Halomonas stevensii sp. nov., *Halomonas hamiltonii* sp. nov. and *Halomonas johnsoniae* sp. nov., isolated from a renal care centre

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A total of 14 *Halomonas* strains were isolated from the blood of two patients and from dialysis machines of a renal care centre. The strains were Gram-negative, halophilic, motile and non-spore-forming rods. They produced cream-coloured colonies and contained Q-9 as the predominant ubiquinone and C_{18:1ω7c} and C_{16:0} as the major fatty acids. Phylogenetic analysis based on 16S rRNA gene sequencing showed that the 14 isolates were most closely related to *Halomonas magadiensis* 21 MI^T with 98.1–98.9% sequence similarity and that they formed three separate lineages among themselves. Combined phenotypic and DNA–DNA hybridization data support the conclusion that they represent three novel species of the genus *Halomonas*, for which the names *Halomonas stevensii* sp. nov. (type strain S18214^T=KCTC 22148^T=DSM 21198^T), *Halomonas hamiltonii* sp. nov. (type strain W1025^T=KCTC 22154^T=DSM 21196^T) and *Halomonas johnsoniae* sp. nov. (type strain T68687^T=KCTC 22157^T=DSM 21197^T) are proposed.

The family *Halomonadaceae* of the class *Gammaproteobacteria* currently comprises ten genera, *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Halomonas*, *Halotalea*, *Kushneria*, *Modicisalibacter*, *Salicola*, *Salinicola* and *Zymobacter*, which accommodate halophilic/halotolerant and non-halophilic bacteria. Since the family *Halomonadaceae* was first described (Franzmann *et al.*, 1988), its members typically have occurred in saline lakes, solar salt facilities, saline soils and marine environments. Since some of its members are also alkaliphilic, they are found in soda lakes and alkaline soils. The exceptions to the above are *Carnimonas nigrificans*, *Chromohalobacter beijerinckii*, *Chromohalobacter canadensis*, *Chromohalobacter japonicus*, *Halomonas alimentaria*, *Halomonas alkaliphila*, *Halomonas campaniensis*, *Halomonas daqingensis*, *Halomonas desiderata*, *Halomonas halodenitrificans*, *Halomonas muralis*, *Halotalea alkalilenta*, *Kushneria aurantia*, *Kushneria avicenniae*, *Modicisalibacter tunisiensis* and *Zymobacter palmae*

(Arahal & Ventosa, 2006; Ben Ali Gam *et al.*, 2007; Ntougias *et al.*, 2007; Peçonek *et al.*, 2006; Romano *et al.*, 2005, 2006; Sánchez-Porro *et al.*, 2007, 2009; Wu *et al.*, 2008). Of these 16 organisms, only *Zymobacter palmae* is neither halophilic nor halotolerant.

Members of the genus *Halomonas* are ubiquitous and moderately halophilic/halotolerant bacteria and, at the time of writing, the genus comprised 56 phylogenetically heterogeneous species. The type species is *Halomonas elongata*. During the screening of bacteria from patients who developed bacteraemia during dialysis and from dialysis machines, 14 halophilic organisms were isolated, which were considered to belong to novel species in the genus *Halomonas*, and subjected to a polyphasic taxonomic investigation.

Strains S18214^T and T49407 were isolated from the blood of two patients and strains F22135, F22157, F22171, H35115, T68674, T68679, T68687^T, W1012, W1017, W1019, W1023 and W1025^T were isolated from dialysis machines of a renal care centre in Santa Clara Valley Medical Center, San Jose, California, USA (Stevens *et al.*, 2008). For most experiments, strains were cultured on solid MH medium (Ventosa *et al.*, 1982) with 5% (w/v) sea-salt solution (Rodríguez-Valera *et al.*, 1981) at 30 °C for 48 h. When strains were cultured on other complex media for phenotypic tests, the final salt concentration was adjusted to 5% (w/v) according to the above. For fatty acid analysis, strains were cultured on tryptic soy agar (TSA; Difco) and on TSA with 5% (w/v) NaCl at 30 °C for 48 h. *Halomonas*

Abbreviations: EPS, exopolysaccharide; PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of *Halomonas stevensii* S18214^T, *Halomonas hamiltonii* W1025^T and *Halomonas johnsoniae* T68687^T are AM941388, AM941396 and AM941399, respectively.

