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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.

Sharee A. McCammon and John P. Bowman

Author for correspondence: John P. Bowman. Tel: +61 3 6226 2776. Fax: +61 3 6226 2642. e-mail: john.bowman@utas.edu.au

School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart, Tasmania 7001, Australia

16S rRNA phylogenetic analysis of a number of yellow- and orange-pigmented strains isolated from a variety of Antarctic habitats including sea ice, lakewater and cyanobacterial mats indicated a close relationship to the genus *Flavobacterium* but distinct from known *Flavobacterium* species. Phenotypic properties, DNA G+C content and whole-cell fatty acid profiles of the Antarctic strains were consistent with those of the genus *Flavobacterium*. DNA–DNA hybridization analysis indicated the presence of two distinct and novel genospecies each isolated from a different Antarctic habitat. From polyphasic taxonomic data it is proposed that the two groups represent new species with the following proposed names: *Flavobacterium gillisiae* (ACAM 601^T) and *Flavobacterium tegetincola* (ACAM 602^T). In addition polyphasic analysis of the species '[*Cytophaga*] *xantha*' (Inoue and Komagata 1976), isolated from Antarctic mud, indicated it was a distinct member of the genus *Flavobacterium* and was thus revived as *Flavobacterium xanthum*. Phylogenetic and fatty acid analyses also indicate that the species [*Flavobacterium*] *salegens* (Dobson *et al.* 1993), from Organic Lake, Antarctica, is misclassified at the genus level. It is proposed that this species belongs to a new genus, *Salegentibacter salegens* gen. nov., comb. nov.

Keywords: *Flavobacterium*, Antarctica, sea ice, psychrophilic bacteria

INTRODUCTION

The genus *Flavobacterium* is widespread in nature and has been isolated from many freshwater and soil habitats. Several *Flavobacterium* species are pathogens of fish; however this genus appears primarily to play a role in remineralization processes and exhibits strong macromolecular hydrolytic capabilities. Until recently the nomenclatural status of the genus was heterogeneous and confused (Holmes *et al.*, 1984; Bernardet *et al.*, 1996). Recent studies have now resolved many of these problems resulting in *Flavobacterium* representing predominantly gliding, pigmented bacteria which have a DNA G+C content of 32–37 mol% and menaquinone-6 as the primary respiratory quinone (Bernardet *et al.*, 1996). Phylogenetic analysis using 16S rRNA sequences and rRNA–DNA hybridization experiments place *Flavobacterium* in the *Flexibacter–Bacteroides–Flavobacterium* phylum (Bauwens & De Ley, 1981; Woese *et al.*, 1990). *Flavobacterium* is the type genus of the family *Flavobacteriaceae* which also encompasses several other genera including *Chryseobacterium*, *Bergeyella*, *Riemerella*, *Ornithobacterium*, *Empedobacter*, *Weeksella*, *Capnocytophaga*, *Myroides*, *Gelidibacter*, *Psychroserpens*, *Polaribacter*, *Psychroflexus* and various generically misclassified *Cytophaga*

The GenBank accession numbers for the 16S rRNA sequences of ACAM strains 81^T, 601^T, 602^T and 603 are AF030380, U85889, U85887 and U85888, respectively.

and *Flexibacter* species (Bernardet *et al.*, 1996; Bowman *et al.*, 1998).

Several strains related to the genus *Flavobacterium* have been isolated from a number of Antarctic habitats. Inoue & Komagata (1976) isolated '[*Cytophaga*] *xantha*' from a mud pool near Syowa Station, Antarctica (68° S 39° E). rRNA–DNA hybridization experiments demonstrated this species was related to *Flavobacterium aquatile* (Bernardet *et al.*, 1996). Organic Lake, a shallow, meromictic, hypersaline waterbody in the Vestfold Hills ice-free zone of Antarctica (68° S 78° E) has yielded the species [*Flavobacterium*] *gondwanense* and [*Flavobacterium*] *salegens* (Dobson *et al.*, 1993). rRNA–DNA hybridization experiments (Bernardet *et al.*, 1996), fatty acids (Skerratt *et al.*, 1991; Bowman *et al.*, 1998) and 16S rRNA sequence analysis (Dobson *et al.*, 1993) indicate that these species do not belong to the genus *Flavobacterium* but instead represent phylogenetically distinct taxa within the *Flavobacteriaceae*. [*Flavobacterium*] *gondwanense* has been subsequently renamed *Psychroflexus gondwanensis* (Bowman *et al.*, 1998). Lactose-utilizing isolates from a highly oligotrophic freshwater lake in the Vestfold Hills were described as *Flavobacterium hibernum* by McCammon *et al.* (1998). Finally a number of yellow- and orange-pigmented isolates from sea ice and marine salinity lake samples (Franzmann *et al.*, 1990; Bowman *et al.*, 1997a) have been found to be phylogenetically related to the genus *Flavobacterium*; however 16S rRNA sequence data suggested these strains represent potentially novel species.

