Aequorivita capsosiphonis sp. nov., isolated from the green alga *Capsosiphon fulvescens*, and emended description of the genus *Aequorivita*

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A marine bacterial strain, designated A71^T, was isolated from marine algae collected from the South Sea, Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain A71^T belonged to the family *Flavobacteriaceae* and was closely related to *Aequorivita antarctica* SW49^T (96.5 % sequence similarity). Cells of strain A71^T were Gram-negative, aerobic, oxidase-negative, catalase-positive, yellow/orange-pigmented and non-motile. The major fatty acids were iso-C_{15:0} (20.6 %), iso-C_{17:1ω9c} (13.3 %), anteiso-C_{15:0} (13.1 %), iso-C_{17:0} 3-OH (12.7 %) and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1ω7c}; 6.6 %). The DNA G+C content was 36.9 mol%. Several phenotypic characteristics served to differentiate the isolate from recognized members of the genus *Aequorivita*. Data from this polyphasic study clearly demonstrated that strain A71^T represents a novel species of the genus *Aequorivita*. The name *Aequorivita capsosiphonis* sp. nov. is proposed, with strain A71^T (=KCTC 22183^T =JCM 15070^T) as the type strain. In addition, an emended description of the genus *Aequorivita* is presented.

Many members of the family *Flavobacteriaceae* are closely associated with living and dead components of the marine phytoplankton (Glöckner *et al.*, 1999; Grossart, 1999; Brown & Bowman, 2001). They can colonize living algae and absorb nutrient-containing exudates excreted by the host. In addition, they have an important role in the breakdown of complex dissolved and particulate organic matter in oceans (Cottrell & Kirchman, 2000; Davey *et al.*, 2001). The genus *Aequorivita* was recently described by Bowman & Nichols (2002) as a novel member of the family *Flavobacteriaceae*. At the time of writing, the genus comprises four species: *Aequorivita antarctica*, *A. lipolytica*, *A. crocea* and *A. sublithicola*. *A. sublithicola* was isolated from a terrestrial cyanobacterial community in Antarctica, while the three other *Aequorivita* species were isolated from seawater. Members of the genus are characterized as

psychrotolerant and non-agarolytic bacteria that are orange or yellow in colour (as a result of the presence of carotenoid pigments) (Bowman & Nichols, 2002).

During the course of a study on the microflora of marine algae, a Gram-negative bacterium, designated strain A71^T, was isolated. A polyphasic approach was used to study this organism, which was found to have high levels of 16S rRNA gene sequence similarity with respect to members of the genus *Aequorivita*. Here, our analysis of this novel member of the genus is described.

Strain A71^T was isolated from a sample of the green alga *Capsosiphon fulvescens* collected, in February 2006, from the South Sea near Yeosu (34° 44' N 127° 44' E) located in the Republic of Korea, using the standard dilution plating technique. Isolation was achieved using MR2A [i.e. R2A agar (Difco) supplemented with 3.5 % artificial sea salts (Sigma)]. The isolate was routinely cultured on marine 2216 agar (MA; Difco) at 25 °C and maintained at –80 °C as a suspension in marine 2216 broth (MB; Difco) containing glycerol (20 %, w/v).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A71^T is EU290153.

Scanning electron and phase-contrast micrographs of cells of strain A71^T are available in a supplementary figure with the online version of this paper.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resulting sequence (1385 nt) was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available in all sequences (positions 39–1447; *Escherichia coli* numbering system) by using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated as described by Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/jphydit/>) and PAUP 4.0 (Swofford, 2002) as described previously (Chun *et al.*, 2000).

Preliminary sequence comparisons with 16S rRNA gene sequences held in GenBank indicated that strain A71^T was closely related to the genus *Aequorivita*. The newly determined sequence was then aligned manually against representatives of the genus *Aequorivita*. Strain A71^T showed the highest 16S rRNA gene sequence similarity with *A. antarctica* SW49^T (96.5 %); the sequence similarities with respect to all other species of the genus *Aequorivita* were in the range 95.6–96.0 %. Also, strain A71^T occupied a distinct position in the neighbour-joining tree (Fig. 1). The tree based on the Fitch–Margoliash method showed essentially similar topology (not shown).

