Aequorivita viscosa sp. nov., isolated from an intertidal zone, and emended descriptions of *Aequorivita antarctica* and *Aequorivita capsosiphonis*

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An aerobic, Gram-stain-negative, short rod-shaped, non-motile and non-sporulating bacterium, designed strain 8-1b^T, was isolated from seaweed collected from the intertidal zone of Zhoushan sea area, East China Sea. Strain 8-1b^T grew at 4–39 °C (optimum, 28–32 °C) and at pH 6.0–9.5 (optimum, 7.0–8.5), and with 0.5–8 % (w/v) NaCl (optimum, 1–3 %) and 0.5–10 % (w/v) sea salts (optimum, 2–3 %). Analysis of 16S rRNA gene sequences revealed that strain 8-1b^T was related closely to *Aequorivita capsosiphonis* JCM 15070^T (96.7 % similarity). The DNA G+C content of strain 8-1b^T was 36.6 mol%. Compared with reference strains, cells of strain 8-1b^T showed positive activities for H_2S production and utilization of D-mannose, DL-lactic acid, L-asparagine and glycyl L-aspartic acid. The major fatty acids of strain 8-1b^T were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G and iso-$C_{17:1}\omega9c$. The main respiratory quinone was menaquinone 6. The polar lipids of strain 8-1b^T consisted of phosphatidylethanolamine (PE), three uncharacterized aminolipids (AL1–3), four uncharacterized glycolipids (GL1–4) and five uncharacterized lipids (L1–5). Based on the phenotypic and genotypic characterization, strain 8-1b^T represents a novel species of the genus *Aequorivita*, for which the name *Aequorivita viscosa* sp. nov. is proposed. The type strain is strain 8-1b^T (=CGMCC 1.11023^T=JCM 18497^T). Emended descriptions of *Aequorivita antarctica* and *Aequorivita capsosiphonis* are also presented.

The family *Flavobacteriaceae* is most closely associated with living and dead phytoplankton, and they can either colonize living algae or assimilate nutrient exudates from phytoplankton (Glöckner *et al.*, 1999; Grossart, 1999; Brown & Bowman, 2001). In addition, it is reported that they play an important role in the degradation of dissolved and particulate complex organic matter (Cottrell & Kirchman, 2000; Davey *et al.*, 2001). At the time of writing, the genus *Aequorivita*, belonging to the family *Flavobacteriaceae*, contains five recognized species: *Aequorivita antarctica*, *A. lipolytica*, *A. crocea*, *A. sublithincola* (Bowman & Nichols, 2002) and *A. capsosiphonis* (Park *et al.*, 2009). They were isolated from Antarctic terrestrial and marine habitats (Bowman & Nichols, 2002) and a green alga collected from the South Sea, Republic of Korea, respectively (Park *et al.*, 2009).

Seaweed samples were collected from the intertidal zone of Zhoushan sea area, East China Sea, in March 2010. The samples were mashed and suspended in sterile seawater. The suspension was appropriately diluted and spread on modified marine 2216 plates which were incubated at 28 °C. The composition of the modified marine 2216 medium was the same as marine broth 2216 (Becton Dickinson) except that 5 g trypticase peptone l^{-1} (Becton Dickinson) and 0.01 g ferric citrate l^{-1} were added. A Gram-stain-negative bacterium that formed yellow colonies was picked out and designed as strain 8-1b^T for further research. Type strains of *A. capsosiphonis* and *A. antarctica* were obtained from the Japan Collection of Microorganisms (JCM), Tsukuba, Japan, and a previous study (Bowman & Nichols, 2002), respectively, and were used as reference strains in this study. Unless otherwise mentioned, strain 8-1b^T and reference strains of *A. antarctica* and *A. capsosiphonis* were all incubated in modified marine 2216 medium.

Growth temperature was tested at 4, 10, 16, 22, 26, 28, 30, 32, 34, 37, 39 and 43 °C. The pH range for growth was determined using different buffering agents: MES (pH 5.5–6.5), Bistris (pH 6.5–9.5) and CAPSO (pH 9.5–10.0). Salt tolerance was tested in modified marine 2216 medium

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 8-1b^T is HM485318.

