***Aequorivita* gen. nov., a member of the family Flavobacteriaceae isolated from terrestrial and marine Antarctic habitats**

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Several strains isolated from Antarctic winter sea water, sea-ice algal assemblages and quartz stone subliths were found to belong to a novel 16S rDNA sequence cluster within the family *Flavobacteriaceae* (*Cytophaga–Flavobacterium–Bacteroides* division). The strains were Gram-negative, non-motile, psychrotolerant, strictly aerobic, chemoheterotrophic rod-shaped cells that contained orange or yellow carotenoid pigments and required yeast extract when grown in defined mineral-salts media. The requirement for sodium ions varied between strains. Results of DNA–DNA hybridization analysis were used to divide the strains into four distinct genospecies, which were differentiated by physiological and nutritional characteristics. The DNA G+C content of the strains was 33–39 mol%. The fatty acid profiles of representative strains were very similar, with major constituents including i15:1 ω 10c, a15:1 ω 10c, i15:0, a15:0, i16:1 ω 6c, i17:1 ω 5c and 3-OH i16:0. The novel genus *Aequorivita* gen. nov., which has widespread distribution in the Antarctic region, is proposed. The genus comprises four species: the type species *Aequorivita antarctica* sp. nov. (type strain SW49^T = ACAM 640^T = DSM 14231^T), *Aequorivita lipolytica* sp. nov. (type strain Y10-2^T = ACAM 641^T = DSM 14236^T), *Aequorivita crocea* sp. nov. (type strain Y12-2^T = ACAM 642^T = DSM 14239^T) and *Aequorivita sublithicola* sp. nov. (type strain 9-3^T = ACAM 643^T = DSM 14238^T).

Keywords: *Aequorivita*, *Flavobacteriaceae*, Antarctica, sea water, sea ice

INTRODUCTION

Members of the *Cytophaga–Flavobacterium–Bacteroides* division (flavobacteria) make up a large proportion (10–20%) of marine bacterioplanktonic communities (Glöckner *et al.*, 1999). Cultivation and molecular studies suggest that flavobacteria are mostly closely associated with living and dead phytoplankton; for example, they can colonize living algae, absorbing nutrient exudates produced during photosynthesis (Glöckner *et al.*, 1999; Grossart, 1999; Brown & Bowman, 2001). In addition, it is now thought that

flavobacteria have an important role in the breakdown of complex dissolved and particulate organic matter (Cottrell & Kirchman, 2000; Davey *et al.*, 2001). The group therefore has an important and central role in remineralization processes in the ocean. South of the Antarctic Convergence, *in situ* fluorescent hybridization analyses indicate that flavobacteria make up 30–40% of the bacterioplankton populations and, on this basis, this bacterial group must have a highly significant impact on carbon and nutrient flow in Southern Ocean waters (Simon *et al.*, 1999).

At present, 19 genera containing species isolated from marine environments belong to the *Cytophaga–Flavobacterium–Bacteroides* division. Of these, several have been isolated originally from Antarctic maritime lakes and the surrounding Southern Ocean, including the genera *Gelidibacter*, *Psychroserpens*, *Polaribacter*, *Psychroflexus* and *Salegentibacter* (Bowman *et al.*, 1997a,

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The GenBank accession numbers for the 16S rDNA sequences of strains SW49^T, G#5, O9-2, Y10-2^T and Y12-2^T are AY027802–AY027806, respectively.

Table 1. Bacterial strains investigated in this study

Strain	Antarctic isolation site	Reference
<i>Aequorivita antarctica</i> sp. nov.		
SW49 ^T (= ACAM 640 ^T)	Under-ice sea water, Prydz Bay	Bowman <i>et al.</i> (1997b)
SW57	Under-ice sea water, Ellis Fjord	Bowman <i>et al.</i> (1997b)
M_15	Sea ice, Mertz Polynya	This study
9-14	Quartz stone sublith, Vestfold Hills	Smith <i>et al.</i> (2000)
9-16	Quartz stone sublith, Vestfold Hills	Smith <i>et al.</i> (2000)
G#1	Quartz stone sublith, Vestfold Hills	This study
G#5	Quartz stone sublith, Vestfold Hills	This study
O9-2	Sea water, Mertz Polynya	This study
O14-2	Sea water, Mertz Polynya	This study
<i>Aequorivita lipolytica</i> sp. nov.		
Y10-2 ^T (= ACAM 641 ^T)	Sea water, Mertz Polynya	This study
1-18	Quartz stone sublith, Vestfold Hills	Smith <i>et al.</i> (2000)
SW55	Under-ice sea water, Ellis Fjord	This study
<i>Aequorivita crocea</i> sp. nov.		
Y12-2 ^T (= ACAM 642 ^T)	Sea water, Mertz Polynya	This study
Y13-2	Sea water, Mertz Polynya	This study
Y6-1	Sea water, Mertz Polynya	This study
M_05	Sea ice, Mertz Polynya	This study
M_09	Sea ice, Mertz Polynya	This study
<i>Aequorivita sublithicola</i> sp. nov.		
9-3 ^T (= ACAM 643 ^T)	Quartz stone sublith, Vestfold Hills	Smith <i>et al.</i> (2000)

ACAM, Australian Collection of Antarctic Micro-organisms, School of Agricultural Science, University of Tasmania, Hobart, Tasmania, Australia.

