14.0	16.7
$C_{17:0}$ cyclo	ND	tr	2.9	ND	tr	ND	ND	ND	ND	ND	ND	ND	1.0
$C_{18:1}\omega7c$	57.4	61.8	33.6	47.2	47.3	48.6	70.5	62.4	46.6	67.6	51.0	61.9	47.3
$C_{18:0}$	ND	tr	1.3	ND	tr	tr	tr	ND	tr	ND	ND	ND	tr
$C_{19:0}$ cyclo $\omega8c$	3.6	6.3	18.3	tr	1.1	ND	1.2	ND	ND	5.1	1.0	ND	6.1

*Summed feature 4 contains $C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH.

Description of *Halomonas gomseomensis* sp. nov.

Halomonas gomseomensis (gom.se.om.en′sis. N.L. fem. adj. *gomseomensis* referring to Gomseom in Anmyeondo, from where the first strains were isolated).

Cells are aerobic, Gram-negative, non-spore-forming rods (0.6–0.8 × 1.8–2.4 μm). Colonies are cream–beige, smooth, translucent and circular with entire edges and stick slightly to solid media. Cells are motile with peritrichous flagella. Oxidase-negative and catalase-positive. Growth occurs at 5–45 °C (optimum 25–30 °C) and at pH 6–10 (optimum pH 7–8). Growth occurs at salinities of 1–20 % NaCl (optimum 8–12 % NaCl). Indole and H_2S are not produced. Voges–Proskauer test is negative. Nitrate and nitrite are not reduced. Aesculin and DNA are hydrolysed, but casein, gelatin, starch, Tween 80 and urea are not. Acid is produced from glycerol, D-arabinose, L-arabinose, D-xylose, galactose, glucose, fructose, mannose, inositol, arbutin, aesculin, maltose, sucrose, trehalose, D-turanose, D-fucose and L-fucose, but not from erythritol, ribose, L-xylose, adonitol, methyl β-D-xylose, sorbose, rhamnose, dulcitol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. The following compounds are utilized as sole carbon sources: D-glucose, *N*-acetylglucosamine, salicin, inositol, L-fucose, sucrose, maltose, L-arabinose, gluconate, propionate, malate, malonate, valerate, acetate, citrate, DL-lactate, histidine, L-alanine, 2-ketogluconate, 3-hydroxybutyrate, 4-hydroxybenzoate and L-proline. The following carbon sources are not utilized: mannitol, rhamnose, D-ribose, D-melibiose, mannose, D-sorbitol, itaconate, suberate, adipate, caprate, phenylacetate, 5-ketogluconate, glycogen, 3-hydroxybenzoate and L-serine. Results from API ZYM tests are available in Supplementary Table S2 in IJSEM Online. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega7c/C_{15:0}$ iso 2-OH are predominant. The G+C content of the DNA is 62.0–63.6 mol% (62.0 mol% for the type strain).

The type strain is M12^T (=KCTC 12662^T=DSM 18042^T), isolated from saline water of Gomseom solar saltern in Anmyeondo, Korea.

Description of *Halomonas janggokensis* sp. nov.

Halomonas janggokensis (jang.gok.en′sis. N.L. fem. adj. *janggokensis* referring to Janggok in Anmyeondo, from where the first strains were isolated).

Cells are aerobic, Gram-negative, non-spore-forming rods (0.5–0.7 × 1.6–2.0 μm). Colonies are white, smooth, translucent and circular with entire edges. Cells are motile with peritrichous flagella. Oxidase-negative and catalase-positive. Growth occurs at 5–45 °C (optimum 25–30 °C) and at pH 6–10 (optimum pH 7–8). Growth occurs at salinities of 1–20 % NaCl (optimum 10–15 % NaCl). Indole and H_2S are not produced. Voges–Proskauer test is negative. Nitrate and nitrite are not reduced. DNA is hydrolysed, but aesculin, casein, gelatin, starch, Tween 80 and urea are not. Acid is produced from glycerol, L-arabinose, adonitol, galactose, glucose, fructose, mannitol, sorbitol, maltose, sucrose, trehalose, xylitol, D-turanose, D-fucose, D-arabitol and

