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.

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A group of strains with potent extracellular enzymic activity were isolated from the surfaces of the chain-forming sea-ice diatom *Melosira* and from an unidentified macrophyte collected from the Eastern Antarctic coastal zone. 16S rDNA sequence analysis indicated that the strains belonged to the genus *Cellulophaga* and showed greatest similarity to the species *Cellulophaga baltica* (sequence similarity 97%). Phenotypic characteristics, DNA base composition and DNA–DNA hybridization values clearly separate the Antarctic strains from *Cellulophaga baltica* and other *Cellulophaga* species. Thus, the strains form a distinct and novel species and have the proposed name *Cellulophaga algicola* sp. nov. (type strain IC166^T = ACAM 630^T). In addition, it was recognized that the species *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989, a species phylogenetically remote from the type species of the genus *Cytophaga*, possessed 16S rDNA sequences and phenotypic and chemotaxonomic traits similar to those of other *Cellulophaga* species. Thus, it was proposed that the species *Cytophaga uliginosa* be renamed as *Cellulophaga uliginosa* comb. nov.

Keywords: *Cellulophaga*, Antarctica, epiphytic bacteria, agarolytic bacteria, *Cytophaga*

INTRODUCTION

Dense algal assemblages within Antarctic sea ice are a haven for a rich diversity of bacteria including many psychrophilic taxa (Bowman *et al.*, 1997b). Many of these ice-dwelling (sympagic) bacteria belong to the family *Flavobacteriaceae* (order *Cytophagales*). Recent fluorescent *in situ* hybridization studies suggest that the order *Cytophagales* can be highly abundant in the coastal pelagic zone of Antarctica, contributing from 20 to 70% of the bacterial biomass in various seawater samples (Glöckner *et al.*, 1999). Owing to this group's large biomass, they conceivably have important roles in secondary mineralization processes, in which organic material formed by primary production (mostly photosynthetic activity) is degraded back into micro-nutrients.

Species belonging to the family *Flavobacteriaceae* that

have been isolated from Antarctic marine and marine-derived ecosystems include *Gelidibacter algens*, *Psychroserpens burtonensis* (Bowman *et al.*, 1997a), *Psychroflexus torquis* (Bowman *et al.*, 1998), *Polaribacter irgensii*, *Polaribacter filamentus*, *Polaribacter glomeratus*, *Polaribacter franzmannii* (Gosink *et al.*, 1998), a variety of *Flavobacterium* spp. (McCammon *et al.*, 1998; McCammon & Bowman, 2000) and several other unclassified isolates (Bowman *et al.*, 1997b). Some of these species are sympagic and pelagic algal epiphytes (J. P. Bowman, unpublished data), while others are planktonic, some of which can form gas vesicles (Gosink *et al.*, 1998). A group of agarolytic Antarctic strains, isolated from masses of the chain-forming pennate diatom *Melosira* (colonizing the lower surface of some areas of coastal sea ice) and from the surfaces of an unidentified macrophyte, were also found to belong to the family *Flavobacteriaceae* (Bowman *et al.*, 1997b). The strains were most similar to the recently described genus *Cellulophaga* (Johansen *et al.*, 1999), isolated from the surfaces of the seaweed

Abbreviation: CMC, carboxymethylcellulose.

Fucus serratus growing in the Baltic Sea. The goal of this study was to determine the taxonomy of the Antarctic agarolytic isolates in relation to the genus *Cellulophaga* and to other species with similar 16S rDNA sequences.

METHODS

Strains and cultivation conditions. The Antarctic agarolytic strains were isolated from strips of ice algal and macrophytic material (1–2 × 3–5 cm). The material was cut with sterile scalpels and placed flat upon the surface of marine 2216 agar (Difco) and incubated at 0–4 °C. The appearance of pale-orange spreading growth from the algal strips onto the agar was noticeable after 2–3 weeks. Cells from the spreading growth were then transferred to marine 2216 agar and incubated at 10 °C. The resultant colonies were selected for further purification. Isolates and reference strains investigated in this study are shown in Table 1. All strains used in the study were routinely cultivated on marine 2216 agar at 20 °C.