A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of the novel *Halomonas* strains among all described *Halomonas* species (Fig. S1), and DNA–DNA hybridization data (Table S1) and fatty acid contents of cells grown on TSA with 5% NaCl (Table S2) of the novel strains and closely related *Halomonas* type strains are available with the online version of this paper.

aquamarina KCTC 22913^T, *Halomonas axialensis* KCTC 22914^T, *Halomonas magadiensis* KCTC 22915^T and *Halomonas meridiana* KCTC 22916^T, grown under identical conditions, were used as reference strains for fatty acid analysis, other phenotypic tests and DNA–DNA hybridization tests.

The Gram reaction was performed as described by Gerhardt *et al.* (1994). Cell morphology and motility were observed under a light microscope (Optiphot-2, Nikon; $\times 1500$ magnification) with cells grown for 1–7 days. Motility was tested by the hanging-drop technique (Skerman, 1967) and the presence of flagella was determined by transmission electron microscopy (JEM-1011; JEOL) after negative staining with 2% (w/v) uranyl acetate. Poly- β -hydroxybutyrate (PHB) staining was done in 5 day cultures by using the method of Ostle & Holt (1982). Exopolysaccharide (EPS) production was examined on solid MH medium after incubation for 3 days (Mata *et al.*, 2002). Oxidase activity was tested by using Bactident-Oxidase strips (Merck) and catalase activity was tested by using 3% H₂O₂. Growth was investigated at temperatures ranging from 5 to 50 °C at intervals of 5 °C and at pH 5–11 at intervals of 1 pH unit. Requirement for and tolerance of salts were determined in liquid MH medium with different sea-salt concentrations (0, 0.5, 1, 2, 3, 5, 7.5, 10, 15, 20, 25 and 30%, w/v). Anaerobic growth was examined on solid MH medium incubated in jars with the GasPak anaerobic system (BBL). Growth on MacConkey agar (Difco) and cetrimide agar (Difco) was investigated. Hydrolysis of casein, DNA, starch and tyrosine was tested on casein agar (Difco), DNase test agar (BBL), starch agar (Difco) and tyrosine agar (PML Microbiologicals). Hydrolysis of Tween 20 and Tween 80 was tested on solid MH medium supplemented with 1% (v/v) Tween 20 or Tween 80. Haemolysis was studied on solid MH medium supplemented with 5% (v/v) sheep blood. The lecithovitellin test was performed on egg yolk agar (McClung & Toabe, 1947). Utilization of glucose by oxidation or fermentation was checked in modified MOF medium (Ventosa *et al.*, 1982) with 5% (w/v) NaCl. Respiration on nitrate, nitrite and fumarate, selenite reduction and gluconate oxidation were studied according to the procedures of Mata *et al.* (2002). H₂S production was tested in liquid MH medium supplemented with 0.01% (w/v) cysteine with a strip of lead acetate paper (Fluka) as an indicator. Methyl red and Voges–Proskauer tests were performed in MR-VP broth (BBL). A nutritional assay was carried out using modified Koser medium (Ventosa *et al.*, 1982) containing (% w/v): NaCl, 5; KCl, 0.2; MgSO₄·7H₂O, 0.02; KNO₃, 0.1; (NH₄)₂HPO₄, 0.1; KH₂PO₄, 0.05. Filter-sterilized substrate (0.1%, w/v) was added to this medium, with the exception of carbohydrates, which were used at 0.2% (w/v). When amino acids were used as substrates, the basal medium contained neither KNO₃ nor (NH₄)₂HPO₄. The organic compounds tested as sole sources of carbon and energy or sole sources of carbon, nitrogen and energy were as follows: aesculin, amygdalin, glycogen, L-arabinose, cellobiose, D-

fructose, D-fucose, D-galactose, D-glucose, lactose, maltose, D-mannose, melezitose, raffinose, L-rhamnose, D-ribose, D-salicin, starch, sucrose, trehalose, D-xylose, adonitol, dulcitol, ethanol, glycerol, *myo*-inositol, D-mannitol, sorbitol, acetate, adipate, citrate, formate, fumarate, gluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, malate, malonate, propionate, succinate, L-alanine, L-cysteine, L-histidine, DL-isoleucine, L-lysine, L-methionine, L-proline, L-serine and L-valine. Acid production tests, enzyme activity tests and additional phenotypic tests were performed by using API 20E, API 20NE, API 50CH and API ZYM galleries according to the instructions of the manufacturer (bioMérieux); the inocula for API test kits were supplemented with 5% (w/v) sea-salt solution. Duplicate antibiotic susceptibility tests were performed by using Sensi-Disks (6 mm; BBL) containing the following: amoxicillin (25 µg), ampicillin (10 µg), carbenicillin (100 µg), cefoxitin (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), nitrofurantoin (300 µg), penicillin G (10 IU), polymyxin B (300 IU), rifampicin (5 µg), streptomycin (10 µg), sulphamide (250 µg), tetracycline (30 µg), tobramycin (10 µg), trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg) and vancomycin (30 µg). Zone diameter interpretive standards of the NCCLS (2003) were used for interpretation.