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

containing 0–12 % NaCl or in 0–12 % sea salts solution supplemented with 5 g trypticase peptone l^{-1} and 1 g yeast extract l^{-1}. Cells grown at 30 °C for 2 days were observed by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) for morphology determination. Anaerobic growth was tested at 30 °C in Hungate tubes filled with N_2.

Various hydrolysis tests were performed on modified marine 2216 agar supplemented with different substrates at certain concentration. Hydrolysis of xylan (1 %, w/v) and xanthine (0.4 %, w/v) were tested as previously described (Barrow & Feltham, 1993; Gordon *et al.*, 1974; Park *et al.*, 2009). Hydrolysis of CM-cellulose (1 %, w/v) and pectin (0.2 %, w/v) were tested by flooding Congo red reagent (0.2 %, w/v) on well-cultured plates. Hydrolysis of alginate (1 %, w/v) was tested by flooding the agar plates with 70 % ethanol after sufficient incubation (Kawasaki *et al.*, 2002). Other biochemical tests were performed with the modified 2216 medium using the methods described by Mata *et al.* (2002). API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate (Biolog) were also used according to the manufacturers' instructions and the description of Park *et al.* (2009). Acid production was tested in modified 2216 medium supplemented with 0.5 % carbon sources and bromocresol purple. Sensitivity to antibiotics was detected on agar plates using different antibiotic discs including (per disc): ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), streptomycin (10 μg), vancomycin (30 μg), gentamicin (10 μg), kanamycin (30 μg), polymyxin B (300 IU), tetracycline (30 μg), nalidixic acid (30 μg), penicillin (10 IU), amikacin (30 μg), carbencillin (100 μg), novobiocin (30 μg), neomycin (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg) and rifampicin (5 μg).

Cells were grown in modified 2216 medium for 3 days at 30 °C for chemotaxonomic characterization. Fatty acid methyl esters were obtained from freeze-dried cells as described by Kuykendall *et al.* (1988), and their identification and quantification were performed using the Sherlock Microbial Identification System (MIDI) with the standard MIS Library Generation Software version 4.5 (Microbial ID). Isoprenoid quinones were identified using LC-MS as described previously (Tindall, 1989; Chung *et al.*, 1997). Polar lipids were analysed by two-dimensional TLC with silica gel 60 F_{254} plates (Merck) as described by Xu *et al.* (2007a). Sulfuric acid, ninhydrin, Zinzadze reagent and α-naphthol were used to detect total lipids, amino lipids, phospholipids and glycolipids, respectively.

The 16S rRNA gene was amplified using the method described by Xu *et al.* (2007b). The resulting sequence (1488 nt) was aligned with sequences obtained from the EzTaxon-e database (Kim *et al.*, 2012). Multiple sequence alignment was performed with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods in the MEGA 5 program package (Tamura *et al.*, 2011). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method. The G+C content of the genomic DNA was determined by reversed-phase HPLC as described by Mesbah & Whitman (1989).

Cells of strain 8-1b^T were Gram-stain-negative, non-spore-forming and non-motile short rods 4.0–4.5 × 1.8–2.2 μm in size (Fig. S1, available in IJSEM Online). No growth was observed under anaerobic conditions. Strain 8-1b^T grew at 4–39 °C (optimum, 28–32 °C) and pH 6.0–9.5 (optimum, 7.0–8.5). The concentration ranges of NaCl and sea salts for growth were 0.5–8 % (optimum, 1–3 %, w/v) and 0.5–10 % (optimum, 2–3 %, w/v), respectively. Detailed physiological and biochemical characteristics are summarized in the species description. Differences between strain 8-1b^T and the reference strains are shown in Table 1, which indicated that strain 8-1b^T could be distinguished from the reference strains based on the utilization of D-mannose, DL-lactic acid, L-asparagine and glycyl L-aspartic acid and H_2S production.