Phenotypic characterization. Most phenotypic tests have been published previously (Bowman *et al.*, 1997a). Gliding motility tests were performed by preparing a light suspension of cells in seawater and then placing a drop on quarter-strength marine 2216 medium solidified with 1% agarose. After 16 h incubation at 10 °C, the inoculated area was covered with a glass coverslip and examined by oil-immersion phase-contrast microscopy. Flexirubin pigments were detected by suspending cells in 20% KOH (Fautz & Reichenbach, 1980). Carboxymethylcellulose (CMC), fibrinogen and elastin hydrolysis were tested by overlaying marine 2216 agar with a thin layer of 0.5% CMC, 0.2% fibrinogen or 0.2% elastin in seawater solidified with 1.2% agar and observing for hydrolysis zones after 14–21 d incubation at 20 °C. Lipase activity was tested with the procedure of Kouker & Jaeger (1987), using marine 2216

plates containing 1 mg rhodamine B ml⁻¹ and 2.5% (w/v) olive oil (Sigma; both filtered-sterilized). Plates were incubated for 7 d at 20 °C and observed under long wavelength (~350 nm) UV light. Production of acid from carbohydrates (at 0.5% w/v) was examined in liquid medium with 5 ml oxidation/fermentation medium (Leifson, 1963) aliquotted into 10 ml screw-cap test tubes and incubated for 14–21 d at 20 °C.

DNA base composition. DNA was extracted by the procedure of Marmur & Doty (1962) and the DNA G + C content was determined from thermal denaturation profiles (Sly *et al.*, 1986).

DNA–DNA hybridization. The spectrophotometric renaturation rate kinetic procedure adapted by Huss *et al.* (1983) was used to determine DNA–DNA reassociation values between genomic DNA of different strains. Genomic DNA was sheared to a mean size of 1 kb by sonication, dialysed overnight at 4 °C in 2 × SSC buffer (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) and adjusted in concentration to approximately 75 µg ml⁻¹. Following denaturation of the DNA samples, hybridization was performed at the optimal temperature for renaturation (T_{OR}), which was 25 °C below the DNA melting temperature and was calculated from the following equation: $T_{OR} = 48.5 + (0.41 \times \%G + C)$, where T_{OR} is in °C and %G + C is the mol% G + C content. The declines in absorbance over a 60 min interval of DNA mixtures and control DNA samples were used to calculate DNA hybridization values from the following equation (Huss *et al.*, 1983):

$$\text{DNA hybridization (\%)} = 100(4AB - A - B/2\sqrt{(A \times B)})$$

where A and B represent the change in absorbance for the two DNA samples being compared and AB represents the change in absorbance for equimolar mixtures of A and B. A DNA hybridization value equal to or below 25% is considered to represent background hybridization and is thus not considered significant.

Table 1. Bacterial strains used in this study

Taxon	Strain*	Isolation site
Antarctic strains		
Agarolytic <i>Cytophaga</i> -like sp.	IC166 ^T = ACAM 630 ^T	<i>Melosira</i> sp., sea ice
Agarolytic <i>Cytophaga</i> -like sp.	IC155 = ACAM 631	<i>Melosira</i> sp., sea ice
Agarolytic <i>Cytophaga</i> -like sp.	IC156 = ACAM 632	Macrophyte
Agarolytic <i>Cytophaga</i> -like sp.	IC167 = ACAM 633	Macrophyte
Agarolytic <i>Cytophaga</i> -like sp.	IC137 = ACAM 634	<i>Melosira</i> sp., sea ice
Agarolytic <i>Cytophaga</i> -like sp.	IC135	<i>Melosira</i> sp., sea ice
Agarolytic <i>Cytophaga</i> -like sp.	IC136	<i>Melosira</i> sp., sea ice
Reference strains		
<i>Cellulophaga lytica</i>	ACAM 74 ^T = ATCC 23178 ^T	Surface sediment
<i>Cellulophaga lytica</i>	NCIMB 1388	Seawater aquarium outflow
<i>Cellulophaga lytica</i>	NCIMB 1395	Seawater aquarium outflow
<i>Cellulophaga lytica</i>	NCIMB 1412	Surface sediment
<i>Cellulophaga baltica</i>	LMG 18535 ^T	Surface of seaweed (<i>Fucus serratus</i>), Baltic Sea
<i>Cellulophaga fucicola</i>	LMG 18536 ^T	Surface of seaweed (<i>Fucus serratus</i>), Baltic Sea
<i>Cytophaga uliginosa</i>	ACAM 538 ^T = ATCC 14397 ^T	Surface sediment
<i>Cytophaga marinoflava</i>	ACAM 75 ^T = LMG 1345 ^T = ATCC 19326 ^T	Seawater aquarium outflow
<i>Cytophaga latercula</i>	ATCC 23177 ^T	Seawater aquarium outflow