1998b; Gosink *et al.*, 1998; Bowman, 2000). All except the genus *Gelidibacter* are exclusive to marine or marine-derived habitats, including marine-salinity and hypersaline lakes, sea ice and sea water. Species of the genera *Polaribacter* and *Psychroserpens* have been isolated or detected in both Arctic and Antarctic sea ice (Gosink *et al.*, 1998; Staley & Gosink, 1999; Brown & Bowman, 2001) and the genus *Gelidibacter* has been isolated from sea ice and from quartz stone subliths of the Vestfold Hills (Bowman *et al.*, 1997a; Smith *et al.*, 2000).

In this study, several similar isolates have been obtained from Antarctic terrestrial and marine habitats, including quartz stone subliths, sea ice and sea water. Polyphasic taxonomic analyses of the isolates indicate that they form a distinct and coherent taxon located within the family *Flavobacteriaceae*. The analyses used in the study demonstrate that the strains group into four distinct species in a novel genus designated *Aequorivita* gen. nov.

METHODS

Samples. Sublithic material was sampled from the undersides of quartz stones collected from feldfield areas of the Vestfold Hills as described previously (Smith *et al.*, 2000). Surface sea water and sea ice samples were collected in August 1999 over a short transect through the Mertz Polynya, an ice-formation zone near the Antarctic coast (66–67° S, 144–145° E). Sea-ice samples were collected using a SIPRE ice corer and

processed as described previously by Bowman *et al.* (1997b). Sea-water samples were collected either by bucket or from an inline shipboard water sampler, which takes samples directly from a 7 m depth. Water samples (1.0–3.0 l sample volumes) and melted sea ice (0.3–2.0 l sample volumes) were filtered through a 1.0 µm filter and then through a 0.2 µm filter. Materials present on the two filter types were then resuspended separately in 10–20 ml of 0.2-µm-filtered natural sea water.

Bacterial isolation. Quartz stone sublithic isolates were obtained by serially diluting sublithic material (suspended in 2.5% NaCl) and plating onto plates of marine agar 2216 (Difco) and R2A agar (supplemented with 2.5% NaCl) (Oxoid) and incubating at 2 °C for 14–28 days. Individual colonies were selected and purified on marine agar 2216 at 10 °C. Sea-water isolates were obtained from Mertz Polynya sea-water filter samples diluted for most-probable number counting. The filtered material was diluted initially 1:100 and then diluted in 1:5 steps in 96-well titre trays. The media used were prepared in natural sea water (pH 7.4–7.9) and included ZoBell's marine medium (7 g total nutrients l⁻¹) and a dilute sea-water nutrient medium (SW medium, 2.8 g nutrients l⁻¹). ZoBell's marine medium contained (l⁻¹) 5 g bacteriological peptone, 2 g yeast extract and 10 mg ferric pyrophosphate. The SW medium contained (l⁻¹) 0.5 g yeast extract, 0.5 g tryptone, 0.5 g bacteriological peptone, 0.5 g D-glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate and 0.3 g K₂HPO₄. Both media were supplemented with 1 ml vitamin solution l⁻¹ (Balch & Wolfe, 1976) and aliquots were then filtered through 0.2 µm disposable filters before being dispensed into the titre trays. The trays were incubated at 2 °C for 21 days. Bacterial isolates were obtained from the highest dilutions of the most-probable number trays and also

Fig. 1. Photographs of *Aequorivita antarctica* sp. nov. SW49^T (a), *Aequorivita sublithicola* sp. nov. 9-3^T (b) and *Aequorivita crocea* sp. nov. Y12-2^T (c) (*Aequorivita lipolytica* sp. nov. has a similar appearance to *A. crocea*). Strains were grown on marine agar 2216 at 2 °C for 14 days and observed under phase-contrast microscopy. Bars, 10 µm.

directly from the sea-water filter samples, serially diluted onto agar media that had been solidified with 1.2% agar. Media included marine agar 2216 and SW agar. SW agar medium had the same ingredients as the SW liquid medium indicated above except that concentrated stocks of filtered-sterilized glucose, K_2HPO_4 and vitamins were added following autoclaving of the remaining medium components, which had been dissolved in a 1 l sea-water salts solution (35 g l⁻¹; Sigma-Aldrich). Agar plates were incubated at 2 °C for 7–21 days before the subsequent colonies were examined and subcultured to obtain pure cultures. Strains were maintained as viable cultures on marine agar slants or plates at 2 °C and also cryopreserved at –80 °C, frozen within marine 2216 liquid media supplemented with 30% glycerol.

Phenotypic analysis. Tests used have been detailed previously in Bowman *et al.* (1998a).