In this study phenotypic, fatty acid analysis and genotypic data were utilized to ascertain the relationship of a number of Antarctic sea ice and lake isolates to known *Flavobacterium* species. From this research it was found the isolates represented two distinct and novel species of *Flavobacterium* with the following proposed names: *Flavobacterium gillisiae* sp. nov. and *Flavobacterium tegetincola*. '[*Cytophaga*] *xantha*' was also recognized as a distinct member of the genus *Flavobacterium*, and is revived as *Flavobacterium xanthum*. In addition the misclassified species [*Flavobacterium*] *salegens* was elevated to genus status to reflect its distinct phylogenetic position and was designated *Salegentibacter salegens* gen. nov., comb. nov.

METHODS

Strains and cultivation conditions. Protocols describing the isolation of the Antarctic strains investigated in this study were previously described (Bowman *et al.*, 1997a). Isolates and reference strains investigated in this study are shown in Table 1. All strains utilized in the study were routinely cultivated on R2A medium (Oxoid) at 20 °C.

Phenotypic characterization. Most phenotypic tests have been published previously (Bowman *et al.*, 1997b). Flexirubin pigments were detected by suspending cells in 20% (w/v) KOH, with a colour change from yellow or orange to brown–red indicative of a positive result (Fautz & Reichenbach, 1980). The detection of extracellular glucans by the Congo red absorption test was performed by flooding plates with 0·01% (w/v) Congo red (McCurdy, 1969). The procedures of Hildebrand (1971) were followed to test for the degradation of pectin. CMC hydrolysis was tested by overlaying nutrient agar with a thin layer of 0·5% (w/v) CMC in tapwater agar and observing for hydrolysis zones after 21 d incubation.

DNA base composition. Genomic DNA was extracted and purified from cells using the Marmur & Doty (1962) procedure. The DNA G+C content was then determined from thermal denaturation profiles using procedures developed by 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 using 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} °C = 48·5 + (0·41 × mol% G+C). The decline in absorbance over a 40 min interval of DNA mixtures and control DNA samples was used to calculate DNA hybridization values from the following equation (Huß *et al.*, 1983):

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

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

Fatty acid analysis. All strains were cultivated on trypticase soya agar (Difco) at 20 °C for 2 d, harvested and then lyophilized using a vacuum freeze-drier (Dynavac). Whole-cell fatty acid profiles were quantitatively determined using gas chromatographic and GC-MS procedures (Nichols *et al.*, 1986). The geometry and position of double bonds in monounsaturated fatty acids was confirmed using dimethyl-disulfide derivatization and analysis using GC-MS. The double-bond positions are numbered from the methyl (ω) end of the fatty acid.

Phylogenetic analysis. The 16S rRNA gene sequences for representatives of the isolates (ACAM 601^T and ACAM 603^T) were obtained in an earlier study (Bowman *et al.*, 1997a). The sequence for '[*Cytophaga*] *xantha*' ACAM 81^T was obtained in this study. Conditions and reagents used for PCR amplification and sequencing of 16S rRNA sequences have been previously published (Bowman *et al.*, 1997a). Sequence reactions were prepared with the Prism dRhodamine terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoresis and sequence reading were performed on a A377 DNA sequencer (Applied Biosystems). The sequences used in the study were compared to the compilation of 16S rRNA genes available in the GenBank nucleotide database. The complete sequences were aligned with hypervariable regions aligned according to secondary structure. Subsequent phylogenetic analyses of

Table 1. Strain designation and sources of Antarctic strains, *Flavobacterium* species and related species compared in this study

Species	Strain*	Isolation site
Antarctic		
Flavobacterium sp.	ACAM 601^T	Coastal sea ice
Flavobacterium sp.	ACAM 602^T	Cyanobacterial mat
Flavobacterium sp.	ACAM 603	Cyanobacterial mat
'[*Cytophaga*] *xantha*'	ACAM 81^T = NCIMB 2069^T	Pool mud
Flavobacterium hibernum	ACAM 376^T	Freshwater lake
Non-Antarctic		
Flavobacterium flevense	ACAM 579^T = NCIMB 12056^T	Freshwater lake
Flavobacterium hydatis	NCIMB 2215^T	Gills of diseased salmon
Flavobacterium saccharophilum	ACAM 581^T = NCIMB 2072^T	River water
Flavobacterium psychrophilum	NCIMB 1947^T	Kidney of salmon
Flavobacterium sp. ('[*Sporocytophaga*] *cauliformans*' type 2)	ACAM 580 = NCIMB 9488	Lakewater

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

the sequence 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 using SEQBOOT and CONSENSE using 250 resamplings of the dataset.