* Abbreviations: ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

Fatty acid analysis. Strains were cultivated in marine 2216 broth at 20 °C, harvested and then lyophilized using a vacuum freeze-drier (Dynavac). Whole-cell fatty acid profiles were determined quantitatively by gas chromatography–mass spectrometry (GC–MS) and the geometry and position of double bonds in monounsaturated fatty acids were confirmed using dimethyldisulfide derivatization and analysis by GC–MS as described by Nichols *et al.* (1986). The double-bond positions are numbered from the methyl (ω) end of the fatty acid.

16S rDNA sequence analysis. The 16S rDNA sequence for a representative agarolytic strain (IC166^T) was obtained in an earlier study (Bowman *et al.*, 1997b). The sequence was compared with the compilation of 16S rDNA sequences available in the GenBank nucleotide library and aligned to sequences of the members of the family *Flavobacteriaceae*. Near-complete sequences (1420–1500 bp) were aligned with hypervariable regions aligned according to secondary structural features (Gutell, 1994). Analyses of the 16S rDNA datasets utilized PHYLIP (version 3.57c) (Felsenstein, 1993). DNADIST was used to determine sequence similarities using the maximum-likelihood algorithm option. Phylogenetic trees were constructed with the neighbour-joining method by using the program NEIGHBOR. Bootstrap analysis was performed with SEQBOOT and CONSENSE using 250 re-samplings of the dataset, utilizing both DNADIST and NEIGHBOR as well as the program DNAPARS, which constructs trees based on the maximum-parsimony method.

RESULTS AND DISCUSSION

Morphological and phenotypic properties of the Antarctic strains

When cultivated on marine 2216 agar (2 d, 20 °C), the Antarctic strains initially formed yellow-orange pigmented, flat, irregular to circular colonies with a butyrous consistency and with diameters of 1–3 mm. The colony edges were much paler in pigmentation than the central mass and were very irregular. The colonies were slightly sunken into the agar, which had softened but not liquefied. Flooding of the agar with a 1% (w/v) KI-sublimed iodine (1:1) solution highlighted extensive clearing zones around the growth, indicating that the agar had been at least partially depolymerized and degraded. The KOH test results indicated that flexirubin pigments were absent. Microscopic investigations revealed that the strains appeared rod-like, with either rounded or tapered ends and with cell lengths and widths ranging from 1.5 to 4 and 0.4 to 0.5 μ m, respectively. All strains examined exhibited rapid gliding motility.

The Antarctic strains grew profusely on a variety of complex seawater agars including marine 2216 agar, R2A (Oxoid) and Anacker–Ordal agar (Bernardet *et al.*, 1996) supplemented with 35 g seawater salts l⁻¹. Some strains were able to grow poorly on nutrient agar. No growth occurred on R2A or Anacker–Ordal agar without added NaCl. The temperature range for growth was between –2 and 28 °C. The optimal temperature for growth was about 15–20 °C on solid media and was slightly higher (20–25 °C) in liquid media. Biochemical test results for the strains are

compared with other *Cellulophaga* species in Table 2 and are also given in the species description below.

Fatty acid profiles

The fatty acid profile of IC166^T (the hypothetical median representative of the Antarctic isolates) has been reported previously along with fatty acid profiles for the species *Cellulophaga lytica* and *Cytophaga uliginosa* (Bowman *et al.*, 1998). Additional strains were analysed in this study in order to confirm the homogeneity of the species groups. Fatty acid data for each *Cellulophaga* species were quite similar and are shown in Table 3. The fatty acids *a*15:1 ω 10c, *i*15:0, *a*15:0, 16:1 ω 7c, *i*17:1 ω 7c and 3-OH *i*17:0 appear to be the most useful for the discrimination of *Cellulophaga* species from other members of the *Flavobacteriaceae* (Bowman *et al.*, 1998).