16S rDNA sequence analysis. The 16S rDNA was amplified using PCR from all of the isolates obtained, as described previously (Bowman *et al.*, 1997b). The amplicons were purified using the Prep-A-Gene kit (Bio-Rad) and sequences were generated using the ABI Prism BigDye Terminator cycle sequencing kits with sequence reactions separated and

analysed on an ABI 377 automated DNA sequencer. Sequences generated were 1420–1430 bp in length. Sequences were analysed initially by gapped BLAST analysis (Altschul *et al.*, 1997) operated through the National Center of Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>). From these searches, selected sequences were downloaded and near-complete sequences were aligned manually to the Antarctic strains. The region of the 16S rDNA aligned and analysed extended from positions 31 to 1470 (*Escherichia coli* equivalent). Subsequent phylogenetic analyses of the sequence datasets utilized PHYLIP (version 3.57c) (Felsenstein, 1993), applying the maximum-likelihood algorithm (DNADIST) option to calculate distances. Phylogenetic trees were then constructed from the distance data using the neighbour-joining method (NEIGHBOR). Bootstrap analysis was performed with the programs SEQBOOT and CONSENSE using 250 resamplings of the dataset.

DNA base composition and DNA–DNA hybridization. Genomic DNA was extracted and purified from cells using the procedure of Marmur & Doty (1962). The DNA G+C content was then determined from thermal denaturation profiles using procedures developed by Sly *et al.* (1986). The spectrophotometric renaturation rate kinetic procedure

adapted from Huß *et al.* (1983) (Bowman *et al.*, 1998a) was used to determine DNA–DNA reassociation values between genomic DNA of different strains.

Fatty acid analysis. All strains were cultivated on marine agar 2216 (Difco) at 20 °C for 2 days and then harvested. Whole-cell fatty acid profiles were determined quantitatively using GC and GC-MS procedures (Nichols *et al.*, 1993). The double-bond positions of the fatty acids are numbered from the methyl (ω) end of the fatty acid.

RESULTS AND DISCUSSION

Phenotypic characteristics

Isolates, listed in Table 1, formed either orange or yellow colonies when cultivated on marine agar 2216 (3–7 days, 20 °C). All strains formed colonies that were compact, circular, convex and smooth with an entire non-spreading edge and a butyrous consistency. Flexi-rubin pigments were not detected by the application of 20% KOH. Microscopic examination revealed that the strains had similar morphologies, with cells appearing rod-shaped with rounded ends. Most orange-pigmented strains possessed cells that were 0.5–20.0 μm long and 0.2–0.3 μm wide (Fig. 1a, b). Younger cultures (< 5 days at 20 °C) had a larger number of short, filamentous cells (5.0–20.0 $\times$

0.2–0.4 μm) than did older cultures. Though similar in length, orange-pigmented strain 9-3^T formed thicker filaments, with widths of 0.4–0.5 μm (Fig. 1b). Yellow-pigmented strains uniformly formed cells that were rod-shaped, 0.5–3.0 μm long and 0.4–0.5 μm wide (Fig. 1c). The motility of the strains was assessed microscopically using the hanging-drop method. For determination of gliding motility, strains were grown for 12–24 h at 20 °C on various dilute media (SW agar and 10% strength marine agar 2216, solidified with 1% agar noble) prepared as a thin layer on ethanol-rinsed microscope slides. Following incubation, the edges of microcolonies were investigated under phase microscopy. All strains were non-motile.

The strains grew profusely on complex media that have been prepared in 35 g sea-water salts l⁻¹, including nutrient agar (Oxoid), trypticase soy agar (Oxoid), Anacker–Ordal agar (Bernardet *et al.*, 1996) and SW agar. All strains could grow on nutrient agar (Oxoid) that contained 2.5% NaCl, indicating that strains did not require high levels of divalent cations (Mg²⁺, Ca²⁺) for growth. Several yellow-pigmented strains (Y12-2^T, Y13-2, Y6-1, M_05, M_09) grew in the absence of sodium ions (Table 2); however, all other strains required at least 0.5–1.0% NaCl in the medium for growth.

Table 2. Phenotypic characteristics that differentiate *Aequorivita* species

All strains produced catalase, alkaline phosphatase and esterase (Tween 80 hydrolysis) and hydrolysed L-tyrosine and gelatin. All strains were negative for the following tests: iron reduction with ferric pyrophosphate/ferric oxide as electron acceptors and lactate and acetate as electron donors; oxidative or fermentative acid production from carbohydrates including D-glucose, DL-arabinose, L-rhamnose, D-mannose, D-ribose, D-fructose, D-galactose, cellobiose, sucrose, trehalose, maltose, lactose, D-raffinose, D-melibiose, N-acetylglucosamine, D-adonitol, *myo*-inositol, D-mannitol, D-sorbitol, dulcitol and glycerol; tolerance of 12% or more NaCl or 1% bile salts; hydrolysis of agar, alginate, carboxymethylcellulose (CMC), cellulose (filter paper), dextran, xylan, urate and xanthine; production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, α -arabinosidase, α -fucosidase, β -glucosidase, β -galactosidase, 6-phospho- β -galactosidase and β -glucuronidase. Tests are scored as: +, all strains positive; v+/v–, result varies between strains, type strain positive/negative; –, all strains negative.