RESULTS AND DISCUSSION

Morphological and phenotypic properties of Antarctic strains

All of the Antarctic strains investigated, including '[*Cytophaga*] *xantha*' ACAM 81^T, formed yellow or yellow–orange pigments; however the KOH test results indicated flexirubin pigments were absent. Gliding motility tests were performed on full- and quarter-strength R2A agar media with growth examined after 7 and 16 h incubation. From microscopic observations only the cyanobacterial mat strains ACAM 602 and ACAM 603 appeared capable of gliding motility. All of the strains were able to grow on seawater agar (marine 2216 agar and R2A agar prepared with 35 g artificial sea salts l^{-1}), could grow at 0 °C but were unable to grow at 30 °C or higher. The isolates were able to form acid oxidatively from D-glucose in Leifson's oxidation/fermentation medium (Leifson, 1963) and in addition could use D-glucose as a sole carbon and energy source. However, none of strains were able to grow fermentatively or grow by anaerobic respiration using ferric iron, nitrate, nitrite or trimethylamine *N*-oxide as electron acceptors. On this basis the isolates are strictly aerobic. The capacity to degrade macromolecules and some other compounds varied considerably between the isolates as indicated in Table 2. None of the isolates were susceptible to vibriostatic agent O/129 or were able to adsorb Congo red dye, indicating the absence of glucan-type polysaccharides. Likewise, none of the strains could hydrolyse ONPG, form brown diffusible pigments on tyrosine agar, form precipitates or clearing on egg-yolk agar or produce acid from L-arabinose, L-rhamnose, D-xylose, melibiose, raffinose, adonitol, D-sorbitol or inositol. The remaining traits investigated vary between the strains studied and are useful for their differentiation (Table 2). '[*Cytophaga*] *xantha*' ACAM 81^T was reinvestigated phenotypically and the results were in general accordance with those reported by Inoue & Komagata (1976). Additional phenotypic tests were performed so that a better comparison could be made with other *Flavobacterium* species (Table 2).

Fatty acid profiles

The Antarctic strains possessed similar whole-cell fatty acid profiles (Table 3), with the major constituents including 15:0 (6–7 %), i15:1ω10*c* (5–9 %), i15:0 (5–11 %), a15:0 (6–15 %), 15:1ω6*c* (2–21 %), 16:1ω7*c* (18–23 %) and 3-OH i15:0 (6–13 %) (Table 3). Overall, the profiles were similar to fatty acid profiles determined for other *Flavobacterium* species (Bernardet *et al.*, 1996) with quantitative differences mostly due to the different lipid extraction and cultivation conditions protocols employed between this and other studies.

Genotypic analysis

The DNA G+C contents of the strains (Table 2) fell within the range typical for *sensu stricto Flavobacterium* species (32–37 mol %). The mol % G+C of ACAM 81^T was 36 mol %, which is similar to the result from Bernardet *et al.* (1996). Inoue & Komagata (1976) originally reported a DNA G+C content of 39 mol % for ACAM 81^T. DNA–DNA hybridization

Table 2. Phenotypic characteristics differentiating the Antarctic and other *Flavobacterium* species

Flavobacterium species: 1, *F. gillisiae*; 2, *F. tegetincola*; 3, *F. xanthum*; 4, *F. flevense*; 5, *F. hibernum*; 6, *F. aquatile*; 7, *F. branchiophilum*; 8, *F. columnare*; 9, *F. psychrophilum*; 10, *F. hydatis*; 11, *F. johnsoniae*; 12, *F. pectinovorum*; 13, *F. saccharophilum*; and 14, *F. succinicans*. Abbreviations: +, test is postive; (+), test positive, weak or delayed response; −, negative test result; v, test results vary between strains of species; ND, data either not available or are unreliable.