Genotypic analysis

The DNA G+C contents of the Antarctic strains (Table 2) ranged from 36 to 38 mol% (mean 37.1 $\pm$ 0.8, *n* = 7), as determined by the thermal denaturation procedure. By comparison, the DNA G+C contents for *Cellulophaga* species and *Cytophaga uliginosa* are 32–34 mol% (Johansen *et al.*, 1999; Reichenbach, 1989) and 42 mol% (Reichenbach, 1989), respectively. DNA–DNA hybridization analyses indicated that DNA of strain IC166^T hybridized at high levels to five other Antarctic strains, with values ranging from 81 to 95% ($\pm$ 7–16%) (Table 4). No significant hybridization (< 25% hybridization) was detected between IC166^T and the species *Cellulophaga lytica*, *Cellulophaga baltica*, *Cellulophaga fucicola*, *Cytophaga marinoflava*, *Cytophaga uliginosa* or *Cytophaga latercula* (Table 4). From these results, it can be concluded that the Antarctic agarolytic strains are a distinct genospecies.

16S rDNA sequence analysis

Analysis of previously determined 16S rDNA sequence data (Bowman *et al.*, 1997b) indicated that IC166^T was most similar to *Cellulophaga baltica* LMG 18535^T. The strains share a sequence similarity of 0.97. IC166^T was comparatively more distant from other *Cellulophaga* species and from *Cytophaga uliginosa*, with sequence similarities ranging from 0.91 to 0.92 (Table 4). 16S rDNA sequence comparison places strain IC166^T, the members of the genus *Cellulophaga* and *Cytophaga uliginosa* into a single group (Fig. 1). The stability of this group is supported by high bootstrap values (range 81–97%).

Classification of the Antarctic strains

The Antarctic strains can be differentiated from the species *Cellulophaga baltica* by a number of phenotypic traits, including growth temperature range, tolerance of NaCl, range of proteolytic activity and carbohydrate oxidation (Table 2). The DNA G+C content of the Antarctic strains was also 3–5 mol% higher

Table 2. Phenotypic characteristics of the Antarctic strains and species of the genus *Cellulophaga*

The following characteristics were positive for all strains tested: growth on marine 2216 agar and R2A seawater agar; growth at 10–25 °C; hydrolysis of agar, CMC, starch, aesculin, *o*-nitrophenyl β -galactopyranoside and L-tyrosine; pigment formation on L-tyrosine agar; and acid production from D-glucose, D-galactose, sucrose, trehalose and cellobiose. The following characteristics were negative for all strains tested: formation of filamentous, helical or circular cells; growth on R2A and Anacker–Ordal agar; fermentation of D-glucose; anaerobic growth on marine 2216 agar; nitrate reduction; denitrification; hydrolysis of xanthine and dextran; arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, alkaline phosphatase and cytochrome *c* oxidase activities; Voges–Proskauer test; indole production; and acid production from adonitol, inositol, D-sorbitol and dextran. Abbreviations: +, test positive for 90–100% of strains; –, test negative for 90–100% of strains; V+, test positive for 11–89% of strains and the type strain is positive; V–, test positive for 11–89% of strains and the type strain is negative. Results in parentheses indicate tests that gave only weak responses.

Characteristic	Antarctic strains (<i>n</i> = 7)	<i>Cellulophaga lytica</i> (<i>n</i> = 4)	<i>Cellulophaga baltica</i> [*]	<i>Cellulophaga fucicola</i> LMG 18536 ^{T*}	<i>Cytophaga uliginosa</i> ACAM 538 ^T
Flexirubin pigment	–	–	–	–	+
Growth on:					
Nutrient agar	(V+)	(V–)	–	–	–
Nutrient agar + 8% NaCl	+	+	–	–	–
Growth at:					
–2 °C	+	–	–	–	–
4 °C	+	–	+	+	(+)
30 °C	–	+	+	+	+
Hydrolysis of:					
Gelatin	+	V+	+	+	+
Chitin	V–	–	[–]†	–	+
DNA	V+	–	+	+	+
Casein	+	V–	+	–	+
Elastin	–	–	+	+	–
Fibrinogen	–	–	+	–	–
Tween 80	+	V–	[+]	+	(+)
Urate	–	–	[+]	+	–
Lipase activity	V–	V–	[–]	–	–
Nitrate reduction	+	–	[–]	+	+
Urease activity	–	–	[–]	+	–
Cytochrome <i>c</i> oxidase activity	+	+	–	–	+
Formation of acid from:					
D-Fructose	V–	+	[+]	+	+
D-Mannose	+	+	[+]	–	+
L-Arabinose	–	V–	[–]	+	+
D-Xylose	–	V–	[+]	–	+
N-Acetylglucosamine	V–	–	[–]	–	+
D-Mannitol	–	+	[–]	–	–
Maltose, lactose	+	+	[–]	–	+
Glycerol	–	V+	[–]	–	–
L-Rhamnose	–	V–	[–]	+	–
DNA G + C content (mol %)	36–38	32–34	33	32	42

* Some data are from Johansen *et al.* (1999).