Test/character	<i>A. antarctica</i>	<i>A. lipolytica</i>	<i>A. crocea</i>	<i>A. sublithincola</i>
No. of strains studied	9	3	5	1
Filamentous cells (> 10 μm)	+	–	–	+
Pigment*	o	y	y	o
Na ⁺ ions required	+	+	–	+
D-Glucose utilized	+	–	–	–
Urease	v–	v+	–	+
α -Galactosidase, α -glucosidase	v–	–	–	–
N-Acetyl- β -glucosaminidase	–	–	–	+
Lecithinase (egg yolk reaction), lipase (olive oil)	–	+	–	–
Tributyryl hydrolysis	–	+	–	–
Extracellular DNase	–	–	v+	–
Hydrolysis of:				
Starch	+	–	–	–
Casein	–	+	+	–
Elastin	v–	+	+	–
Aesculin	+	+	+	–
G + C content (mol%) (T_m method)	38–39	35–36	33–34	36–37

* o, Orange; y, yellow.

Fig. 2. Phylogenetic relationship of *Aequorivita* species and related species within the family *Flavobacteriaceae* based on 16S rDNA sequences (positions 31–1470, *E. coli* equivalent). The tree was created using maximum-likelihood distances clustered by the neighbour-joining method. Sequences for strains 9-14 and O14-2 were identical. Bar, 0.1 changes per mean nucleotide position. Percentage bootstrap support is indicated next to the branch nodes. Numbers in parentheses are GenBank nucleotide accession numbers. The outgroup used for the analysis was *Capnocytophaga ochracea*.

The temperature range for growth of the strains was very similar and was -2 to 25 °C on agar media. No growth occurred on agar media at 30 °C. Optimal growth on agar media was observed at approximately 20 °C. Biochemical tests that differentiate the genospecies defined by DNA–DNA hybridization data (Table 3) are given in Table 2.

16S rDNA sequences

Nearly complete 16S rDNA sequences, 1420–1430 bp long (nt 31–1470, *E. coli* equivalent), were obtained for representative strains selected after comparisons of phenotypic data. The strains formed a distinct lineage (100% bootstrap supported) within the family *Flavobacteriaceae*, with the most closely related species including those of the genera *Gelidibacter* (sequence similarity 0.88–0.89) and *Cellulophaga* (0.87–0.89) and the species *[Cytophaga] marinoflava* (0.89–0.90) (Fig. 2). Eight strains formed a shallow cluster that included two subgroups. Five strains, including SW49^T, formed one subgroup (sequence similarity > 0.99), whereas strains Y10-2^T and Y12-2^T formed the other subgroup (sequence similarity 0.987). Strain 9-3^T formed a more

distinct lineage, sharing 0.94–0.95 sequence similarity with the other strains (Fig. 2).

DNA base composition and DNA–DNA hybridization

The DNA G+C contents of the test strains were 33–39 mol%. Values for the individual genospecies defined by DNA–DNA hybridization are given in Table 2 and values for individual strains are given in Table 3. Strain selection for DNA–DNA hybridization analyses was determined by DNA G+C content and 16S rDNA sequence similarity. Four genospecies were clearly defined from the analysis (Table 3). The first group included orange-pigmented strains represented by SW49^T, which possessed a G+C content of 38–39 mol%. DNA–DNA hybridization between SW49^T and five representative strains of this group exceeded 78% (Table 3). Strain SW49^T had only 29–39% DNA hybridization with yellow-pigmented strains, although they were closely related by 16S rDNA sequencing (Fig. 2). The yellow-pigmented strains consisted of two genospecies sharing 38–57% DNA–DNA hybridization (Table 3). One group was represented by Y10-2^T (G+C content 35–36 mol%), whereas the second group was represented by Y12-2^T (G+C content 33–34 mol%). Within each of these groups, DNA hybridization levels exceeded 79%. Strain 9-3^T, which formed a distinct branch in the 16S rDNA cluster, was genetically different from all other strains, sharing only 8–17% DNA hybridization (Table 3).