As the 16S rRNA gene sequence similarities between strain A71^T and strains of *Aequorivita* species with validly published names were below the level indicative of

relatedness at the species level (97 %; Stackebrandt & Goebel, 1994), a DNA–DNA hybridization test was not needed to define a novel species.

Cells grown for 5 days at 25 °C in MB were observed with phase-contrast (TMS-F; Nikon) and scanning electron (S-4800; Hitachi) microscopy. Sea-salt requirements [final concentration 0–12 % (Sigma), in increments of 1 %] and tolerance of NaCl (0–12 %, in increments of 1 %) for growth were tested on nutrient agar (Difco), trypticase soy agar (Difco), R2A agar and ZoBell's agar (ZoBell, 1941; 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate and 15 g Bacto agar, per litre distilled water). All of the media used in the physiological tests were supplemented with 2 % sea salt, with the exception of MA and MB. The pH range for growth was determined in ZoBell's liquid medium. The media were adjusted to various pH values (pH 3–11, in increments of 1 pH unit) by the addition of HCl and NaOH, which had been sterilized by filtration. Growth was tested (on MA after incubation for up to 1 week) at various temperatures (4–50 °C) and in an anaerobic chamber (CO₂/H₂/N₂, 10 : 10 : 80; Sheldon Manufacturing). Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovács' reagent (Kovács, 1956), respectively. Acid production from glucose and maltose were tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was tested in MB containing 0.2 % KNO₃ (Skerman, 1967). H₂S production was determined on Kligler's iron agar (Difco). Hydrolysis of CM-cellulose (1 %, w/v), casein (2 % skimmed milk, w/v), dextran (1 %, w/v), egg yolk (10 %, w/v), elastin (0.5 %, w/v), starch (0.2 %, w/v) and Tween 80 (1 %, w/v) was tested as described by Smibert & Krieg (1994), using MA as the basal medium. Hydrolysis of L-tyrosine (0.5 %, w/v) and xylan

Fig. 1. Neighbour-joining tree phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain A71^T and members of the family Flavobacteriaceae. Numbers at nodes are bootstrap percentages (based on 1000 resampled datasets); only values greater than 50 % are shown. The sequence of *Capnocytophaga ochracea* ATCC 33596 was used as an outgroup. Bar, 0.01 nucleotide substitutions per position.

(1%, w/v) was tested using MA as the basal medium (Barrow & Feltham, 1993). Decomposition of xanthine (0.4%, w/v) was checked, using MA, as described by Gordon *et al.* (1974). DNase activity was determined on DNase test agar (Difco). Other biochemical tests and enzyme assays were performed using the API 20E and API ZYM kits (bioMérieux) and the GN2 MicroPlate (Biolog) according to the manufacturers' instructions except that bacterial strains were suspended in distilled water supplemented with 2% sea salt. Antibiotic resistance was determined using the disc-diffusion method, with commercial antibiotic-impregnated discs (BBL Becton Dickinson). After 5 days incubation at 25 °C on MA, the results were interpreted according to the guidelines set forth by the CLSI (2003). The presence of flexirubin-type pigments was assessed as described by Bernardet *et al.* (2002).

Strain A71^T grew well on MA and weakly on trypticase soy agar, but did not grow on R2A or nutrient agar without sea salt or NaCl. Sea salt (2–10%) supported growth of the cells on ZoBell's medium, but NaCl did not. Cells in MB were rod-shaped, but sometimes appeared to be filamentous or helical (Supplementary Fig. S1, available in IJSEM Online). On MA, colonies were yellow but turned orange after incubation for 6 days. Physiological characteristics are described in Table 1 and in the species description.

For fatty acid analysis, cells of strain A71^T grown on MA for 2 days at 20 °C were analysed using GLC according to the instructions of the Microbial Identification System (MIDI, 1999). The G+C content of the DNA was determined by using the thermal denaturation method of Marmur & Doty (1962).