† Data available only for the type strain of *C. baltica* (LMG 18535^T) are shown in square brackets.

than that of *Cellulophaga baltica* (Table 2). The Antarctic isolates possess a common relatedness, as indicated by DNA–DNA hybridization values, while a representative strain, IC166^T, was genetically distinct from other *Cellulophaga* species (Table 4). The Antarctic isolates thus represent a distinct species within the genus *Cellulophaga* and the name *Cellulophaga algicola* sp. nov. is proposed, in recognition of the

isolation sites of the species and their presumed epiphytic mode of existence.

Reclassification of *Cytophaga uliginosa*

In this study, it is also proposed that *Cytophaga uliginosa* belongs to the genus *Cellulophaga* and should be renamed *Cellulophaga uliginosa* comb. nov. *Cyto-*

Table 3. Whole-cell fatty acid profiles of the Antarctic strains and species of the genus *Cellulophaga*

Fatty acid data are from Bowman *et al.* (1998) and this study. The values are mean percentages; the values in parentheses are standard deviations. *a*, anteiso; *i*, iso.

Fatty acid	Antarctic strains (<i>n</i> = 7)	<i>Cellulophaga lytica</i> (<i>n</i> = 4)	<i>Cellulophaga baltica</i> LMG 18535 ^T	<i>Cellulophaga</i> <i>fucicola</i> LMG 18536 ^T	<i>Cytophaga</i> <i>uliginosa</i> ACAM 538 ^T
14:0	0.3 (0.2)	0.6 (0.3)	0.8	0.5	0.8
<i>i</i> 15:1 ω 10c	7.5 (2.0)	10.3 (1.7)	9.8	9.0	10.4
<i>a</i> 15:1 ω 10c	tr	tr	–	0.3	–
<i>i</i> 15:0	7.5 (1.8)	18.9 (3.6)	13.6	21.4	31.3
<i>a</i> 15:0	2.5 (1.0)	1.0 (0.6)	2.6	1.2	3.3
15:1 ω 6c	2.6 (0.6)	2.5 (0.8)	2.3	1.4	1.0
15:0	14.3 (2.4)	9.3 (2.0)	12.2	9.8	10.4
<i>i</i> 16:1 ω 6c	2.2 (1.1)	1.4 (1.0)	1.1	1.3	0.5
<i>i</i> 16:0	2.7 (1.3)	0.9 (0.3)	0.6	1.4	0.1
16:1 ω 7c	19.2 (3.0)	9.0 (1.5)	16.9	13.8	11.7
16:0	1.3 (0.5)	1.7 (1.0)	3.4	1.2	6.2
<i>i</i> 17:1 ω 7c	6.1 (1.5)	5.1 (0.9)	5.2	6.7	6.2
<i>a</i> 17:1 ω 7c	0.7 (0.3)	1.5 (0.5)	0.9	1.0	0.5
<i>i</i> 17:0	tr	0.1 (0.1)	0.3	0.3	0.9
17:1 ω 6c	–	0.1 (0.1)	0.2	tr	0.6
3-OH <i>i</i> 15:0	8.6 (2.3)	6.2 (1.9)	6.1	3.2	0.8
3-OH <i>a</i> 15:0	1.5 (1.0)	1.1 (0.7)	0.4	1.8	1.0
3-OH 15:0	0.7 (0.5)	0.3 (0.2)	0.1	tr	–
3-OH <i>i</i> 16:0	6.5 (1.9)	5.2 (3.4)	7.3	5.0	6.1
3-OH 16:0	1.8 (0.9)	0.3 (0.3)	1.1	1.9	2.1
3-OH <i>i</i> 17:0	4.5 (2.0)	20.8 (4.0)	14.0	16.9	11.7
3-OH <i>a</i> 17:0	0.9 (0.6)	0.2 (0.2)	0.3	0.2	0.4
Other	1.3 (0.5)	1.5 (0.8)	0.8	1.7	0.9

tr, Trace fatty acid, present at 0.1 % or less of total fatty acid.