Fatty acid analysis

Whole-cell fatty acid profiles were generated to determine whether there were any distinct differences between the strains and to compare them to related genera of the family *Flavobacteriaceae*. One strain from each genospecies was selected for analysis. The major fatty acids present in all strains included i15:1 ω 10c, a15:1 ω 10c, i15:0, a15:0, i16:1 ω 6c, i17:1 ω 5c, a17:1 ω 7c and 3-OH i16:0 (Table 4). The fatty acid compositions were very similar between the representative strains, with variations in fatty acid content mostly present amongst minor 3-hydroxy fatty acid components, as well as some differences in the proportion of fatty acids such as palmitoleic acid (16:1 ω 7c) (Table 4). The distinct clustering of the test strains by 16S rDNA sequences was also supported by the fatty acid composition. Strains SW49^T, Y10-2^T and Y12-2^T were characterized by an i15:1 ω 10c:a15:1 ratio of approximately 1:2; in comparison, this ratio is reversed for strain 9-3^T. In comparison to related genera including *Gelidibacter* and *Cellulophaga* and the species *[Cytophaga] marinoflava* (Table 4), the fatty acid profiles of the test strains included various quantitative and qualitative differences, in particular the percentage of 15:0 and some monounsaturated branched-chain fatty acids. For example, compared with *Cellulophaga* species and *[Cytophaga] marinoflava*, the test strains had much higher levels of a15:1 ω 10c, a15:0 and i16:1 ω 6c (Table 4).

Table 3. DNA base composition and DNA–DNA hybridization of strains of the genus *Aequorivita*

DNA G + C contents and DNA hybridization values are means of three to five experimental replicates.

Strain	G + C content (mol %)	Hybridization (%) with DNA from:			
		SW49 ^T	Y10-2 ^T	Y12-2 ^T	9-3 ^T
<i>A. antarctica</i>					
SW49 ^T	38.5	100	39	34	12
M_15	37.8	83	33		
9-16	39.1	94	32		
G#5	38.3	87			
O9-2	38.0	78	29		
O14-2	38.0	81			
<i>A. lipolytica</i>					
Y10-2 ^T	35.6	39	100	44	17
1-18	35.3	29	89	57	
<i>A. crocea</i>					
Y12-2 ^T	33.8	34	44	100	8
A290	33.5		54	103	
M_05	34.1		41	79	
M_09	33.9		38	86	
<i>A. sublithincola</i>					
9-3 ^T	36.5	12	17	8	100

Polyphasic taxonomy of the novel strains

Polyphasic analyses demonstrated that the strains belong to a coherent and novel genus within the family *Flavobacteriaceae*. Phenotypic data (Table 2, Fig. 1), DNA G + C content (Table 3) and DNA–DNA hybridization data (Table 3) clearly demonstrated that the strains formed four distinct subgroups equivalent to species. Differences between the species are recorded in Tables 2 and 3. Phylogenetic analysis indicated that three of the species, represented by strains SW49^T, Y10-2^T and Y12-2^T, were closely related (Fig. 2). This was confirmed by the relatively high levels of DNA–DNA hybridization found between genomes of these and other strains (Table 3); however, the hybridization levels were below the 60–70% DNA hybridization level used to define bacterial species (Wayne *et al.*, 1987). Strain 9-3^T was comparatively more distinct from the other genospecies in terms of DNA hybridization values (Table 3). This was also clearly reflected in terms of 16S rDNA sequence similarity, which indicates that strain 9-3^T is an outlier species of the genus (Fig. 1). Fatty acid data confirmed that the strains were similar to each other, but exhibited some differences from related species (Table 4).

On the basis of the data described above, it can be concluded that the strains examined in this study represent a novel genus within the family *Flavobacteriaceae*. It is proposed that the name *Aequorivita* gen. nov. be given for this novel taxon, referring to their

marine and marine-derived habitats. With the increasing number of taxa present with the *Flavobacteriaceae*, there is increasing difficulty in identifying strains at the genus level on the basis of phenotype alone. Phenotypically, the genus *Aequorivita* can be generally differentiated from other marine members of the family *Flavobacteriaceae* (Table 5) as long as sufficient phenotypic tests are carried out and the data are supported by 16S rDNA sequence information.

The genus *Aequorivita* is represented by four species, as demonstrated by phenotypic and genotypic data, designated *Aequorivita antarctica* sp. nov. (type species, type strain SW49^T), *Aequorivita lipolytica* sp. nov. (type strain Y10-2^T), *Aequorivita crocea* sp. nov. (type strain Y12-2^T) and *Aequorivita sublithincola* sp. nov. (type strain 9-3^T).

Description of *Aequorivita* gen. nov.

Aequorivita [Ae. quo. ri. vi. ta. L. neut. n. *aequor* the flat surface of the sea; L. fem. n. *vita* life; N.L. fem. n. *Aequorivita* life (living being) at the sea surface].

Cells are non-motile, Gram-negative rods or filaments that occur singly or in pairs. Gas vesicles and helical or ring-shaped cells are not formed. Metabolism is strictly aerobic and chemoheterotrophic. Strains are catalase-positive. Non-agarolytic. Strains possess orange or yellow carotenoid pigments, but flexirubin pigments are absent. Strains may require sodium ions for growth

Table 4. Phospholipid fatty acid profiles of members of the genus *Aequorivita* and related species

Taxa are identified as: 1, *A. antarctica* SW49^T; 2, *A. lipolytica* Y10-2^T; 3, *A. crocea* Y12-2^T; 4, *A. sublithincola* 9-3^T; 5, *Gelidibacter algens*; 6, *Cellulophaga* spp.; 7, [*Cytophaga*] *marinoflava*. Data in columns 5–7 were taken from Bowman *et al.* (1998b). tr, Trace.