Fatty acid methyl esters were prepared and analysed as described by Klatte *et al.* (1994) by using the standard Microbial Identification System (MIDI) for automated GC analysis (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by using TLC on Kieselgel 60 F₂₅₄ plates (20 × 20 cm, 0.5 mm thickness; Merck) with petroleum ether/diethyl ether (9:1, v/v) as the solvent. The identities of the quinones were determined by using reversed-phase HPLC analysis as described by Shin *et al.* (1996).

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 GenBank/EMBL/DDBJ by using CLUSTAL_X (Thompson *et al.*, 1997) and edited via the BIOEDIT program (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) by using MEGA version 3.0 (Kumar *et al.*, 2004). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. DNA G + C contents were determined by HPLC, following hydrolysis, as described by Tamaoka & Komagata (1984), and non-methylated λ DNA (Sigma) was used as a standard. DNA–DNA hybridization was performed fluorometrically (Ezaki *et al.*, 1989) by using DNA probes labelled with photobiotin (A1935; Sigma) and 96-well microdilution plates (Greiner Bio-One) at 50 °C.

The novel *Halomonas* strains formed visible colonies (about 2 mm in diameter) on solid MH medium at 30 °C within 48 h. Good growth occurred at temperatures ranging from 20 to 35 °C. Colonies were cream-coloured, translucent and circular with entire edges. Cells were aerobic, Gram-negative, motile and non-spore-forming rods.

The almost-complete 16S rRNA gene sequences (approx. 1500 nt) of the 14 isolates were determined and compared with those of existing species within the family *Halomonadaceae*. Strains S18214^T, F22135, F22157, F22171, H35115, T49407, T68674 and T68679, which shared 99.7–100.0 % sequence similarity with each other and clustered as group I, showed the highest similarities to strain W1025^T, strain T68687^T, *H. magadiensis* 21 MI^T, *H. aquamarina* DSM 30161^T, *H. axialensis* Althf1^T and *H. meridiana* DSM 5425^T (99.4, 99.0, 98.2, 98.0, 98.0 and 97.9 %, respectively). Strains W1025^T, W1017 and W1023, which shared 99.9–100.0 % similarity with each other and clustered as group II, showed the highest similarities to strain T68687^T, strain S18214^T, *H. magadiensis* 21 MI^T, *H.*

aquamarina DSM 30161^T, *H. axialensis* Althf1^T and *H. meridiana* DSM 5425^T (99.5, 99.4, 98.6, 98.5, 98.5 and 98.4 %, respectively). Strains T68687^T, W1012 and W1019, which shared 99.9–100.0 % similarity with each other and clustered as group III, showed the highest similarities to strain W1025^T, strain S18214^T, *H. magadiensis* 21 MI^T, *H. aquamarina* DSM 30161^T, *H. axialensis* Althf1^T and *H. meridiana* DSM 5425^T (99.5, 99.0, 98.9, 98.5, 98.5 and 98.5 %, respectively). The 14 strains occupied three distinct positions among members of *Halomonas* rRNA group 2 (Arahal *et al.*, 2002) and formed a stable branch with *H. magadiensis*, *H. aquamarina*, *H. axialensis* and *H. meridiana* in the phylogenetic tree (Fig. 1). An extended version of the 16S rRNA gene phylogenetic tree, including all recognized species of the genus *Halomonas*, is available as Supplementary Fig. S1 in IJSEM Online.

DNA–DNA hybridization tests were performed among the novel *Halomonas* strains and related taxa to confirm the taxonomic status of the novel isolates. According to the DNA–DNA hybridization levels (see Supplementary Table S1 in IJSEM Online), members of group I shared 90–95 %

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences constructed according to the neighbour-joining method showing the positions of the novel *Halomonas* strains among members of *Halomonas* rRNA group 2 (Arahal *et al.*, 2002). Filled circles indicate branches that were also found with the maximum-parsimony treeing algorithm. Numbers at branch points refer to bootstrap percentages (from 1000 resamplings; only values ≥ 50 % are shown). Bar, 1 substitution per 100 nt positions.

