***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.**

John P. Bowman, Sharee A. McCammon, Tom Lewis,
Jennifer H. Skerratt, Janelle L. Brown, David S. Nichols
and Tom A. McMeekin

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

Antarctic CRC and
School of
Agricultural Science,
University of Tasmania,
GPO Box 252-80, Hobart,
Tasmania 7001, Australia

A group of sea-ice-derived psychrophilic bacterial strains possessing the unusual ability to synthesize the polyunsaturated fatty acids eicosapentaenoic acid (20:5 ω 3) and arachidonic acid (20:4 ω 6) belong to the Family *Flavobacteriaceae* (*Flexibacter*–*Bacteroides*–*Flavobacterium* phylum), according to 16S rRNA sequence analysis. Surprisingly, the isolates were also found to cluster closely to the moderately halophilic and psychrotrophic species [*Flavobacterium*] *gondwanense* (sequence similarity 97.8–98.1%). The whole-cell fatty acid profiles of this group and [*Flavobacterium*] *gondwanense* were very similar and distinct from other related flavobacteria. The sea ice strains and [*Flavobacterium*] *gondwanense* differed substantially in terms of ecophysiology, possibly representing divergent adaptations to sympagic and planktonic marine habitats, respectively. Evidence based on phylogeny and fatty acid profiles supports the conclusion that the taxa are close relatives distinct from other bacterial groups. It is thus proposed that the sea ice strains represent a novel taxon designated *Psychroflexus torquis* gen. nov., sp. nov. (type strain ACAM 623^T) while [*Flavobacterium*] *gondwanense* becomes *Psychroflexus gondwanense* gen. nov., comb. nov.

Keywords: *Psychroflexus* gen. nov., Antarctica, polyunsaturated fatty acids, psychrophilic bacteria, halophilic bacteria

INTRODUCTION

A partitioning phenomenon has been observed to occur between the bacterial communities of sympagic (sea ice) and coastal pelagic environments of Antarctica. Sea ice diatom assemblages provide a niche for the proliferation of a wide variety of epiphytic and free-living psychrophilic bacteria (Bowman *et al.*, 1997a, b). The reasons for this are not completely clear but are thought to be primarily due to nutrient concentration enhancement and increased availability of surfaces for colonization (Bowman *et al.*, 1997b; Delille, 1996; Helmke & Weyland, 1995; Priddle *et al.*, 1996; Sullivan

& Palmisano, 1984). Within the sea ice/seawater interface zone, especially areas with platelet ice populated heavily by ice diatoms, a number of psychrophilic, gas-vacuolate bacteria have been isolated (Gosink & Staley, 1995; Gosink *et al.*, 1998; Irgens *et al.*, 1996). Often sympagic (ice-dwelling) psychrophiles have fastidious growth requirements and only utilize a relatively narrow range of substrates (Bowman *et al.*, 1997a). Many of these nutrients are probably derived from exudates of sea ice diatoms (Malinsky-Rushansky & Legrand, 1996) and extensive bacterial colonization of the surfaces of various diatom species has been observed frequently (Sullivan & Palmisano, 1984; Palmisano & Garrison, 1993). By comparison, the reduction in the availability of organic compounds and lack of surfaces for colonization is at least partially responsible for the

Abbreviation: PUFA, polyunsaturated fatty acid.

bacterioplanktonic community being mostly psychrotrophic, nutritionally versatile and non-exacting (Delille, 1996), but with a distinct capacity for cold adaptation (Christian & Karl, 1995). In this context it is possible that within a single genus, species which inhabit each of these habitats may express substantially different ecophysiological traits, reflecting adaptations to their respective habitats. In this study a group of psychrophilic sea ice strains with the unusual capacity to form polyunsaturated fatty acids (PUFAs) (Nichols *et al.*, 1997) were found to be closely related to the psychrotrophic, moderately halophilic, planktonic species [*Flavobacterium*] *gondwanense* (Dobson *et al.*, 1993). The two taxa represent distinct species within a novel genus, *Psychroflexus* gen. nov. The ecophysiological differences between the species of *Psychroflexus* provide an example of the capacity of bacterial species to become highly adapted to a range of specific habitats but still retain common chemotaxonomic properties and phylogenetic relatedness.