Character	*Flavobacterium* species:*													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Growth on:														
Seawater agar	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Nutrient agar	+	+	+	+	+	−	−	−	−	+	+	+	+	+
Trypticase soy agar	+	+	+	+	+	(+)	−	−	−	+	+	+	+	+
Growth at 25 °C on agar	(+)	(+)	(+)	+	+	+	+	+	−	+	+	+	+	+
Gliding motility	−	+	−	+	+	+	−	+	(+)	+	+	+	+	+
Flexirubins	−	−	−	−	+	−	−	+	+	+	+	+	+	−
Glucose utilization	+	+	+	+	+	ND	ND	−	−	+	+	+	+	+
Acid from carbohydrates	+	+	+	+	+	+	+	−	−	+	+	+	+	+
Degradation of:														
Gelatin	−	−	+	−	+		+	+	+	+	+	+	+	(+)
Casein	+	−	+	−	+	+	+	+	+	+	+	+	+	+
Starch	+	−	+	ND	+		+	−	−	+	+	+	+	+
CMC	−	−	−	−	−	−	−	−	−	ND	+	+	+	ND
Agar	−	−	−	+	−	−	−	−	−	−	−	−	+	−
Pectin	−	−	−	+	−	ND	ND	ND	−	+	+	+	+	ND
Chitin	+	−	−	−	−	−	−	−	−	(+)	+	+	−	−
Aesculin	+	−	+	+	+		−	−	−	+	+	+	+	+
DNA	−	−	−	−	+	−	−	+	(+)	+	+	+	−	+
Tyrosine	−	−	−	−	+		+	−	v	+	+	+	+	−
Precipitate on egg yolk medium	−	−	−	−	−	+	+	+	+	−	−	−	−	−
ONPG hydrolysis	−	−	−	+	+	ND	+	−	−	+	+	+	+	+
H_2S production	−	−	+	−	−	−	−	+	−	−	−	ND	+	+
$NO_3 \rightarrow NO_2$	−	−	+	ND	+	ND	−	ND	−	+	+	+	+	v
Mol% G+C (mean)	32	32	36	35	36	33	33	32	35	34	34	35	33	36

* Data from Bernardet *et al.* (1996), McCammon *et al.* (1998) and this study.

analyses indicated strains ACAM 602 and ACAM 603 were closely related, sharing a reassociation level of 87 (±10)%, corresponding to the close phylogenetic affiliation of these strains. ACAM 601^T was genotypically distinct, sharing reassociation levels at less than 25% with its closest phylogenetic relatives, ACAM 81^T and *F. psychrophilum*. Likewise, no significant DNA hybridization was found for any of the Antarctic strains with *Flavobacterium flevense* ACAM 582^T, *Flavobacterium saccharophilum* ACAM 580^T or *Flavobacterium hibernum* ACAM 376^T.

Phylogeny

Surveys of bacteria from a variety of Antarctic habitats have revealed a rich diversity of novel organisms as well as interesting bacterial associations such as pronounced enrichments of psychrophilic bacteria in sea-ice diatom assemblages and within maritime lakes (Bowman *et al.*, 1997a). From these studies, a number of yellow to orange pigmented strains were found to be related phylogenetically to the genus *Flavobacterium*. The phylogenetic relationship of all *sensu stricto Flavobacterium* species and the Antarctic strains are shown in Fig. 1. ACAM 601^T was 92·9–95·2% similar to the 16S rRNA of other *Flavobacterium* species. The 16S rRNA sequences of cyanobacterial mat strains ACAM 602^T and ACAM 603 were very similar (99·3%) forming a distinct branch within the *Flavobacterium* clade, sharing a 93·0–94·5% similarity to other *Flavobacterium* 16S rRNA sequences. In this study, the 16S rRNA sequence of '[*Cytophaga*] *xantha*' ACAM 81^T was generated and found to cluster loosely with ACAM 601^T. Phylogenetically, ACAM 81^T was

Table 3. Whole-cell fatty acid profiles (percentage composition) of Antarctic *Flavobacterium* species

Fatty acid	*F. gillisiae* ACAM 601^T	*F. tegetincola* ACAM 602^T	'[*Cytophaga*] *xantha*' ACAM 81^T
i13:0	–	0·2	–
a13:0	–	0·3	–
i14:1ω9*c*	0·2	–	–
i14:0	0·7	0·8	0·3
14:0	0·4	0·5	0·4
15:1ω6*c*	21·5	1·8	7·9
i15:1ω10*c*	5·1	8·9	7·3
a15:1ω10*c*	1·3	5·7	2·2
i15:0	5·1	8·0	10·6
a15:0	5·8	15·4	10·5
15:0	7·4	6·5	6·9
16:1ω9*c*	–	–	–
16:1ω7*c*	21·7	18·4	23·1
16:1ω5*c*	0·6	0·6	0·4
i16:1ω6*c*	3·2	3·8	0·5
16:2*	–	0·3	–
i16:0	3·5	4·8	1·3
16:0	2·9	1·9	2·1
17:1ω6*c*	0·9	2·3	0·3
i17:1ω7*c*	4·4	3·1	4·0
i17:1ω5*c*	1·0	3·5	2·6
i17:0	0·2	–	0·3
a17:0	1·5	–	–
18:0	0·6	–	–
3-OH i15:0	10·0	5·7	13·1
3-OH a15:0	0·2	1·0	0·8
3-OH 15:0	0·3	0·5	1·2
3-OH i16:0	0·8	2·8	2·0
3-OH a16:0	0·4	0·1	0·2
3-OH 16:0	0·4	1·1	1·7
3-OH i17:0	0·5	3·0	0·3