Table 1. Differential characteristics of the novel strains and closely related type strains of the genus *Halomonas*

Species/strains: 1, *H. stevensii* sp. nov. ($n=8$); 2, *H. hamiltonii* sp. nov. ($n=3$); 3, *H. johnsoniae* sp. nov. ($n=3$); 4, *H. magadiensis* KCTC 22195^T; 5, *H. aquamarina* KCTC 22193^T; 6, *H. axialensis* KCTC 22194^T; 7, *H. meridiana* KCTC 22196^T. n , Number of strains tested; +, positive; –, negative; v, variable; data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6	7
Pigmentation	Cream	Cream	Cream	Cream	Cream	Cream	White
Cell length (µm)	2.0–3.0	2.0–3.0	1.5–4.0	4.0–6.0*	4.0–6.0*	2.0–3.0†	1.5–4.0*
Cell width (µm)	0.9–1.1	0.9–1.0	0.7–0.9	0.6–0.8*	0.4–0.8*	0.9–1.0†	0.6–1.0*
Flagella arrangement‡	Pe	L	L-P	Pe	Pe*	Pe†	L*
Salt tolerance (% total salts):							
Range	0–20	0–20	0–20	0–20§	0.5–20§	0.5–24†	0–20§
Optimum	3.0–7.5	5.0–7.5	0–5	0–7§	7.5–10.0§	4†	1–3§
Temperature tolerance (°C):							
Range	10–40	10–40	10–40	20–50†	5–40†	–1 to 35†	–5 to 45†
Optimum	30–35	30–35	30–35	37†	20–25†	30†	28–40†
pH tolerance:							
Range	7–11	6–10	7–10	5–11§	5–10§	5–12†	5–10§
Optimum	8–9	8–9	8–9	9–10	7–8	7–8†	7–8
Voges–Proskauer test	–	+	–	–	–	–	–
H ₂ S production	+	+	+	+	–	–	+
Nitrate reduction	–	–	+	+	+	+	–
Urease	+	v (–)	–	+	–	–	+
Growth on cetrimide agar	v (+)	+	v (–)	–	–	+	–
Respiration on:							
Nitrate	–	–	–	–	–	+	–
Nitrite	–	–	–	–	–	+	–
Acid production from:							
L-Arabinose	–	+	–	+	–	–	+
D-Fucose	–	+	–	+	–	–	–
D-Galactose	–	+	–	+	–	–	+
Maltose	+	–	–	+	–	–	+
Methyl α-D-glucoside	+	+	–	+	+	–	–
Glycerol	+	–	+	+	+	–	–
Inositol	+	–	–	–	–	–	–
D-Mannitol	–	–	+	+	+	–	+
D-Sorbitol	–	–	–	+	+	–	+
Growth on:							
L-Arabinose	–	–	–	+	+	+	–
D-Fucose	–	–	–	+	–	–	+
D-Galactose	–	+	–	+	–	–	+
Lactose	–	–	–	+	+	–	–
Melezitose	–	+	+	–	+	–	+
Amygdalin	–	–	–	+	–	–	+
Glycogen	–	–	–	–	+	+	+
Starch	v (+)	–	–	+	+	–	–
Dulcitol	–	–	–	+	–	–	+
myo-Inositol	+	–	–	+	+	–	+
D-Mannitol	–	+	+	+	+	–	+
D-Sorbitol	–	+	+	+	–	–	–
Adipate	–	+	–	+	–	+	+
4-Hydroxybenzoate	–	+	+	+	–	–	–
Malonate	–	+	–	–	+	–	–
L-Cysteine	+	–	–	–	–	–	–
DL-Isoleucine	–	–	+	+	+	+	+
L-Lysine	–	+	–	+	+	–	+
L-Methionine	+	–	–	+	–	–	–
L-Valine	+	–	+	+	–	–	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
Hydrolysis of:							
DNA	–	–	–	+	+	–	–
Tyrosine	+	+	+	–	–	–	–
L-Valyl 2-naphthylamide	–	–	–	+	+	+	+
Susceptibility to:							
Chloramphenicol	+	–	v (–)	+	+	+	+
Neomycin	v (+)	+	–	–	+	+	+
Tetracycline	+	+	+	–	–	–	–
Trimethoprim/sulfamethoxazole	–	–	–	+	+	+	+
Vancomycin	v (+)	–	–	–	–	–	–
DNA G + C content (mol%)	61.0–63.2	63.0–64.6	64.4–65.2	62.2	58.0†	57.6†	59.5†

*Data from Arahal *et al.* (2002).†Data from Kaye *et al.* (2004).