METHODS

Cultivation of bacterial strains. The sea ice strains, including *Polaribacter* strains IC054, IC066 and 23-P, [*Flectobacillus*] *glomeratus* (brackets indicate species is generically misclassified), *Gelidibacter algens*, *Psychroserpens burtonensis* and 'Melosira-colonizing' strain IC166 were grown at 5–10 °C on marine 2216 agar (Difco). [*Flavobacterium*] *gondwanense*, [*Flavobacterium*] *salegens*, [*Cytophaga*] *latercula*, [*Cytophaga*] *lytica*, [*Cytophaga*] *marinoflava* and [*Flexibacter*] *maritimus* were cultivated on marine 2216 agar at 20 °C. The sea ice isolates were preserved by freezing cell suspensions at –80 °C in marine 2216 broth to which 30% (v/v) glycerol was added.

Phenotypic characterization. Most phenotypic tests used in the study have been described previously (Bowman *et al.*, 1997a, c). Additional enzymic tests were performed using the following chromogenic substrates (Smibert & Krieg, 1994): *p*-nitrophenyl α -L-arabinopyranoside (α -arabinosidase), *p*-nitrophenyl α -L-fucopyranoside (α -fucosidase), *p*-nitrophenyl α -D-galactopyranoside (α -galactosidase), *o*-nitrophenyl β -D-galactopyranoside (ONPG; β -galactosidase), *p*-nitrophenyl α -D-glucopyranoside (α -glucosidase), *p*-nitrophenyl β -D-glucopyranoside (β -glucosidase), *o*-nitrophenyl β -D-galactopyranoside 6-phosphate (streptococcal β -galactosidase), *p*-nitrophenyl β -D-glucuronide (β -glucuronidase), α -D-mannopyranoside (α -mannosidase) and *p*-nitrophenyl phosphate (alkaline phosphatase, in 0.4 M glycine buffer, pH 10.0).

Growth rate analysis. The temperature/growth rate response curve of strain ACAM 623^T was determined in a temperature gradient incubator (Toyokagaku Sangyo). The temperature gradient ranged from 0 to 25 °C. Tubes containing 10 ml marine 2216 broth, were inoculated with 0.5 ml cells taken from late-exponential-phase cultures grown at 10 °C. Growth was measured by an increase in OD₅₅₀ for up to 20 d. Growth was considered to have occurred if the OD₅₅₀ increased by more than 20%. The data were fitted to the Ratkowsky square root temperature growth model (Ratkowsky *et al.*, 1983) and cardinal temperatures were determined.

Whole-cell fatty acid analysis. Strains were cultivated on marine 2216 agar at 10 °C, harvested into a small amount of artificial seawater and then lyophilized using a vacuum freeze-

drier (Dynavac). Whole-cell fatty acid profiles were quantitatively determined using GC and GC-MS procedures (Nichols *et al.*, 1997). The geometry and position of double bonds in monounsaturated fatty acids was confirmed using dimethyldisulfide derivatization and GC-MS analysis (Nichols *et al.*, 1986). The double bond positions are numbered from the methyl (*ω*) end of the fatty acid.

Genotypic analysis. Genomic DNA was extracted and purified using the procedure of Marmur & Doty (1962). The G+C content was then determined from thermal denaturation profiles (Sly *et al.*, 1986). The spectrophotometric renaturation rate kinetic procedure (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 1 × SSC buffer (0.15 M NaCl, 0.015 M Na citrate, pH 7.0) and adjusted in concentration to approximately 75 µg ml⁻¹. Following denaturation of the DNA samples, 20 × SSC was added to increase the total sodium concentration to 0.5 M; hybridization was then performed at the optimal temperature for renaturation (*T*_{or}) which was 25 °C below the DNA melting temperature and was calculated from the equation *T*_{or} (°C) = 51.5 + (0.41 × %G + C). The decline in absorbance of DNA mixtures and control DNA samples over a 40 min period was used to calculate DNA hybridization values from the following equation DNA hybridization (%) = (4AB – A – B/2√(A × B)) × 100 (Huss *et al.*, 1983), where A and B represent the changes in absorbance for the two DNA samples being compared and AB represents the change in absorbance for equimolar mixtures of the two DNA samples. DNA hybridization values equal to or below 25% are considered to represent background hybridization and are thus not considered significant (Huss *et al.*, 1983).