Fatty acid	1	2	3	4	5	6	7
15:0	0.6	0.6	0.7	3.1	8.8	9.3–14.3	8.7
16:0	1.9	1.2	2.9	0.9	1.4	1.2–6.2	1.6
i14:0	0.2	0.5	1.0	0.9	0.5	–	0.1
i15:1 ω 10c	9.5	10.5	8.9	12.9	11.3	7.5–10.4	17.4
a15:1 ω 10c	16.0	23.0	13.8	8.5	8.8	0.0–0.3	1.6
i15:0	7.6	16.3	15.9	16.8	7.9	7.5–31.3	15.0
a15:0	15.7	20.7	19.2	17.8	13.2	1.0–3.3	4.6
i16:1 ω 6c	3.0	7.4	6.2	6.7	7.7	0.5–2.2	0.4
i16:0	1.4	1.2	4.7	5.3	8.0	0.1–2.7	1.2
i17:1 ω 5c	5.8	4.8	8.6	9.7	4.9	5.1–6.7	16.6
a17:1 ω 7c	5.0	4.2	3.2	2.5	5.2	0.5–1.5	1.7
a17:0	–	–	–	–	1.5	–	–
15:1 ω 11c	–	–	–	–	3.5	–	–
15:1 ω 6c	0.6	–	–	0.9	–	1.0–2.6	2.0
15:1 ω 4c	–	–	–	–	1.3	–	–
16:1 ω 7c	7.8	1.2	2.9	0.8	5.9	9.0–19.2	7.0
16:1 ω 5c	–	–	–	–	1.5	–	–
17:1 ω 6c	–	–	–	–	1.5	0.0–0.6	–
3-OH i15:0	5.4	1.7	2.9	2.1	0.5	0.8–8.6	1.6
3-OH a15:0	5.8	1.6	1.6	tr	–	0.4–1.8	1.0
3-OH i16:0	9.2	2.1	4.4	5.6	0.8	0.0–0.7	2.0
3-OH i17:0	2.0	2.3	–	4.5	0.4	4.5–20.8	9.4
3-OH a17:0	2.9	–	4.2	4.0	0.4	0.2–0.9	4.7
Other	2.7	0.6	2.5	0.9	0.9	1.7–2.4	3.2

and require yeast extract when grown in defined mineral-salts media. Psychrotolerant, with growth occurring between -2 and 25 °C; optimal growth at about 20 °C but no growth at 30 °C (on agar media). Major fatty acids are i15:1 ω 10c, a15:1 ω 10c, a15:0, i15:0, i16:1 ω 6c, i17:1 ω 5c, a17:1 ω 7c and 3-OH i16:0. Member of the family *Flavobacteriaceae*, order *Cytophagales*. The type species is *Aequorivita antarctica*.

Description of *Aequorivita antarctica* sp. nov.

Aequorivita antarctica (ant.arc'ti.ca. N.L. fem. n. *antarctica* from Antarctica).

The description is the same as that of the genus unless specified otherwise. Cells appear as rods or short filaments in young cultures (0.5 – 20.0×0.2 – 0.3 μ m). Forms bright-orange colonies. Requires sodium ions for growth (at least 0.5 % NaCl). Tolerates 6 % NaCl and grows weakly in 10 % NaCl. No growth occurs in the presence of 1 % ox-bile salts. Detectable levels of acid are not produced from carbohydrates; however, most strains can utilize D-glucose as a sole carbon and energy source in the presence of 0.02 % yeast extract. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are negative. Nitrate is not reduced. Hydrogen sulfide is not formed from L-

cysteine. Hydrolyses starch, aesculin, L-tyrosine (no diffusible pigment), Tween 80 and gelatin. Strains may also hydrolyse urea and elastin. Unable to hydrolyse agar, cellulose, carboxymethylcellulose (CMC), xylan, alginate, dextran, DNA, tributyrin, urate or xanthine. Lipase (olive oil) and lecithinase are not produced. Produces alkaline phosphatase. Some strains can form α -glucosidase or α -galactosidase, but do not produce α -arabinosidase, β -galactosidase, 6-phospho- β -galactosidase, β -glucosidase, β -glucuronidase, α -fucosidase or *N*-acetyl- β -glucosaminidase. The G + C content of cells is 38 – 39 mol %. The type strain is strain SW49^T (= ACAM 640^T = DSM 14231^T), which was isolated from under-ice sea water of Prydz Bay, Antarctica.

Description of *Aequorivita lipolytica* sp. nov.

Aequorivita lipolytica (lip.o.ly'ti.ca. Gr. n. *lipos* fat; Gr. adj. *lytikos* dissolving; N.L. fem. adj. *lipolytica* fat-dissolving).