‡L, Lateral; L-P, lateral/polar; Pe, peritrichous.

§Data from Mata *et al.* (2002).

relatedness and strain S18214^T, the type of group I, showed 17–42 % hybridization with strain W1025^T, strain T68687^T, and the type strains of *H. magadiensis*, *H. aquamarina*, *H. axialensis* and *H. meridiana*. Members of group II shared 88–90 % relatedness and strain W1025^T, the type of group II, showed 35–52 % hybridization with strain T68687^T, strain S18214^T, and the type strains of *H. magadiensis*, *H. aquamarina*, *H. axialensis* and *H. meridiana*. Members of group III shared 90–93 % relatedness and strain T68687^T, the type of group III, showed 32–52 % hybridization with strain W1025^T, strain S18214^T, and the type strains of *H. magadiensis*, *H. aquamarina*, *H. axialensis* and *H. meridiana*. Consequently, DNA–DNA hybridization results confirmed the proposal of three novel *Halomonas* species (Wayne *et al.*, 1987).

The physiological, biochemical and chemotaxonomic characteristics of the novel *Halomonas* species are given in the species descriptions and those that distinguish them from related taxa and from each other are listed in Table 1. The novel *Halomonas* species could be distinguished from related taxa by differences in cell morphology, cell growth ranges, hydrolysis of tyrosine and L-valyl 2-naphthylamide and susceptibility to tetracycline and trimethoprim/sulfamethoxazole, as well as by differences in acid production and substrate utilization profiles. Furthermore, significant differences in Voges–Proskauer test results, nitrate reduction, urease activity, acid production from L-arabinose, D-fucose, D-galactose, maltose, inositol and D-mannitol, growth on D-galactose, starch, *myo*-inositol, adipate, malonate, L-cysteine, DL-isoleucine, L-lysine and L-methionine, and susceptibility to chloramphenicol, neomycin and vancomycin supported the proposal of three novel species of the genus *Halomonas*.

The fatty acid profiles of the novel *Halomonas* species were similar to those of related taxa; the fatty acids C_{18:1}ω7c and

C_{16:0} were predominant. However, they showed slight differences, e.g. higher amounts of C_{18:1}ω7c and the absence of C_{17:0} cyclo and C_{19:0} cyclo ω8c, to related taxa except *H. magadiensis*. When cells were grown on media with different NaCl concentrations, i.e. TSA and TSA with 5 % (w/v) NaCl, no significant change in fatty acid composition was observed for all tested strains. Detailed fatty acid compositions are shown in Table 2 and Supplementary Table S2 (available in IJSEM Online).

Combined phenotypic and genotypic data support the proposal that the 14 strains represent three novel species in the genus *Halomonas*, for which the names *Halomonas stevensii* sp. nov., *Halomonas hamiltonii* sp. nov. and *Halomonas johnsoniae* sp. nov. are proposed.

Description of *Halomonas stevensii* sp. nov.

Halomonas stevensii (ste.ven'si.i. N.L. gen. n. *stevensii* of Stevens, named after Dr David A. Stevens, a physician/epidemiologist who isolated and characterized the first strains).

Cells are aerobic, Gram-stain-negative and non-spore-forming rods (0.9–1.1 × 2.0–3.0 μm). Cells are motile with peritrichous flagella. PHB is accumulated. EPS is not produced. Colonies are cream-coloured, smooth, translucent and circular with entire edges. Catalase- and oxidase-positive. Growth occurs at 10–40 °C (optimum 30–35 °C) and at pH 7–11 (optimum pH 8–9). Growth occurs at sea-salt concentrations of 0–20 % (w/w) (optimum 3.0–7.5 %, w/v). Growth is observed on MacConkey agar. Anaerobic growth is not observed on solid MH medium. Indole is not produced. H₂S is produced from L-cysteine. Nitrate is not reduced. Selenite is reduced. Methyl red and Voges–Proskauer tests are negative. Utilization of glucose is oxidative. Gluconate is

Table 2. Cellular fatty acids of the novel strains and closely related type strains of the genus *Halomonas* grown on TSA