* Double-bond positions not determined.

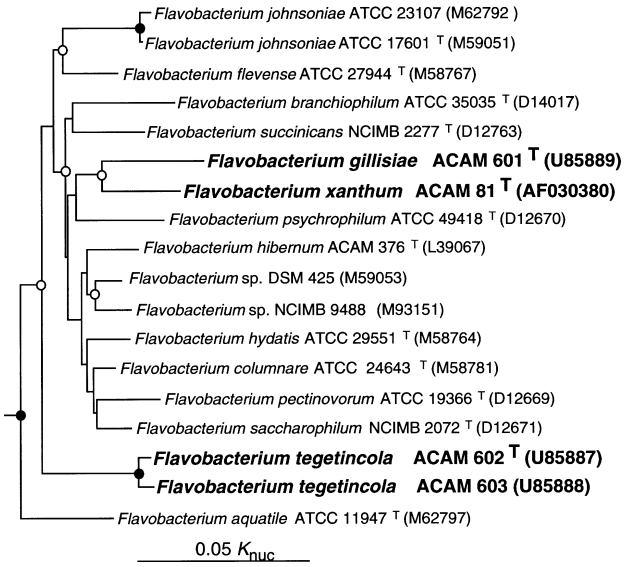

Fig. 1. Phylogenetic relationships of *Flavobacterium* species based on 16S rRNA sequence comparison. Species in bold type are new species. The branch lengths and branching pattern were generated by maximum-likelihood and neighbour-joining methods. Outgroup sequence used was *Myroides odoratus* (M58777). The circles at the branch nodes represent bootstrap values, with filled circles indicating bootstrap values of 76–100%, while open circles indicate bootstrap values of 50–75%. The numbers in parentheses are accession numbers for the GenBank nucleotide sequence database.

distinct from all other *Flavobacterium* species with the most similar 16S rRNA being that of *Flavobacterium psychrophilum* (sequence similarity 95·7%).

On the basis of polyphasic analysis, the Antarctic strains represent two distinct and novel *Flavobacterium* species. Each species is from a different habitat and possess different phenotypic traits (Table 2). The Antarctic species differ from 'mainstream' *Flavobacterium* species in not possessing flexirubin pigments, are tolerant to seawater and most lack gliding motility. However, these traits are not totally conserved within *Flavobacterium*. Some species, including *F. aquatile*, *F. branchiopilum*, *F. flevense* and *F. succinicans* also lack flexirubin pigments while *F. branchiophilum* is unable to glide. *F. flevense* is able to grow quite well on seawater agar. This suggests the genus *Flavobacterium* is phenotypically diverse but other data indicates that it is conserved at the chemotaxonomic and phylogenetic levels. The degree of phenotypic diversity in *Flavobacterium* may only be revealed following the isolation and description of new species. A consequence of this diversity is that a degree of reliance on phylogenetic and chemotaxonomic analysis is necessary for the definitive identification of flavobacteria. The following names (and type strains) are proposed as two new Antarctic species: *Flavobacterium gillisiae* ACAM 601^T and *Flavobacterium tegetincola* ACAM 602^T. Our results also indicate that '[*Cytophaga*] *xantha*' ACAM 81^T is a distinct species in the genus *Flavobacterium* and thus the species is revived as *Flavobacterium xanthum* comb. nov.

Description of *Flavobacterium gillisiae* sp. nov.

Flavobacterium gillisiae (gil.is.i'ae. M.L. gen. *gillisiae* of Gillis, named after Monique Gillis, a microbiologist who pioneered new techniques for bacterial taxonomy).