The fatty acid compositions of strain 8-1b^T and the reference strains are shown in Table 2. The major fatty acids of strain 8-1b^T were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G, iso-$C_{17:1}\omega9c$, anteiso-$C_{15:0}$ and unknown 13.565. The component of unknown 13.565 was first detected in members of the genus *Aequorivita*. Compared with *A. antarctica* SW49^T and *A. capsosiphonis* JCM 15070^T, strain 8-1b^T had lower amounts of summed feature 3 and iso-$C_{17:1}\omega9c$ but higher amounts of iso-$C_{15:1}$. Strain 8-1b^T contained menaquinone 6 (MK-6) as the main respiratory quinone, which was in accordance with *A. antarctica* SW49^T and *A. capsosiphonis* JCM 15070^T. The polar lipids of strain 8-1b^T consisted of phosphatidylethanolamine (PE), three uncharacterized aminolipids (AL1–3), four uncharacterized glycolipids (GL1–4) and five uncharacterized lipids (L1–5) (Fig. S2). PE, AL1, L1 and L2 were detected in all tested strains. However, L5 and AL3 were only detected in strain 8-1b^T, GL5 was only detected in the two reference strains, and L6 was only found in *A. antarctica* SW49^T. The results indicated that there are some minor differences between the polar lipid profiles of strain 8-1b^T and the reference strains.

Strain 8-1b^T showed highest 16S rRNA gene sequence similarity to *A. capsosiphonis* JCM 15070^T (96.7 %). Both the neighbour-joining and maximum-likelihood trees indicated that strain 8-1b^T belonged to the genus *Aequorivita*, in which the new isolate clustered with *A. antarctica* SW49^T, *A. capsosiphonis* JCM 15070^T, *A. crocea* Y12-2^T and *A. lipolytica* Y10-2^T and showed shorter evolutionary distances to *A. antarctica* SW49^T and *A. capsosiphonis* JCM 15070^T (Fig. 1). The DNA G+C content of strain 8-1b^T was 36.6 mol% (HPLC).

In contrast to the reference strains, strain 8-1b^T formed viscous colonies, utilized D-mannose, DL-lactic acid, L-asparagine and glycyl L-aspartic acid, and showed positive activity for H_2S production. Regarding distinguishing chemotaxonomic characteristics, strain 8-1b^T had lower levels of summed feature 3 and iso-$C_{17:1}\omega9c$ and a higher

Table 1. Differential characteristics between strain 8-1b^T and related type strains of species of the genus *Aequorivita*

Strains: 1, 8-1b^T; 2, *A. capsosiphonis* JCM 15070^T; 3, *A. antarctica* SW49^T. All data are from this study. All strains are positive for catalase activity and hydrolysis of gelatin, Tween 20 and Tween 80. All strains are negative for oxidase activity, hydrolysis of aesculin, alginate, casein, CM-cellulose, DNA, pectin, starch, xanthine and xylan, indole production, methyl red and Voges–Proskauer tests, and nitrate reduction. +, Positive; w, weakly positive; −, negative.

Characteristic	1	2	3
H_2S production	+	−	−
Hydrolysis of:			
Tween 40	−	−	+
Tween 60	−	−	+
Tyrosine	+	+	−
Urease	+	−	w
Utilization of:			
L-Asparagine	+	−	−
L-Aspartic acid	+	+	−
L-Fucose	−	+	−
Glycogen	+	+	−
Glycyl L-aspartic acid	+	−	−
DL-Lactic acid	+	−	−
D-Lactose	−	+	−
D-Mannose	+	−	−
D-Sorbitol	−	+	−
Sucrose	−	+	−
Enzyme activities (API ZYM)			
N-Acetyl-β-glucosaminidase	−	w	w
α-Chymotrypsin	−	−	w
Cystine arylamidase	−	w	w
α-Galactosidase	−	w	−
Lipase (C14)	−	w	−
Trypsin	w	−	w
Antibiotic resistance			
Ampicilin (10 μg)	−	−	+
Nalidixic acid (30 μg)	+	+	−
Neomycin (30 μg)	+	−	+
Novobiocin (30 μg)	−	+	−
Penicillin (10 IU)	−	−	+
DNA G+C content (mol%)	36.6	36.9	38.5

level of iso-$C_{15:1}$ G. Moreover, polar lipids L5 and AL3 were detected only in strain 8-1b^T.