The description is the same as that of the genus unless specified otherwise. Cells appear as straight rods (0.5 – 3.0×0.4 – 0.5 μ m), which occur singly or in pairs. Forms bright-yellow colonies. Requires sodium ions for growth (at least 0.5 % NaCl). Tolerates 6 % NaCl, but not 10 % NaCl. No growth occurs in the presence

Table 5. Characteristics that differentiate the genus *Aequorivita* from other marine genera and species of the family Flavobacteriaceae

+, Positive; v, present or absent in species of the genus; —, absent.

Characteristic	<i>Aequorivita</i>	<i>Cytophaga</i> <i>mariniflava</i>	<i>Gelidibacter</i>	<i>Cellulophaga</i>	<i>Psychroserpens</i>	<i>Polaribacter</i>	<i>Tenacibaculum</i>	<i>Psychroflexus</i> <i>torquus</i>	<i>Psychroflexus</i> <i>gondwanensis</i>	<i>Saiegentibacter</i>	<i>Cytophaga</i> <i>luteocaula</i>
Isolation site*	I, SW, QSS	SW	I, SW, QSS	I, SW, BR	SW, MLW	I, SW	SW, DF	I	HLW	HLW	SW
Pigmentation†	O or Y	O-Y	Y	O-Y (F)	Y	O	Y	O	O	Y	O-R (F)
Morphology	Rods	Rods	Rod-filament	Rod	Rod-filament, helices	Rod-filament, gas vesicles	Rod-filaments	Filaments	Rods	Rods	Rods
Gliding motility	—	—	+	+	—	—	+	+	—	—	+
Salt requirement‡	Marine or none	Marine	Marine	Marine	Marine	Marine	Marine	Marine	Moderately halophilic	Moderately halophilic	Marine
Growth at 4/25 °C	+ / +	- / +	+ / v	v / +	+ / -	+ / -	- / +	+ / -	+ / +	+ / +	- / +
Agarase	—	—	—	+	—	—	—	—	—	—	+
Amylase	v	+	+	+	+	v	—	+	+	—	+
Amino acid requirement§	+	—	—	—	+	+	+	+	—	—	—
G + C content (mol %)	33–39	36	36–38	32–42	27–29	30–34	29–33	31–32	36	37–38	32

* Known isolation sites: I, sea ice; SW, sea water (includes marine macrophyta and algae); QSS, quartz stone subliths; MLW, marine-salinity lake water; DF, diseased fish and eggs; HLW, hypersaline lake water; BR, brackish and estuarine waters.

† O, Orange; Y, yellow; R, red; (F) flexirubin pigment formed.

‡ Marine. Strains require at least 0.5–1.0 % NaCl, optimum is usually about 2–3 % NaCl, may require divalent cations; none, no salt requirement; moderately halophilic, optimal growth is at a salinity greater than that of sea water (for species listed, it is about 5 % NaCl).

§ Requirement for added yeast extract (and, in some cases, vitamins) to grow in a defined mineral-salts medium.

of 1 % ox-bile salts. Detectable levels of acid are not produced from carbohydrates nor do strains utilize D-glucose as a sole carbon and energy source in the presence of 0.02 % yeast extract. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are negative. Nitrate is not reduced. Hydrogen sulfide is not formed from L-cysteine. Hydrolyses aesculin, L-tyrosine (no diffusible pigment), Tween 80, gelatin, casein and elastin. Strains may also hydrolyse urea. Unable to hydrolyse agar, starch, cellulose, CMC, xylan, dextran, DNA, urate or xanthine. Tributyrin is hydrolysed and lipase (olive oil) and lecithinase are also produced. Produces an alkaline phosphatase. The following glycosidic enzymes are not produced: α -glucosidase, α -galactosidase, α -arabinosidase, β -galactosidase, 6-phospho- β -galactosidase, β -glucosidase, β -glucuronidase, α -fucosidase and N-acetyl- β -glucosaminidase. The G + C content of cells is 35–36 mol %. The type strain is strain Y10-2^T (= ACAM 641^T = DSM 14236^T), which was isolated from sea water of the Mertz Polynya, Antarctica.

Description of *Aequorivita crocea* sp. nov.

Aequorivita crocea (cro'ce.a. L. fem. adj. *crocea* yellow).

The description is the same as that of the genus unless specified otherwise. Cells appear as straight rods (0.5–3.0 × 0.4–0.5 μ m), which occur singly or in pairs. Forms bright-yellow carotenoid pigment. Does not require sodium ions for growth. Tolerates 6 % NaCl, but not 10 % NaCl. No growth occurs in the presence of 1 % ox-bile salts. Detectable levels of acid are not produced from carbohydrates nor do strains utilize D-glucose as a sole carbon and energy source in the presence of 0.02 % yeast extract. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are negative. Nitrate is not reduced. Hydrogen sulfide is not formed from L-cysteine. Hydrolyses aesculin, L-tyrosine (no diffusible pigment), Tween 80, gelatin, casein and elastin. Strains may also hydrolyse DNA. Unable to hydrolyse agar, starch, cellulose, CMC, xylan, dextran, DNA, urea, urate or xanthine. Unable to hydrolyse tributyrin and negative for lipase (olive oil) and lecithinase. Produces an alkaline phosphatase. The following glycosidic enzymes are not produced: α -glucosidase, α -galactosidase, α -arabinosidase, β -galactosidase, 6-phospho- β -galactosidase, β -glucosidase, β -glucuronidase, α -fucosidase and N-acetyl- β -glucosaminidase. The G + C content of cells is 33–34 mol %. The type strain is strain Y12-2^T (= ACAM 642^T = DSM 14239^T), which was isolated from sea water of the Mertz Polynya, Antarctica.