Gram-negative rods, 2–5 μm in length and 0·4–0·5 μm in width. Non-motile. Cell masses from aerobic cultures are orange. Flexirubin pigments not detected. Colonies have a butyrous consistency, are circular and convex in shape, and possess an entire edge. Sodium nitrate, ammonium chloride, L-glutamate, peptone and Casamino acids serve as nitrogen sources. Vitamins are not required for growth but are stimu-

latory. Does not require NaCl for growth however grows well on seawater-containing media and tolerates up to 5% NaCl. On solid media growth occurs at 0–27 °C, with optimal growth at about 20 °C while no growth occurs at 30 °C or above. Strictly aerobic chemoheterotroph. Produces acid from D-glucose, D-mannose, D-galactose, D-fructose, sucrose, trehalose, cellobiose, maltose, D-mannitol and glycerol, but not from L-arabinose, L-rhamnose, D-xylose, lactose, melibiose, raffinose, adonitol, D-sorbitol or inositol. Degrades casein, starch, aesculin, chitin and Tween 80, but not gelatin, agar, alginate, CMC, pectin, DNA, urea, uric acid or xanthine. The arginine dihydrolase test is positive. Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activity is absent. Nitrate reduction, indole from L-tryptophan, H_2S production, Voges–Proskauer, Simmons' citrate and oxidase tests are negative. Catalase-positive. Can utilize D-glucose as a sole carbon source. DNA G+C content is 32 mol% (determined by the thermal denaturation method). Type strain is ACAM 601^T, isolated from sea ice from Prydz Bay, Antarctica.

Description of *Flavobacterium tegetincola* sp. nov.

Flavobacterium tegetincola (te.get′in.col.a. L. n. *teges* a mat or rug; L. gen. n. L. *incola* an inhabitant; M.L. gen. n. *tegetincola* the mat-inhabitant, pertaining to its cyanobacterial mat habitat).

Gram-negative rods, 2–5 μm in length and 0·4–0·5 μm in width. Motile by gliding. Cell masses from aerobic cultures are yellow. Flexirubin pigments not detected. Colonies have a butyrous consistency, are circular and convex in shape, and possess an entire edge. Peptone and Casamino acids, but not sodium nitrate, ammonium chloride or L-glutamate, serve as nitrogen sources. Vitamins are not required for growth. Non-halophilic but can grow on seawater-containing media and tolerates up to 5% NaCl. On solid media growth occurs at 0–27 °C with optimal growth at about 20 °C while no growth occurs at 30 °C or above. Strictly aerobic chemoheterotroph. Produces acid from D-glucose, D-fructose and D-mannitol, but not from L-arabinose, D-galactose, D-fructose, L-rhamnose, D-xylose, sucrose, trehalose, cellobiose, maltose, melibiose, raffinose, adonitol, D-sorbitol, inositol or glycerol. Degrades Tween 80, but not gelatin, casein, aesculin, starch, agar, alginate, chitin carboxymethylcellulose, pectin, DNA, urea, uric acid or xanthine. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activity are absent. Nitrate reduction, indole from L-tryptophan, H_2S production, Voges–Proskauer, oxidase and Simmons' citrate tests are negative. Catalase-positive. Can utilize D-glucose as a sole carbon source. DNA G+C content is 34 mol% (determined by the thermal denaturation method). Type strain is ACAM 602^T, isolated from cyanobacterial mat material collected from Ace Lake, a marine salinity meromictic lake located in the Vestfold Hills area of Antarctica.

Description of *Flavobacterium xanthum* sp. nov., nom. rev. (basonym '*Cytophaga xantha*' Inoue and Komagata 1976)

The description of the species is the same as given previously by Reichenbach (1989), with the additional information: flexirubin pigments not detected. Ammonium chloride, L-glutamate, peptone and Casamino acids serve as nitrogen sources. Does not require vitamins for growth. On solid media growth occurs at 0 °C, grows optimally at about 20 °C while no growth occurs at 30 °C or above. Produces acid from D-glucose, D-fructose, D-mannose, maltose, cellobiose, sucrose, trehalose and D-mannitol, but not from L-arabinose, D-galactose, L-rhamnose, D-xylose, lactose, melibiose, raffinose, adonitol, D-sorbitol, inositol or glycerol. Degrades casein, aesculin and Tween 80, but not agar, alginate, chitin, pectin, DNA, urea, uric acid or xanthine. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activity are absent. Nitrate reduction and hydrogen sulfide production is positive. Denitrification, indole from L-tryptophan, Simmons' citrate tests and Voges–Proskauer tests are negative. Acid from D-glucose in anaerobic media is not detected. Catalase and oxidase-positive. Can utilize D-glucose as a sole carbon source. DNA G+C content is 36 mol% (determined by the thermal denaturation method). Type strain is ACAM 81^T, isolated from a mud pool near Syowa Station, Antarctica.