The fatty acid profiles of strain A71^T and of the type strains of *Aequorivita* species with validly published names are shown in Table 2. The predominant fatty acids of strain A71^T were iso-C_{15:0}, iso-C_{17:1}ω9c, anteiso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). Fatty acids iso-C_{15:1}ω10c, anteiso-C_{15:1}ω10c, iso-C_{16:1}ω6c, anteiso-C_{17:1}ω7c and iso-C_{17:1}ω5c, found as major components in recognized *Aequorivita* species, were not detected in strain A71^T even though all strains were grown under the same conditions. However, the method used for the fatty acid analysis of strain A71^T differed from that used for the recognized *Aequorivita* strains. Fatty acids iso-C_{17:1}ω9c, C_{17:0} 2-OH and iso-C_{15:0} G were found only in strain A71^T. The DNA G+C content of strain A71^T was 36.9 mol%, a value that is within the range reported for *Aequorivita* species.

A number of phenotypic characteristics clearly distinguish strain A71^T from known *Aequorivita* species (Table 1). Therefore, strain A71^T represents a novel species within the genus *Aequorivita*, for which the name *Aequorivita capsosiphonis* sp. nov. is proposed. On the basis of new data from this study, an emended description of the genus *Aequorivita* is also proposed.

Table 1. Characteristics that serve to differentiate strain A71^T from recognized *Aequorivita* species

Strains: 1, A71^T; 2, *A. antarctica* SW49^T; 3, *A. lipolytica* Y10-2^T; 4, *A. crocea* Y12-2^T; 5, *A. sublithincola* 9-3^T. Data for reference type strains are from Bowman & Nichols (2002). All of the strains are positive for catalase and alkaline phosphatase activities and gelatin hydrolysis. All are negative for oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and β-glucuronidase activities and for hydrolysis of agar, CM-cellulose, dextran, xanthine and xylan. +, Positive; –, negative.

Characteristic	1	2	3	4	5
Na ⁺ ion requirement	+	+	+	–	+
Hydrolysis of:					
Aesculin	–	+	+	+	–
Casein	+	–	+	+	–
DNA	–	–	–	+	–
Egg yolk	–	–	+	–	–
Elastin	–	–	+	+	–
Starch	–	+	–	–	–
Tween 80	–	+	+	+	+
L-Tyrosine	–	+	+	+	+
Urea	–	–	+	–	+
Utilization of carbon sources					
Glucose	–	+	+	+	–
D-Lactose	+	–	–	–	–
D-Sorbitol	+	–	–	–	–
Sucrose	+	–	–	–	–
N-Acetyl-β-glucosaminidase (API ZYM)	–	–	–	–	+
DNA G+C content (mol%)	36.9	38.5	35.6	33.8	36.5

Emended description of *Aequorivita* Bowman and Nichols 2002

The description of the genus *Aequorivita* remains as given by Bowman & Nichols (2002), with the following modifications. Oxidase-negative (this study; J. Bowman, personal communication). Filamentous or helical cells may occur in some species. Some species grow between 4 and 40 °C: optimal growth occurs at approximately 25 °C. The major fatty acids are anteiso-C_{15:0} and iso-C_{15:0}. The DNA G+C contents are in the range 33.5–39.1 mol%.

Description of *Aequorivita capsosiphonis* sp. nov.

Aequorivita capsosiphonis (cap.so.si.pho'nis. N.L. gen. n. *capsosiphonis* of *Capsosiphon*, the scientific name of the marine alga from which the type strain was isolated).

Gram-negative, strictly aerobic bacterium. Oxidase-negative, catalase-positive and non-motile. In MB, cells are rod-shaped and 0.3–0.4 × 2.0–3.0 µm in size. In MB, cells sometimes appear filamentous or helical and reach up to 15 µm in size. Produces non-diffusible yellow- to orange-coloured pigments that are not of the flexirubin type. On MA, colonies are opaque, convex, circular, smooth and

Table 2. Fatty acid compositions (%) of strain A71^T and type strains of recognized *Aequorivita* species

Strains: 1, A71^T; 2, *A. antarctica* SW49^T; 3, *A. lipolytica* Y10-2^T; 4, *A. crocea* Y12-2^T; 5, *A. sublithincola* 9-3^T. Data for reference type strains are from Bowman & Nichols (2002). Fatty acids amounting to less than 1 % of the total fatty acids in all strains listed are not shown. All strains were grown under the same conditions, but different methods were used for the fatty acid analyses. tr, Trace amount (<1 %); –, not detected.