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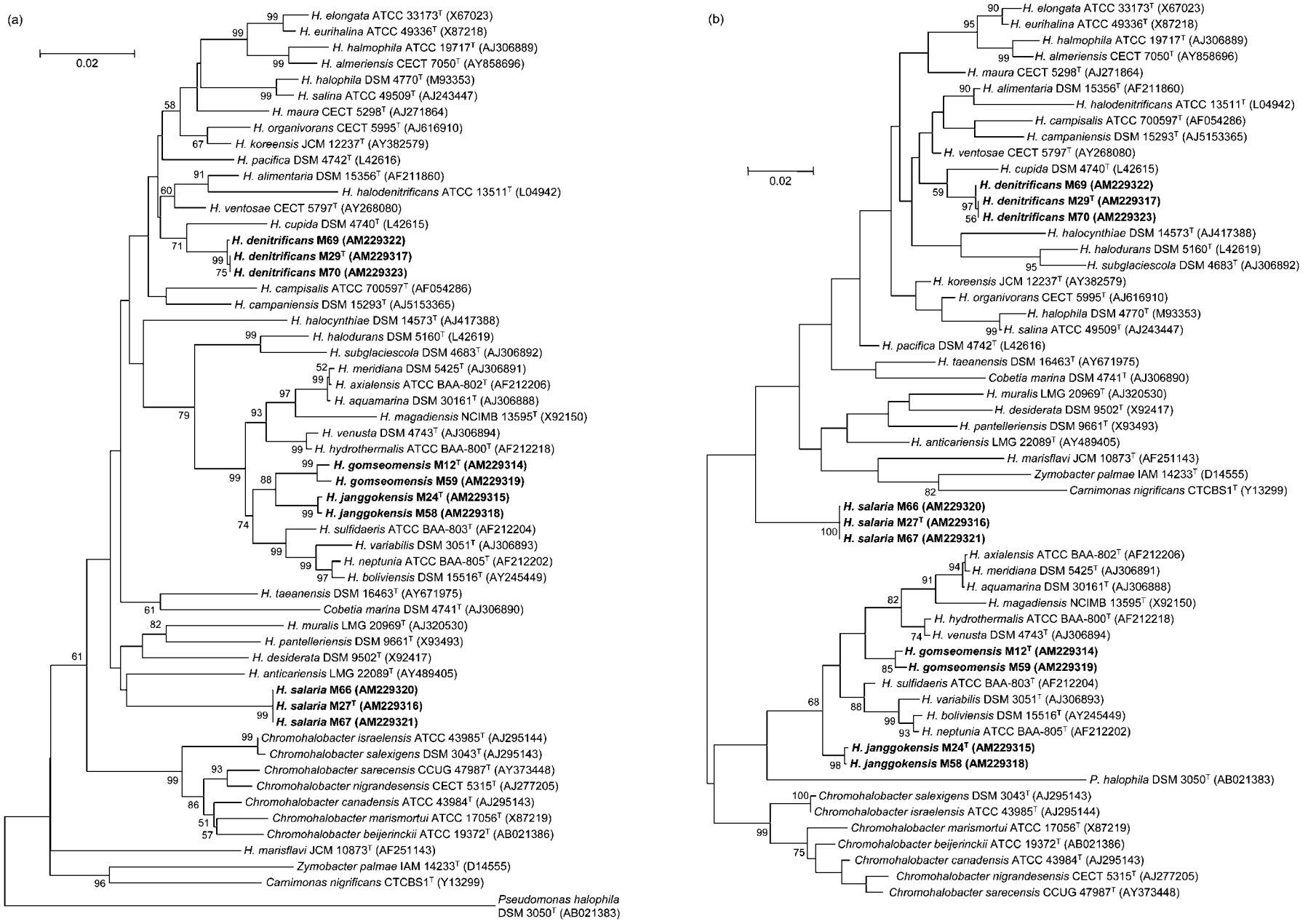

Fig. 1. Phylogenetic trees based on 16S rRNA gene sequences using the neighbour-joining (Saitou & Nei, 1987) (a) and maximum-parsimony (Fitch, 1971) (b) methods, showing the positions of the novel *Halomonas* strains among species of genera within the family *Halomonadaceae*. Numbers at branching points refer to bootstrap values (1000 resamplings; only values above 50 % are shown). Bars, 2 substitutions per 100 nucleotide positions.

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L-arabitol, but not from erythritol, D-arabinose, ribose, D-xylose, L-xylose, methyl β-D-xylose, mannose, sorbose, rhamnose, dulcitol, inositol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, D-lyxose, D-tagatose, L-fucose, gluconate, 2-ketogluconate and 5-ketogluconate. The following compounds are utilized as sole carbon sources: mannitol, D-glucose, inositol, sucrose, maltose, D-sorbitol, L-arabinose, gluconate, propionate, malate, malonate, valerate, acetate, adipate, citrate, DL-lactate, L-alanine, 2-ketogluconate, 3-hydroxybutyrate and L-proline. The following carbon sources are not utilized: rhamnose, *N*-acetylglucosamine, salicin, D-ribose, D-melibiose, L-fucose, mannose, itaconate, suberate, caprate, histidine, phenylacetate, 5-ketogluconate, glycogen, 4-hydroxybenzoate, 3-hydroxybenzoate and L-serine. Results from API ZYM tests are available as Supplementary Table S2. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega7c/C_{15:0}$ iso 2-OH are predominant. The G+C content of the DNA is 60.2–61.0 mol% (61.0 mol% for the type strain).

The type strain is M24^T (=KCTC 12663^T=DSM 18043^T), isolated from saline water of Janggok solar saltern in Anmyeondo, Korea.

Description of *Halomonas salaria* sp. nov.

Halomonas salaria (sa.la′ri.a. L. fem. adj. *salaria* of or belonging to salt).