Species/strains: 1, *H. stevensii* sp. nov. ($n=8$); 2, *H. hamiltonii* sp. nov. ($n=3$); 3, *H. johnsoniae* sp. nov. ($n=3$); 4, *H. magadiensis* KCTC 22195^T; 5, *H. aquamarina* KCTC 22193^T; 6, *H. axialensis* KCTC 22194^T; 7, *H. meridiana* KCTC 22196^T. n , Number of strains tested. Fatty acids are listed using standard abbreviations (number of carbon atoms: number of double bonds). Means $\pm$ SD are given where appropriate. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7
C _{10:0}	1.5 $\pm$ 0.2	1.1 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6	2.3	0.5	0.5
C _{12:0}	ND	0.7 $\pm$ 0.1	0.5 $\pm$ 0.1	0.7	1.2	1.0	1.0
C _{12:0} 3-OH	7.7 $\pm$ 0.1	8.7 $\pm$ 0.4	7.4 $\pm$ 0.1	9.5	10.3	11.1	6.3
C _{14:0}	3.6 $\pm$ 0.1	3.7 $\pm$ 0.0	3.5 $\pm$ 0.1	4.3	4.5	3.7	3.3
Summed feature 4*	6.5 $\pm$ 0.1	7.0 $\pm$ 0.1	6.1 $\pm$ 0.1	7.2	10.1	9.6	10.4
C _{16:0}	13.5 $\pm$ 0.3	14.1 $\pm$ 0.6	13.7 $\pm$ 0.4	12.7	17.1	14.6	14.8
C _{17:0} cyclo	ND	ND	ND	ND	1.7	0.3	0.7
C _{18:1} ω 7c	65.9 $\pm$ 0.5	63.6 $\pm$ 1.0	66.0 $\pm$ 0.3	62.6	48.5	57.9	60.5
C _{18:0}	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1	1.2 $\pm$ 0.2	0.4	0.5	0.4	0.2
11-Methyl C _{18:1} ω 7c	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	ND	0.8	ND	ND	ND
C _{19:0} cyclo ω 8c	ND	ND	ND	ND	3.7	0.8	2.2

*Summed feature 4 contains C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH.

oxidized. Respiration on nitrate, nitrite or fumarate is negative. Tween 20, Tween 80 and tyrosine are hydrolysed, but aesculin, casein, DNA, gelatin, starch and *o*-nitrophenyl- β -D-galactopyranoside are not. Blood is not haemolysed. Urease is produced, but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, β -galactosidase, lecithinase and phosphatase are not. Acetate, citrate, ethanol, D-fructose, fumarate, gluconate, D-glucose, glycerol, *myo*-inositol, malate, maltose, propionate, succinate, sucrose and trehalose are utilized as sole sources of carbon and energy, but adipate, adonitol, aesculin, amygdalin, L-arabinose, cellobiose, dulcitol, formate, D-fucose, D-galactose, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, lactose, malonate, D-mannitol, D-mannose, melezitose, raffinose, L-rhamnose, D-ribose, D-salicin, sorbitol and D-xylose are not. L-Alanine, L-cysteine, L-proline, L-serine, L-methionine and L-valine are utilized as sole sources of carbon, nitrogen and energy, but L-histidine, DL-isoleucine and L-lysine are not. In the API 50CH gallery, acid is produced from D-fructose, D-glucose, maltose, glycerol, methyl α -D-glucoside, sucrose, trehalose and turanose, but not from *N*-acetylglucosamine, aesculin, D-adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, D-mannitol, D-mannose, melezitose, melibiose, methyl α -D-mannoside, methyl β -D-xyloside, raffinose, L-rhamnose, D-ribose, D-salicin, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol, D-xylose or L-xylose. According to results from API ZYM tests, L-leucyl 2-naphthylamide, 2-naphthyl butyrate and 2-naphthyl α -D-glucopyranoside are hydrolysed, but *N*-benzoyl-DL-arginine 2-naphthylamide, 6-bromo-2-naphthyl α -D-galactopyranoside, 6-bromo-2-naphthyl β -D-glucopyranoside, 6-bromo-2-naphthyl α -D-mannopyranoside, L-cystyl 2-

naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI- β -D-glucuronide, naphthol-AS-BI-phosphate, 1-naphthyl *N*-acetyl- β -D-glucosaminide, 2-naphthyl caprylate, 2-naphthyl α -L-fucopyranoside, 2-naphthyl β -D-galactopyranoside, 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), 2-naphthyl phosphate (pH 8.5) and L-valyl 2-naphthylamide are not. Susceptible to amoxicillin, ampicillin, carbenicillin, cefotaxime, cefoxitin, chloramphenicol, nalidixic acid, nitrofurantoin, penicillin G, polymyxin B, rifampicin, sulphamide, tetracycline and tobramycin. Resistant to kanamycin, streptomycin and trimethoprim/sulfamethoxazole. Susceptibility to erythromycin, neomycin and vancomycin is strain-dependent. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids C_{18:1} ω 7c and C_{16:0} are predominant. The G + C content of the DNA is 61.0–63.2 mol%.