Table 4. DNA–DNA hybridization between the Antarctic strains, *Cellulophaga* species and other related species

DNA–DNA hybridization and 16S rDNA sequence similarity are shown relative to strain IC166^T.

Strain	DNA–DNA hybridization (%)	16S rDNA sequence similarity
IC166 ^T	100	1.00
IC155	95	–
IC156	90	–
IC167	81	–
IC137	92	–
<i>Cellulophaga baltica</i> LMG 18535 ^T	30	0.97
<i>Cellulophaga lytica</i> ACAM 74 ^T	21	0.91
<i>Cellulophaga fucicola</i> LMG 18536 ^T	19	0.92
<i>Cytophaga uliginosa</i> ACAM 538 ^T	18	0.91
<i>Cytophaga marinoflava</i> ACAM 75 ^T	12	0.88
<i>Cytophaga latercula</i> ATCC 23177 ^T	20	0.89

phaga uliginosa was originally isolated by ZoBell & Upham (1944) and initially called ‘*Agarbacterium uliginosum*’, until Weeks (1974) transferred it into the

genus *Flavobacterium*. Subsequently, the species was transferred to the genus *Cytophaga* by Reichenbach (1989). The genus *Cytophaga* is now made up of only

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, depicting species of the family *Flavobacteriaceae* including IC166^T and the species of the genus *Cellulophaga*. The numbers at the branch points are bootstrap values (only those > 60% are shown), with the values above the line calculated by the maximum-likelihood/neighbour-joining methods while the values below the line were calculated by maximum-parsimony analysis.

two species, *Cytophaga hutchinsonii* and *Cytophaga aurantiaca*, which are remote from *Cytophaga uliginosa* in terms of 16S rDNA sequences (Nakagawa & Yamasato, 1996). 16S rDNA sequence analyses instead indicate that *Cellulophaga* species and *Cytophaga uliginosa* form a common group (Fig. 1). In addition, *Cytophaga uliginosa* possesses phenotypic characteristics and fatty acids similar to those of other *Cellulophaga* species (Tables 2 and 3). A number of characteristics, including the ability to form flexirubin pigments and a relatively high G + C content, are useful for the differentiation of the species from other *Cellulophaga* species (Table 2).

Description of *Cellulophaga algicola* sp. nov.

Cellulophaga algicola (al.gi.co'la. M.L. n. *algae* algae; L. n. *incola* inhabitant, dweller; M.L. fem. n. *algicola* algae-dweller).

Cells are rod-shaped with either rounded or tapered ends and with cells lengths and widths ranging from 1.5 to 4 and 0.4 to 0.5 µm, respectively. Gliding motility

present. Colonies on marine 2216 agar have yellow-orange pigmentation and a compact centre, with a spreading edge possessing lighter pigmentation, are slimy in consistency and are slightly sunken into the agar. Flexirubin pigments are not formed. Agar around and beneath colonies is softened but not liquefied. Strictly aerobic chemoheterotroph. Does not require elevated CO₂ levels for growth. Strains grow between 0.5 and 10% NaCl, with best growth in the presence of about 2% NaCl. Growth on nutrient agar is poor, while no growth occurs on R2A or Anacker-Ordal agar. Grows between -2 and 28 °C, with most-rapid growth at about 15–20 °C on agar media and at about 20–25 °C in liquid media. Optimal pH for growth is about 7.5. Can hydrolyse agar, starch, CMC, casein, Tween 80, tributyrin and L-tyrosine but not urate, xanthine or dextran. Forms a reddish-brown diffusible pigment when grown in the presence of 1% L-tyrosine. Nitrate reduction, denitrification, H₂S production and indole production are negative. Acid is formed oxidatively from D-galactose, D-glucose, D-fructose, sucrose, cellobiose, lactose and mannitol. Other characteristics are shown in Table 2. G + C

content of the DNA ranges from 36 to 38 mol% (T_m method). Isolated from the surfaces of algal species from the Antarctic marine coast and sea ice. The type strain is IC166^T (= ACAM 630^T).