Cells are aerobic, Gram-negative, non-spore-forming rods (0.8–0.9 × 1.3–1.7 μm). Colonies are yellow, smooth, translucent and circular with entire edges. Cells are motile with lateral/polar flagella. Oxidase-positive and catalase-positive. Growth occurs at 10–45 °C (optimum 25–30 °C) and at pH 5–10 (optimum pH 7–8). Growth occurs at salinities of 0–25 % NaCl (optimum 10–20 % NaCl). Indole and H_2S are not produced. Voges–Proskauer test is negative. Nitrate is reduced, but nitrite is not reduced. Casein and DNA are hydrolysed, but aesculin, gelatin, starch, Tween 80 and urea are not. Acid is produced from glycerol, erythritol, L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, rhamnose, mannitol, cellobiose, maltose, lactose, melibiose, trehalose, D-lyxose, D-fucose and D-arabitol, but not from D-arabinose, L-xylose, adonitol, methyl β-D-xylose, sorbose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-tagatose, L-fucose, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. The following compounds are utilized as sole carbon sources: mannitol, D-glucose, D-ribose, L-fucose, maltose, mannose, L-arabinose, itaconate, gluconate, propionate, malate, valerate, acetate, adipate, citrate, DL-lactate, 2-ketogluconate, 5-ketogluconate, L-proline and L-serine. The following carbon sources are not utilized: rhamnose, *N*-acetylglucosamine, salicin, D-melibiose, inositol, sucrose, D-sorbitol, suberate, caprate, malonate, phenylacetate, histidine, L-alanine, 3-hydroxybutyrate, glycogen, 4-hydroxybenzoate and 3-hydroxybenzoate. Results from API ZYM tests are available as Supplementary Table S2. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{19:0}$ cyclo $\omega8c$ are predominant. The G+C content of the DNA is 58.8–60.1 mol% (58.8 mol% for the type strain).

The type strain is M27^T (=KCTC 12664^T=DSM 18044^T), isolated from seawater in Anmyeondo, Korea.

Description of *Halomonas denitrificans* sp. nov.

Halomonas denitrificans (de.ni.tri′fi.cans. N.L. v. *denitrifico* to denitrify; N.L. part. adj. *denitrificans* denitrifying).

Cells are aerobic, Gram-negative, non-spore-forming rods (0.6–0.8 × 1.2–1.6 μm). Colonies are brown–yellow, smooth, translucent and circular with entire edges. Cells are motile with peritrichous flagella. Oxidase-positive and catalase-positive. Growth occurs at 5–50 °C (optimum 25–35 °C) and at pH 7–10 (optimum pH 8–9). Growth occurs at salinities of 2–20 % NaCl (optimum 8–10 % NaCl). Indole and H_2S are not produced. Voges–Proskauer test is negative. Nitrate and nitrite are reduced. Aesculin, casein, DNA, gelatin, starch, Tween 80 and urea are not hydrolysed. Acid is produced from fructose, but not from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-D-xylose, galactose, glucose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. The following compounds are utilized as sole carbon sources: propionate, malate, valerate, malonate, acetate, citrate, DL-lactate, L-alanine, 3-hydroxybutyrate, L-proline and L-serine. The following carbon sources are not utilized: mannitol, D-glucose, rhamnose, *N*-acetylglucosamine, salicin, D-ribose, D-melibiose, inositol, L-fucose, sucrose, D-sorbitol, maltose, mannose, L-arabinose, itaconate, suberate, adipate, caprate, gluconate, phenylacetate, histidine, 2-ketogluconate, 5-ketogluconate, glycogen, 4-hydroxybenzoate and 3-hydroxybenzoate. Results from API ZYM tests are available as Supplementary Table S2. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega7c/C_{15:0}$ iso 2-OH are predominant. The G+C content of the DNA is 53.8–55.2 mol% (53.8 mol% for the type strain).

The type strain is M29^T (=KCTC 12665^T=DSM 18045^T), isolated from seawater in Anmyeondo, Korea.

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References

Arahal, D. R., García, M. T., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2001). Transfer of *Halomonas canadensis* and *Halomonas israelensis* to the genus *Chromohalobacter* as *Chromohalobacter canadensis* comb. nov. and *Chromohalobacter israelensis* comb. nov. *Int J Syst Evol Microbiol* **51**, 1443–1448.

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.

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

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 microdilution 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. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

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.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

Hall, T. A. (1999). BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

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.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiensis* comb. nov. and *Gordona amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.

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

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.

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.

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.

Peçonek, J., Gruber, C., Gallego, V., Ventosa, A., Busse, H.-J., Kämpfer, P., Radax, C. & Stan-Lotter, H. (2006). Reclassification of *Pseudomonas beijerinckii* Hof 1935 as *Chromohalobacter beijerinckii* comb. nov., and emended description of the species. *Int J Syst Evol Microbiol* **56**, 1953–1957.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a district actinomycete lineage; proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Romano, I., Giordano, 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.

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

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*. Technical Note 101. Newark, DE: MIDI Inc.

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

Tarrand, J. J. & Groschel, D. H. M. (1982). Rapid, modified oxidase test for oxidase-variable bacterial isolates. *J Clin Microbiol* **16**, 772–774.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tindall, B. J. (1990). A comparative study of the lipid composition of *Halobacterium saccarovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

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