Phylogenetic analysis. The 16S rRNA sequence for strains ACAM 624, ACAM 625, ACAM 623^T and ACAM 628 have been determined previously (Bowman *et al.*, 1997b). GenBank accession numbers for these sequences and for reference sequences are shown in Fig. 3. Sequence data were manually aligned to various 16S rRNA sequences of representatives of the family *Flavobacteriaceae* obtained from the GenBank nucleotide library. Software from PHYLIP version 3.57c (Felsenstein, 1993) was used to further analyse the sequence data set. DNADIST (using the maximum-likelihood option) was used to determine sequence similarities and NEIGHBOR (using the neighbourliness option) to create a phylogenetic tree. The 16S rRNA sequence of *Bacteroides fragilis* was used as the outgroup for the phylogenetic tree.

RESULTS

Cellular and colonial morphology

The sea ice isolates (ACAM 623^T, ACAM 624, ACAM 625, ACAM 626 and ACAM 628) examined in this study (Table 1) produced colonies on marine agar 2216 which were pigmented bright orange, elastic in consistency, circular and convex in shape with slightly spreading edges and 5–10 mm in diameter after 21 d incubation at 5 °C. Colonies could be lifted entirely from the surface of the medium with a loop. The strains were similar in terms of cellular morphology, occurring as filaments, rods and in older cultures as coccoidal bodies. In young cultures (24–72 h) filaments of indeterminate length were formed which slowly glided on R2A-seawater agar. In older cultures and in flask cultures during

Table 1. Source of strains studied

Species/strain designation	Strain no.*	Isolation site
Sea ice isolates:		
651	ACAM 623 ^T	Sea ice, Prydz Bay, Antarctica
IC055	ACAM 624	Sea ice, Prydz bay, Antarctica
IC132	ACAM 625	Sea ice, Ellis Fjord, Antarctica
EFDMS10	ACAM 626	Sea ice, Ellis Fjord, Antarctica
IC168	ACAM 628	Sea ice, Long Fjord, Antarctica
Reference strains:		
[<i>Cytophaga</i>] <i>latercula</i>	ACAM 539 ^T	Aquarium outflow
[<i>Cytophaga</i>] <i>lytica</i>	ACAM 74 ^T	Beach mud
[<i>Cytophaga</i>] <i>marinoflava</i>	ACAM 75 ^T	Seawater
' <i>Melosira</i> -colonizing' strain	IC166	<i>Melosira</i> sp., sea ice
[<i>Cytophaga</i>] <i>uliginosa</i>	ACAM 538 ^T	Marine sediment
[<i>Flavobacterium</i>] <i>gondwanense</i>	ACAM 48 ^T	Hypersaline lake
[<i>Flavobacterium</i>] <i>gondwanense</i>	ACAM 43	Hypersaline lake
[<i>Flavobacterium</i>] <i>gondwanense</i>	ACAM 46	Hypersaline lake
[<i>Flavobacterium</i>] <i>gondwanense</i>	ACAM 55	Hypersaline lake
[<i>Flavobacterium</i>] <i>gondwanense</i>	ACAM 62	Hypersaline lake
[<i>Flavobacterium</i>] <i>salegens</i>	ACAM 44 ^T	Hypersaline lake
[<i>Flexibacter</i>] <i>maritimus</i>	NCIMB 2154 ^T	Red sea bream
<i>Psychroserpens burtonensis</i>	ACAM 188 ^T	Marine salinity lake
<i>Gelidibacter algens</i>	ACAM 536 ^T	Sea ice
[<i>Flectobacillus</i>] <i>glomeratus</i>	ACAM 171 ^T	Marine salinity lake
<i>Polaribacter</i> sp.	23-P	Sea ice
<i>Polaribacter</i> sp.	IC054	Sea ice
<i>Polaribacter</i> sp.	IC066	Sea ice