Based on our phenotypic, phylogenetic and chemotaxonomic characterization, strain 8-1b^T is considered to represent a novel species of the genus *Aequorivita*, for which the name *Aequorivita viscosa* sp. nov. is proposed. Emended descriptions of the species *Aequorivita antarctica* and *Aequorivita capsosiphonis* are also provided.

Emended description of *Aequorivita antarctica* Bowman and Nichols 2002

The description of *A. antarctica* is as given by Bowman & Nichols (2002) with the addition that the main respiratory quinone is menaquinone 6 (MK-6) and the polar lipid profile contains phosphatidylethanolamine, two uncharacterized aminolipids, five uncharacterized glycolipids and five uncharacterized lipids.

Table 2. Fatty acid compositions of strain 8-1b^T and related type strains of species of the genus *Aequorivita*

Strains: 1, 8-1b^T; 2, *A. capsosiphonis* JCM 15070^T; 3, *A. antarctica* SW49^T. All data are from the present study. Values are percentages of total fatty acids. tr, Trace amount (<1 %); −, not detected. ECL, equivalent chain length.

Fatty acid	ECL	1	2	3
Unknown 13.565	13.566	7.1	5.3	4.3
iso-$C_{15:1}$ G	14.442	6.4	3.7	4.1
iso-$C_{15:0}$	14.626	34.4	34.0	30.5
anteiso-$C_{15:0}$	14.715	5.0	6.1	14.2
$C_{15:0}$	15.000	3.6	1.3	3.0
iso-$C_{16:1}$ H	15.460	tr	tr	1.1
iso-$C_{16:0}$	15.627	tr	1.0	1.3
Summed feature 3*	15.847	3.5	6.0	7.3
$C_{16:0}$	15.998	tr	tr	1.4
iso-$C_{15:0}$ 3-OH	16.134	3.5	2.8	3.1
$C_{15:0}$ 2-OH	16.223	tr	tr	2.3
iso-$C_{17:1}\omega 9c$	16.418	8.7	13.7	12.7
Summed feature 4*	16.493	−	1.1	−
iso-$C_{16:0}$ 3-OH	17.149	1.5	1.1	tr
iso-$C_{17:0}$ 3-OH	18.162	18.3	17.4	6.2
$C_{17:0}$ 2-OH	18.256	tr	1.6	1.2

*Summed feature 3 consists of $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 4 consists of iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

Emended description of *Aequorivita capsosiphonis* Park *et al.* 2009

The description of *A. capsosiphonis* is as given by Park *et al.* (2009) with the addition that the main respiratory quinone is menaquinone 6 (MK-6)and the polar lipid profile contains phosphatidylethanolamine, two uncharacterized aminolipids, five uncharacterized glycolipids and four uncharacterized lipids.

Description of *Aequorivita viscosa* sp. nov.

Aequorivita viscosa (vis.co′sa. L. fem. adj. *viscosa* viscous, referring to its adherent colonies).

Cells are Gram-stain-negative, non-spore-forming and non-motile short rods 4.0–4.5 × 1.8–2.2 μm in size. No growth is observed under anaerobic conditions. After incubation at 30 °C for 2–3 days, colonies formed on modified 2216 agar plates are yellow, viscous, circular, opaque and smooth. Grows at 4–39 °C (optimum, 28–32 °C) and pH 6.0–9.5 (optimum, 7.0–8.5). Requires 0.5 % NaCl or 0.5 % sea salts for growth and tolerates up to 8 % (w/v) NaCl or 10 % (w/v) sea salts (optimum, 1–3 % NaCl