Fatty acid	1	2	3	4	5
C _{15:0}	tr	tr	tr	tr	3.1
C _{16:0}	3.9	1.9	1.2	2.9	tr
C _{18:0}	2.8	–	–	–	–
C _{15:0} 2-OH	1.5	–	–	–	–
C _{17:0} 2-OH	5.3	–	–	–	–
iso-C _{15:0}	20.6	7.6	16.3	15.9	16.8
anteiso-C _{15:0}	13.1	15.7	20.7	19.2	17.8
iso-C _{15:0} 3-OH	1.5	5.4	1.7	2.9	2.1
anteiso-C _{15:0} 3-OH	–	5.8	1.6	1.6	tr
iso-C _{16:0}	3.3	1.4	1.2	4.7	5.3
iso-C _{16:0} 3-OH	1.6	9.2	2.1	4.4	5.6
iso-C _{17:0} 3-OH	12.7	2.0	2.3	–	4.5
anteiso-C _{17:0} 3-OH	–	2.9	–	4.2	4.0
iso-C _{15:1} ω10c	–	9.5	10.5	8.9	12.9
anteiso-C _{15:1} ω10c	–	16.0	23.0	13.8	8.5
iso-C _{15:1} G	5.0	–	–	–	–
iso-C _{16:1} ω6c	–	3.0	7.4	6.2	6.7
iso-C _{16:1} H	1.0	–	–	–	–
iso-C _{17:1} ω5c	–	5.8	4.8	8.6	9.7
iso-C _{17:1} ω9c	13.3	–	–	–	–
anteiso-C _{17:1} ω7c	–	5	4.2	3.2	2.5
anteiso-C _{17:1} ω9c	2.0	–	–	–	–
C _{16:1} ω7c	–	7.8	1.2	2.9	tr
Summed feature 3*	6.6	–	–	–	–

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the Microbial Identification System. Summed feature 3 consists of C_{16:1} ω7c and/or iso-C_{15:0} 2-OH.

approximately 2 mm in diameter after 5 days at 25 °C (pH 7). Growth occurs at pH 7–9 (optimum, pH 7) and at 4–40 °C (optimum, 25 °C). Requires sodium ions (at least 0.5 % NaCl). Tolerates 8 % NaCl and grows weakly in the presence of 10 % NaCl on R2A agar and trypticase soy agar. Does not produce indole (API 20E). A positive reaction is obtained for H₂S production in the API 20E gallery, but no H₂S is detected on Kligler's iron agar. Acid is not produced from D-glucose or maltose (each at 0.5 %, w/v). Nitrate is not reduced. Produces acetoin (API 20E). Gelatin is hydrolysed, but aesculin, agar, CM-cellulose, casein, dextran, DNA, egg yolk, starch, Tween 80, tyrosine, xanthine and xylan are not hydrolysed. In the API ZYM gallery, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activities are present, weak cystine arylamidase activity is detected

and *N*-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin activities are absent. In the API 20E system, amygdalin, arabinose, glucose, inositol, mannitol, melibiose, rhamnose, sorbitol and sucrose are not assimilated. The following substrates in the GN2 MicroPlate gallery are utilized: L-aspartic acid, L-fucose, gentiobiose, D-glucosaminic acid, glycogen, inosine, α-ketoglutaric acid and L-threonine. The following substrates in the GN2 MicroPlate gallery are utilized only weakly: adonitol, L-alanine, L-asparagine, citric acid, D-galactonic acid lactone, DL-lactic acid, malonic acid, propionic acid, raffinose, turanose and uridine. The remaining substrates in the GN2 MicroPlate gallery are not utilized. Sensitive to the following antibiotics (μg per disc, unless otherwise indicated): ampicillin (10), chloramphenicol (30), erythromycin (15), nalidixic acid (30), penicillin (10 IU), streptomycin (10) and vancomycin (30). Resistant to the following antibiotics (μg per disc, unless otherwise indicated): amikacin (30), gentamicin (10), kanamycin (30), polymyxin B (300 IU) and tetracycline (30). The predominant fatty acids are iso-C_{15:0}, iso-C_{17:1} ω9c, anteiso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω7c). The DNA G+C content of the type strain is 36.9 mol%. Other physiological and biochemical characteristics are given in Tables 1 and 2.

The type strain, A71^T (=KCTC 22183^T =JCM 15070^T), was isolated from *Capsosiphon fulvescens*, a green alga collected from the South Sea, Republic of Korea.

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