Reclassification of [*Flavobacterium*] *salegens*

[*Flavobacterium*] *salegens* (ACAM 48^T = ATCC 51522^T) remains one of the last validly described *Flavobacterium* species with a misplaced phylogeny. This species is able to grow from 0 to 30 °C and from 0 to 20% NaCl and has been isolated from various meromictic hypersaline lakes in the Vestfold Hills, East Antarctica (Dobson *et al*., 1993; James *et al*., 1994). Studies suggest it is an epiphyte of centric diatoms CUand chlorophytes which proliferate at the ice:water interface and in the surface waters of these lakes (James *et al*., 1994; J. P. Bowman, unpublished data). Phenotypically [*F.*] *salegens* is most similar to *Psychroflexus gondwanensis* (ACAM 44^T = ATCC 51278^T) which also occupies the same habitat. Both species possess a very similar morphology, ecophysiology and are non-motile. By comparison, *Psychroflexus torquis* (ACAM 623^T) is highly adapted to sympagic (sea-ice) habitats and differs considerably from *Psychroflexus gondwanensis* in terms of morphology and ecophysiology (Bowman *et al*., 1988), but they are very similar in terms of chemotaxonomy and 16S rRNA sequence (Fig. 2). Fatty acid analysis indicates [*F.*] *salegens* is quite distinct from most other members of the *Flavobacteriaceae* including *Psychroflexus* species. The fatty acid pattern of [*F.*] *salegens* in qualitative terms is most similar to that of *Gelidibacter algens* (Bowman *et al*., 1998). The profiles of the two species differ by the presence of a15:1ω10*c*,

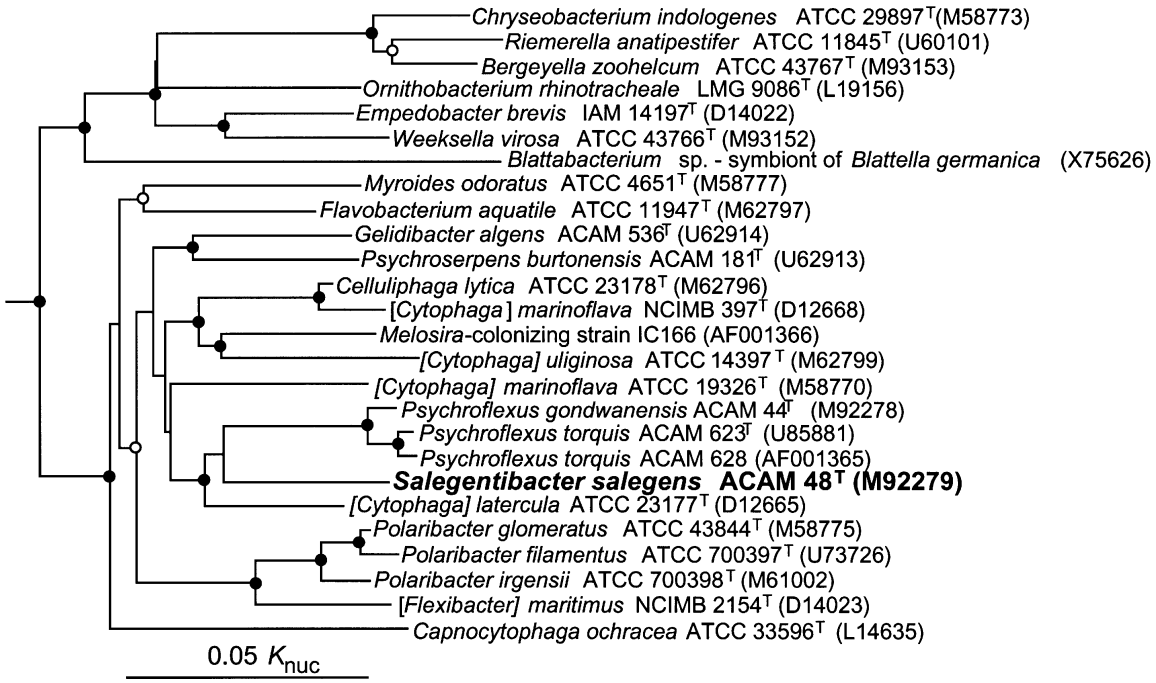

Fig. 2. Phylogenetic relationships of *Salegentibacter salegens* ([*Flavobacterium*] *salegens*) to other members of the family *Flavobacteriaceae* on the basis of 16S rRNA sequence comparison The branch lengths and branching pattern were generated by maximum-likelihood and neighbour-joining methods. Outgroup sequence used was *Bacteroides fragilis* (X83935). The circles at the branch nodes represent bootstrap values, with filled circles indicating bootstrap values of 76–100 %, while open circles indicate bootstrap values of 50–75 %. The numbers in parentheses are accession numbers for the GenBank nucleotide sequence database.