* Abbreviations: ACAM, Australian Collection of Antarctic Micro-organisms, Antarctic CRC, University of Tasmania, Tasmania, Australia; ATCC, American Type Culture Collection, Rockville, MD, USA; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

exponential-phase growth, the filaments were generally shorter (5–50 µm in length, 0.4–1.5 µm in width) with rod-shaped and coccoidal cells much more prevalent (Fig. 1a). Filaments were often coiled or twisted in a somewhat variable fashion (Fig. 1b).

Physiology and metabolism

Some physiological, biochemical and nutritional properties of the sea ice strains are shown in Table 2. The strains were strictly aerobic, seawater-requiring chemoheterotrophs which failed to grow in liquid media at temperatures higher than 20 °C. On solid medium several of the strains also failed to grow at 15 °C while some grew poorly at 10 °C. The optimum growth temperature (T_{opt}) in marine 2216 broth was estimated to be 12 °C for ACAM 623^T. The maximum growth temperature (T_{max}) was estimated to be about 19 °C while the theoretical minimum growth temperature (T_{min}) of ACAM 623^T was estimated to be –16 °C (Fig. 2). The maximum specific growth rate (μ_{max}) was 0.037 h⁻¹.

The sea ice strains were stenohaline, exhibiting optimal growth in the presence of seawater. Many of the strains exhibited either poor or no growth when the seawater

concentration was reduced to 50 % or when increased to double strength. Little or no growth occurred when the medium was only supplemented with NaCl (at concentrations ranging from 2 to 5 %). The strains required yeast extract for growth in a chemically defined medium. The strains were unable to grow anaerobically either by fermentation or anaerobic respiration. Only oxidative acid production from carbohydrates was observed and in general these reactions were weak and slow, taking up to 10 d to form on Leifson's oxidation/fermentation (O/F) medium. The isolates were also able to hydrolyse starch, Tween 80 and DNA and were positive for catalase and cytochrome-*c* oxidase activity (Table 2). The strains possessed alkaline phosphatase, α -glucosidase and β -glucosidase activity, indicated by the hydrolysis of chromogenic substrates (Table 2).

DNA base composition and DNA–DNA hybridization

The strains had G+C contents ranging from 32 to 33 mol % (mean 32.6 ± 0.6, $n = 5$). Strains ACAM 623^T, ACAM 624 and ACAM 625 shared high DNA–DNA reassociation levels ranging from 84 to 96 % while exhibiting only low hybridization levels with [*Flavobacterium*] *salegens* ACAM 44^T (8–12 %), [*Flavo-*

Fig. 1. (a) Phase-contrast micrograph of strain ACAM 623^T grown on marine 2216 agar at 5 °C for 14 d. (b) Phase-contrast micrograph of strain ACAM 623^T grown in marine 2216 broth at 5 °C for 7 d. Bars, 10 μm.

bacterium] *gondwanense* ACAM 48^T (17–28 %) and [*Cytophaga*] *latercula* ACAM 539^T (11–21 %).

Fatty acid profiles

The sea ice strains possessed very similar whole-cell fatty acid profiles, with the major constituents (>5 % of total fatty acids) including a15:1ω10c, a15:0, i16:0,

3-OH i16:0 and 3-OH a17:0 (Table 3). Straight-chain monounsaturated fatty acids were completely absent. However, the strains have the unusual capacity to synthesize PUFAs including 20:5ω3c (2–9 %) and 20:4ω6c (1–3 %). A number of reference flavobacterial species were also analysed for direct comparison as shown in Tables 1 and 3. The fatty acid profiles of a number of strains of [*Flavobacterium*] *gondwanense*

Table 2. Phenotypic characteristics differentiating sea ice strains from [*Flavobacterium*] *gondwanense*