The type strain, S18214^T (=KCTC 22148^T=DSM 21198^T), was isolated from blood of a renal care patient. The DNA G + C content of the type strain is 62.0 mol%.

Description of *Halomonas hamiltonii* sp. nov.

Halomonas hamiltonii (ha.mil.to'ni.i. N.L. gen. n. *hamiltonii* of Hamilton, named after Dr John R. Hamilton, a microbiologist who isolated and characterized the first strains).

Cells are aerobic, Gram-stain-negative and non-spore-forming rods (0.9–1.0 $\times$ 2.0–3.0 μ m). Cells are motile with lateral flagella. PHB is accumulated. EPS is not produced. Colonies are cream-coloured, smooth, translucent and circular with entire edges. Catalase- and oxidase-positive. Growth occurs at 10–40 °C (optimum 30–35 °C) and at pH 6–10 (optimum pH 8–9). Growth occurs at sea-salt concentrations of 0–20 % (w/w) (optimum 5.0–7.5 % w/

v). Good growth is observed on MacConkey agar. Anaerobic growth is not observed on solid MH medium. Indole is not produced. H₂S is produced from L-cysteine. Nitrate is not reduced. Selenite is reduced. Methyl red test is negative, but Voges–Proskauer test is positive. Utilization of glucose is oxidative. Gluconate is oxidized. Respiration on nitrate, nitrite or fumarate is negative. Tween 20, Tween 80 and tyrosine are hydrolysed, but aesculin, casein, DNA, gelatin, starch and *o*-nitrophenyl- β -D-galactopyranoside are not. Blood is not haemolysed. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, β -galactosidase, lecithinase and phosphatase are not produced. Acetate, adipate, citrate, ethanol, D-fructose, fumarate, D-galactose, gluconate, D-glucose, glycerol, 4-hydroxybenzoate, malate, malonate, maltose, D-mannitol, melezitose, propionate, sorbitol, succinate, sucrose and trehalose are utilized as sole sources of carbon and energy, but adonitol, aesculin, amygdalin, L-arabinose, cellobiose, dulcitol, formate, D-fucose, glycogen, 3-hydroxybenzoate, *myo*-inositol, lactose, D-mannose, raffinose, L-rhamnose, D-ribose, D-salicin, starch and D-xylose are not. L-Alanine, L-lysine, L-proline and L-serine are utilized as sole sources of carbon, nitrogen and energy, but L-cysteine, L-histidine, DL-isoleucine, L-methionine and L-valine are not. In the API 50CH gallery, acid is produced from L-arabinose, D-fructose, D-fucose, D-galactose, D-glucose, methyl α -D-glucoside, sucrose, trehalose and turanose, but not from *N*-acetylglucosamine, aesculin, D-adonitol, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, L-fucose, gentiobiose, gluconate, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, maltose, D-mannitol, D-mannose, melezitose, melibiose, methyl α -D-mannoside, methyl β -D-xyloside, raffinose, L-rhamnose, D-ribose, D-salicin, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol, D-xylose or L-xylose. According to results from API ZYM tests, L-leucyl 2-naphthylamide, 2-naphthyl butyrate and 2-naphthyl α -D-glucopyranoside are hydrolysed, but *N*-benzoyl-DL-arginine 2-naphthylamide, 6-bromo-2-naphthyl α -D-galactopyranoside, 6-bromo-2-naphthyl β -D-glucopyranoside, 6-bromo-2-naphthyl α -D-mannopyranoside, L-cystyl 2-naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI- β -D-glucuronide, naphthol-AS-BI-phosphate, 1-naphthyl *N*-acetyl- β -D-glucosaminide, 2-naphthyl caprylate, 2-naphthyl α -L-fucopyranoside, 2-naphthyl β -D-galactopyranoside, 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), 2-naphthyl phosphate (pH 8.5) and L-valyl 2-naphthylamide are not. Susceptible to amoxicillin, ampicillin, carbenicillin, cefotaxime, cefoxitin, nalidixic acid, neomycin, nitrofurantoin, penicillin G, polymyxin B, rifampicin, sulphamide, tetracycline and tobramycin. Resistant to chloramphenicol, erythromycin, kanamycin, streptomycin, trimethoprim/sulfamethoxazole and vancomycin. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids C_{18:1 ω 7c} and C_{16:0} are predominant. The G + C content of the DNA is 63.0–64.6 mol%.