Table 4. Phenotypic characteristics that differentiate the genus *Salegentibacter* from other halophilic members of the *Flavobacteriaceae*

Abbreviations: −, negative; +, positive; (+), weakly positive; v, trait varies between species of the genus; v, trait varies between strains of the genus; O, orange; Y, yellow; R, red; SH, slightly halophilic; MH, moderately halophilic; NH, non-halophilic.

Character	*Salegenti-bacter*	*Psychro-flexus*	[*Cytophaga*] *latercula*	*Psychro-serpens*	*Gelidi-bacter*	*Polari-bacter*	[*Flexibacter*] *maritimus* group*	*Celluliphaga* group†
Gliding motility	−	v	−	−	+	−	+	+
Pigments	Y	O	O–R	Y	Y	O	Y	O–Y
Growth at 25 °C	+	v	+	−	v	−	+	+
Tolerates >10 % NaCl	+	v	−	−	−	−	−	−
Requires yeast extract	−	v	−	+	−	+	+	−
Starch hydrolysis	+	+	−	−	+	v	−	+
Agar hydrolysis	−	−	+	−	−	−	−	v
ONPG test	+	−	+	v	−	v	−	v
Nitrate reduction	+	−	−	−	−	−	+	v
Mol% G+C (T_m)	37–38	32–36	32	27–29	36–38	31–34	29–32	32–42

* Includes [*Flexibacter*] *maritimus* and [*Flexibacter*] *ovolyticus*.

† Includes *Celluliphaga lytica*, [*Cytophaga*] *marinoflava* and [*Cytophaga*] *uliginosa*.

15:1ω11*c* and 16:1ω5*c* in the fatty acid profile of *Gelidibacter algens*. These fatty acid components are absent in [*F.*] *salegens* (Skerratt *et al.*, 1991). 16S rRNA sequence analyses also indicates [*F.*] *salegens* has a distinct position within the family *Flavobacteriaceae* separate from the genus *Flavobacterium* and various halophilic genera (Fig. 2). From these results the differentiation of superficially similar taxa such as [*F.*] *salegens* and *Psychroflexus gondwanensis* is quite dependent on chemotaxonomic and phylogenetic analysis. However, a combination of phenotypic, chemotaxonomic and phylogenetic traits indicate [*F.*] *salegens* forms a taxon within the family *Flavobacteriaceae* distinct at the genus level. Thus it is proposed that [*F.*] *salegens* becomes *Salegentibacter salegens* gen. nov., comb. nov. in recognition of its distinct phylogenetic position. Table 4 provides tests useful in differentiating *Salegentibacter* from other halophilic members of the *Flavobacteriaceae*.

Description of *Salegentibacter* gen. nov.

Salegentibacter (Sal.e.gent′i.bact.er. L. n. *salis* salt; L. part. adj. *egentis* needy; Gr. n. *bakterion* rod; L. n. *Salegentibacter* salt-needy rod; referring to the high level of salt requirement).

Cells are rod-shaped and occur as single cells, pairs and occasionally chains. Non-motile. Do not form spores or other resting stages. Does not form gas vesicles, helical or coiled cells. Colonies are pigmented yellow, however flexirubin-type pigments are not formed. Possesses a strictly aerobic, chemo-organotrophic metabolism. Can utilize inorganic nitrogen sources including ammonia and nitrate. Does not require growth factors. Catalase- and oxidase-positive. Moderately halophilic and highly halotolerant (grows at 0–20 % NaCl, optimal growth at about 5 % NaCl). Psychrotolerant, growing between 0–30 °C. Major whole-cell fatty acids are i15:0, a15:0, i16:0, i17:1ω7*c* and a17:1ω7*c*. The major respiratory lipoquinone is menaquinone-6. The G+C content of the DNA is 37–38 mol % (thermal denaturation method). Member of the family *Flavobacteriaceae*, *Cytophagales* division. Only known habitats are the surface waters of hypersaline Antarctic lakes. The type species is *Salegentibacter salegens*.

Description of *Salegentibacter salegens* (*Flavobacterium salegens* Dobson *et al.* 1993) comb. nov.

The species description is the same as that for the genus description and as previously described by Dobson *et al.* (1993). Type strain is ACAM 48^{T} (= ATCC 51522^{T} = DSM 5424^{T}).

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