Description of *Cellulophaga uliginosa* comb. nov. ('*Agarbacterium uliginosum*' (ZoBell and Upham 1944) Breed 1957; *Flavobacterium uliginosum* (ZoBell and Upham) Weeks 1974; *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989)

The species *Cellulophaga uliginosa* was described as *Cytophaga uliginosa* by Reichenbach (1989). Starch hydrolysis was listed as negative in the original description; however, it was listed as positive in Table 23.2 of Reichenbach (1989). It was confirmed in this study that the strain actively degrades starch. The description of the species is otherwise the same as that given previously. In addition, the strain grows between 1 and 4% NaCl and between 4 and 35 °C. CMC is hydrolysed slowly. Extracellular deoxyribonucleases are formed. Acid is formed oxidatively from D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, trehalose, maltose, sucrose, cellobiose, lactose and N-acetylglucosamine. Acid is not formed from L-rhamnose, adonitol, glycerol, inositol, D-mannitol, D-sorbitol or dextran. Only a single strain is known, and was isolated from surface sediment by ZoBell & Upham (1944). The type strain is ACAM 538^T (= ATCC 14397^T = NCIMB 1863^T = DSM 2061^T = CECT 4277^T = CIP 104808^T).

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REFERENCES

- 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., 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., Lewis, T., Skerratt, J. H., Brown, J. L., Nichols, D. S. & McMeekin, T. A. (1998). *Psychroflexus*

torquis 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.

Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments, *FEMS Microbiol Lett* **8**, 87–91.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.57c. 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. igransii* 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.

Gutell, R. R. (1994). Collection of small subunit (16S and 16S-like) ribosomal RNA structures: 1994, *Nucleic Acids Res* **22**, 3502–3507.

Huss, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridisation from renaturation rates, *Syst Appl Microbiol* **4**, 184–192.

Johansen, J. E., Nielsen, P. & Sjøholm, C. (1999). Description of *Cellulophaga baltica* gen. nov., sp. nov. and *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of [*Cytophaga*] *lytica* to *Cellulophaga lytica* gen. nov., comb. nov, *Int J Syst Bacteriol* **49**, 1231–1240.

Kouker, G. & Jaeger, K.-E. (1987). Specific and sensitive plate assay for bacterial lipases, *Appl Environ Microbiol* **53**, 211–213.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria, *J Bacteriol* **85**, 1183–1184.

McCammon, S. A. & Bowman, J. P. (2000). Taxonomy of Antarctic *Flavobacterium* species: description of *Flavobacterium gillisiae* sp. nov., *Flavobacterium tegetincola* sp. nov. and *Flavobacterium xanthum* sp. nov., nom. rev. and reclassification of [*Flavobacterium*] *salegens* as *Salegentibacter salegens* gen. nov., comb. nov, *Int J Syst Evol Microbiol* **50**, 1055–1063.

McCammon, S. A., Innes, B. H., Bowman, J. P., Franzmann, P. D., Dobson, S. J., Holloway, P. E., Skerratt, J. H., Nichols, P. D. & Rankin, L. M. (1998). *Flavobacterium hibernum* sp. nov., a lactose-utilizing bacterium from a freshwater Antarctic lake, *Int J Syst Bacteriol* **48**, 1405–1412.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature, *J Mol Biol* **5**, 109–118.

Nakagawa, Y. & Yamasato, K. (1996). Emendation of the genus *Cytophaga* and transfer of *Cytophaga agarovorans* and *Cytophaga salmonicolor* to *Marinilabilia* gen. nov.: phylogenetic analysis of the *Flavobacterium*–*Cytophaga* complex, *Int J Syst Bacteriol* **46**, 599–603.

Nichols, P. D., Guckert, J. B. & White, D. C. (1986). Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyldisulphide adducts, *J Microbiol Methods* **5**, 49–55.

Reichenbach, H. (1989). Genus I. *Cytophaga* Winogradsky 1929, 577, ^{AL} emend. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2015–2050. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

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.

Weeks, O. B. (1974). Genus *Flavobacterium* Bergey *et al.*, 1923, 97. In *Bergey's Manual of Determinative Bacteriology*, 8th edn.,

pp. 357–364. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

ZoBell, C. E. & Upham, H. C. (1944). A list of marine bacteria including descriptions of sixty new species, *Bull Scripps Inst Oceanogr Univ Calif* **5**, 239–292.