The type strain, W1025^T (=KCTC 22154^T=DSM 21196^T), was isolated from a dialysis machine drain. The DNA G + C content of the type strain is 64.6 mol%.

Description of *Halomonas johnsoniae* sp. nov.

Halomonas johnsoniae (john.so'ni.ae. N.L. gen. n. *johnsoniae* of Johnson, named after Ms. Nancy Johnson, a registered nurse who isolated and characterized the first strains).

Cells are aerobic, Gram-stain-negative and non-spore-forming rods (0.7–0.9 $\times$ 1.5–4.0 μ m). Cells are motile with lateral/polar flagella. PHB is accumulated. EPS is not produced. Colonies are cream-coloured, smooth, translucent and circular with entire edges. Catalase- and oxidase-positive. Growth occurs at 10–40 °C (optimum 30–35 °C) and at pH 7–10 (optimum pH 8–9). Growth occurs at sea-salt concentrations of 0–20 % (w/w) (optimum 0–5 %, w/v). Good growth is observed on MacConkey agar. Anaerobic growth is not observed on solid MH medium. Indole is not produced. H₂S is produced from L-cysteine. Nitrate is reduced, but nitrite is not. Selenite is reduced. Methyl red and Voges–Proskauer tests are negative. Utilization of glucose is oxidative. Gluconate is oxidized. Respiration on nitrate, nitrite or fumarate is negative. Tween 20, Tween 80 and tyrosine are hydrolysed, but aesculin, casein, DNA, gelatin, starch and *o*-nitrophenyl- β -D-galactopyranoside are not. Blood is not haemolysed. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, β -galactosidase, lecithinase, phosphatase and urease are not produced. Acetate, citrate, ethanol, D-fructose, fumarate, gluconate, D-glucose, glycerol, 4-hydroxybenzoate, malate, maltose, D-mannitol, melezitose, propionate, sorbitol, succinate, sucrose and trehalose are utilized as sole sources of carbon and energy, but adipate, adonitol, aesculin, amygdalin, L-arabinose, cellobiose, dulcitol, formate, D-fucose, D-galactose, glycogen, 3-hydroxybenzoate, *myo*-inositol, lactose, malonate, D-mannose, raffinose, L-rhamnose, D-ribose, D-salicin, starch and D-xylose are not. L-Alanine, DL-isoleucine, L-proline, L-serine and L-valine are utilized as sole sources of carbon, nitrogen and energy, but L-cysteine, L-histidine, L-lysine and L-methionine are not. In the API 50CH gallery, acid is produced from D-fructose, D-glucose, glycerol, D-mannitol, melezitose, sucrose, trehalose and turanose, but not from *N*-acetylglucosamine, aesculin, D-adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, maltose, D-mannose, melibiose, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-xyloside, raffinose, L-rhamnose, D-ribose, D-salicin, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol, D-xylose or L-xylose. According to results from API ZYM tests, L-leucyl 2-naphthylamide, 2-naphthyl butyrate and 2-naphthyl α -D-glucopyranoside are hydrolysed, but

N-benzoyl-DL-arginine 2-naphthylamide, 6-bromo-2-naphthyl α -D-galactopyranoside, 6-bromo-2-naphthyl β -D-glucopyranoside, 6-bromo-2-naphthyl α -D-mannopyranoside, L-cystyl 2-naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI- β -D-glucuronide, naphthol-AS-BI-phosphate, 1-naphthyl *N*-acetyl- β -D-glucosaminide, 2-naphthyl caprylate, 2-naphthyl α -L-fucopyranoside, 2-naphthyl β -D-galactopyranoside, 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), 2-naphthyl phosphate (pH 8.5) and L-valyl 2-naphthylamide are not. Susceptible to amoxicillin, ampicillin, carbenicillin, cefotaxime, cefoxitin, nalidixic acid, nitrofurantoin, polymyxin B, rifampicin, sulphamide, tetracycline and tobramycin. Resistant to erythromycin, kanamycin, neomycin, streptomycin, trimethoprim/sulfamethoxazole and vancomycin. Susceptibility to chloramphenicol and penicillin G is strain-dependent. The predominant quinone is ubiquinone Q-9; a small amount of Q-8 is also present. The fatty acids C_{18:1} ω 7c and C_{16:0} are predominant. The G + C content of the DNA is 64.4–65.2 mol%.

The type strain, T68687^T (=KCTC 22157^T=DSM 21197^T), was isolated from a waste handling option port of a dialysis machine. The DNA G + C content of the type strain is 64.4 mol%.

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