Description of *Aequorivita sublithicola* sp. nov.

Aequorivita sublithicola (sub.lith.in'co.la. L. pref. *sub* below; Gr. n. *lithos* stone; L. nom. n. *incola* an inhabitant; N.L. n. *sublithicola* an inhabitant below stone).

The description is the same as that of the genus unless specified otherwise. Cells appear as rods or filaments in young cultures (0.5–20 × 0.4–0.5 µm). Forms orange colonies. Requires sodium ions for growth (at least 1.0 % NaCl). Weakly tolerates 6 % NaCl, but not 10 % NaCl. No growth occurs in the presence of 1 % ox-bile salts. Detectable levels of acid are not produced from carbohydrates nor can strains utilize D-glucose as a sole carbon and energy source in the presence of 0.02 % yeast extract. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are negative. Nitrate is not reduced. Hydrogen sulfide is not formed from L-cysteine. Produces a strong urease and can hydrolyse Tween 80, gelatin and L-tyrosine (no diffusible pigment). Unable to hydrolyse starch, aesculin, agar, cellulose, CMC, xylan, dextran, DNA, tributyrin, urate, xanthine, casein or elastin. Lipase (olive oil) and lecithinase are not produced. Produces an alkaline phosphatase. Strains produce N-acetyl-β-glucosaminidase, but not α-glucosidase, α-galactosidase, α-arabinosidase, β-galactosidase, 6-phospho-β-galactosidase, β-glucosidase, β-glucuronidase or α-fucosidase. The G + C content of cells is 36–37 mol %. The type and only available strain is strain 9-3^T (= ACAM 643^T = DSM 14238^T), which was isolated from a quartz stone sublithic cyanobacterial community found in the Vestfold Hills, Antarctica.

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REFERENCES

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Balch, W. E. & Wolfe, R. S. (1976). New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl Environ Microbiol* **32**, 781–791.
- Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.
- Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.
- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997a). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997b). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Bowman, J. P., McCammon, S. A., Brown, J. L. & McMeekin, T. A. (1998a). *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. *Int J Syst Bacteriol* **48**, 1213–1222.
- Bowman, J. P., McCammon, S. A., Lewis, T., Skerratt, J. H., Brown, J. L., Nichols, D. S. & McMeekin, T. A. (1998b). *Psychroflexus torquus* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**, 1601–1609.
- Brown, M. V. & Bowman, J. P. (2001). A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). *FEMS Microbiol Ecol* **35**, 267–275.
- Cottrell, M. T. & Kirchman, D. L. (2000). Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66**, 1692–1697.
- Davey, K. E., Kirby, R. R., Turley, C. M., Weightman, A. J. & Fry, J. C. (2001). Depth variation of bacterial extracellular enzyme activity and population diversity in the northeastern North Atlantic Ocean. *Deep-sea Res Part II* **48**, 1003–1017.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package), version 3.57c. Department of Genetics, University of Washington, Seattle, WA, USA.
- Glöckner, F. O., Fuchs, B. M. & Amann, R. (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl Environ Microbiol* **65**, 3721–3726.
- Gosink, J. J., Woese, C. R. & Staley, J. T. (1998). *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of '*Flectobacillus glomeratus*' as *Polaribacter glomeratus* comb. nov. *Int J Syst Bacteriol* **48**, 223–235.
- Grossart, H. P. (1999). Interactions between marine bacteria and axenic diatoms (*Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii*) incubated under various conditions in the lab. *Aquat Microb Ecol* **19**, 1–11.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **4**, 109–118.
- Nichols, D. S., Nichols, P. D. & McMeekin, T. A. (1993). Polyunsaturated fatty acids in Antarctic bacteria. *Antarct Sci* **5**, 149–160.
- Simon, M., Glöckner, F. O. & Amann, R. (1999). Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. *Aquat Microb Ecol* **18**, 275–284.
- Sly, L. I., Blackall, L. L., Kraat, P. C., Tian-Shen, T. & Sangkhobol, V. (1986). The use of second derivative plots for the determination of mol% guanine plus cytosine of DNA by the thermal denaturation method. *J Microbiol Methods* **5**, 139–156.
- Smith, M. C., Bowman, J. P., Scott, F. J. & Line, M. A. (2000). Sublithic bacteria associated with Antarctic quartz stones. *Antarct Sci* **12**, 177–184.
- Staley, J. T. & Gosink, J. J. (1999). Poles apart: biodiversity and biogeography of sea ice bacteria. *Annu Rev Microbiol* **53**, 189–215.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.