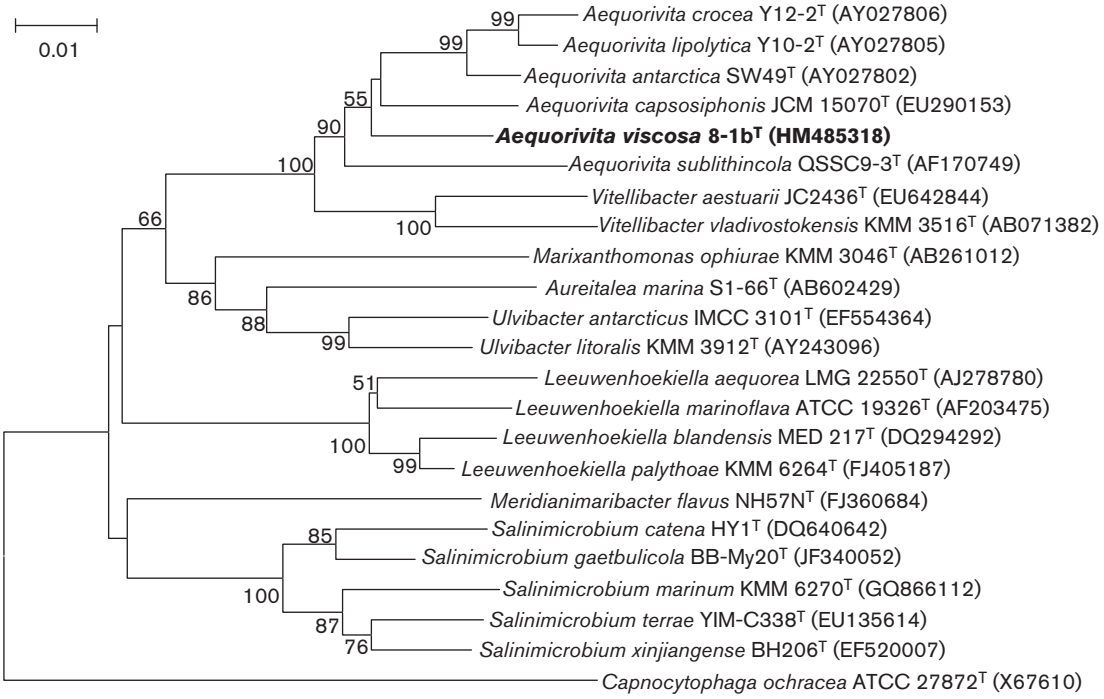

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain 8-1b^T and related taxa. *Capnocytophaga ochracea* ATCC 27872^T was used as an outgroup. Bootstrap percentages are based on 1000 replicates; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

or 2–3 % sea salts). Positive for catalase and urease activities, H_2S production and hydrolysis of gelatin, Tween 20, Tween 80 and tyrosine. Negative for oxidase activity, nitrate reduction, indole production, methyl red and Voges–Proskauer tests, and hydrolysis of starch, casein, Tween 40, Tween 60, xanthine, DNA, aesculin, xylan, CM-cellulose, pectin and alginate. No acid production from carbohydrates. In the API ZYM system, positive result in tests for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase; weakly positive result for valine arylamidase, trypsin and naphthol-AS-BI-phosphohydrolase; negative result in tests for lipase (C14), cysteine arylamidse, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In the API 20NE system, the β-galactosidase test is positive. The following substrates are utilized in GN2 MicroPlate tests: glycogen, D-mannose, DL-lactic acid, L-asparagine, L-aspartic acid and glycyl L-aspartic acid. Sensitive to the following antibiotics (per disc): ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), vancomycin (30 μg), penicillin (10 IU), carbencillin (100 μg), novobiocin (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg) and rifampicin (5 μg). The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G, iso-$C_{17:1}\omega9c$, anteiso-$C_{15:0}$ and unknown 13.565. The main respiratory quinone is menaquinone 6 (MK-6). The polar lipid profile consists of phosphatidylethanolamine (PE), three uncharacterized aminolipids (AL1–3), four uncharacterized glycolipids (GL1–4) and five uncharacterized lipids (L1–5).

The type strain, 8-1b^T (=CGMCC 1.11023^T=JCM 18497^T), was isolated from seaweed collected from the intertidal zone of Zhoushan sea area, East China Sea. The DNA G+C content of the type strain is 36.6 mol% (HPLC).

Ackonwledgements

We thank John P. Bowman for providing the type strain of *Aequorivita antarctica*. This work was supported by the Zhoushan Scientific and Technological Program (10323).

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