Data from Bowman *et al.* (1997b), Dobson *et al.* (1993) and this study. Abbreviations: +, test is positive for all strains; v+, test varies between strains, type strain is positive; v–, test varies between strains, type strain is negative; –, test is negative for all strains. Parentheses indicate a weak and/or delayed reaction. The following tests were positive for both species: production of catalase, cytochrome-*c* oxidase, alkaline phosphatase, α -glucosidase, β -glucosidase and acid from D-mannose; hydrolysis of Tween 80, starch and DNA. The following tests were negative for all strains: hydrolysis of casein, agar, chitin, urate and xanthine; reduction of nitrate to nitrite; denitrification; production of hydrogen sulfide, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, tryptophan deaminase, phenylalanine deaminase, α -arabinosidase, α -fucosidase, α -galactosidase, β -galactosidase, streptococcal β -galactosidase, β -glucuronidase, α -mannosidase; production of indole from tryptophan; production of acid from rhamnose, fructose, galactose, lactose, melibiose, raffinose, sucrose, trehalose, dextran, arabinol, adonitol, sorbitol, mannitol, inositol, N-acetylglucosamine and chitin.

Characteristic	<i>Psychroflexus torquis</i>	[<i>Flavobacterium</i>] <i>gondwanense</i>
No. of strains	5	5
Filaments > 100 μ m	+	–
Gliding motility	+	–
T_{min} (°C)	–16	–5
T_{opt} (°C)	10–15	20–25
T_{max} (°C)	15–20	30
Optimum salinity (M NaCl)	0.4	1.0
Salinity range (M NaCl)	0.25–0.8	0–3.0
Yeast extract requirement	+	–
Hydrolysis of:		
Aesculin	–	+
Urea	–	v+
Gelatin	–	v–
Production of acid from:		
D-Glucose, maltose	(v+)	+
L-Arabinose, D-xylose, cellobiose	–	+
Glycerol	v–	–
DNA G + C content (mol %)	32–33	36

were similar to the profiles of the sea ice isolates but differed in that PUFAs were absent. In addition, the sea ice strains contained higher ratios of anteiso- to iso-branched fatty acids (2.5:1) while for [*Flavobacterium*] *gondwanense* these classes of fatty acids occurred in approximately equimolar ratios.

Phylogeny

The phylogenetic relationships of four representatives of the sea ice strains (ACAM 623^T, ACAM 624, ACAM 625 and ACAM 628) were examined by distance and maximum-likelihood methods. 16S rRNA sequences were 1439–1455 nt in length and extended from nucleotide positions 35 to 1508 (*Escherichia coli* equivalent). The sequences for ACAM 623^T, ACAM 624 and ACAM 625 were identical while the similarity of these strains to ACAM 625 was 99.1%. [*Flavobacterium*] *gondwanense* ACAM 48^T was found to be closely related to the sea ice strains with a sequence similarity of 97.8–98.1% (Table 1). Together the sea ice

strains and [*Flavobacterium*] *gondwanense* formed a distinct branch in the Family *Flavobacteriaceae* (Bernardet *et al.*, 1996) (Fig. 3). The closest relatives were [*Flavobacterium*] *salegens* and [*Cytophaga*] *latercula* which were approximately equidistant from [*Flavobacterium*] *gondwanense* and the sea ice strains.

DISCUSSION

Fatty acid profiles (Table 3), phylogenetic data (Fig. 3) and phenotypic data (Table 4) support a close relationship between the sea ice isolates and the moderately halophilic species [*Flavobacterium*] *gondwanense*. The major differences between the groups are ecophysiological. The sea ice strains are psychrophilic, slow-growing rod-like to coiled filamentous cells capable of gliding motility and with fastidious growth requirements. So far this group has only been isolated from sea ice (Bowman *et al.*, 1997a, b) and so far have not been isolated from under-ice seawater samples (Bowman *et al.*, 1997b) or from the marine-salinity

Fig. 2. Temperature/growth rate response curve of strain ACAM 623^T based on the square root growth model of Ratkowsky *et al.* (1983).

Burton Lake which has a noticeably enriched psychrophilic population (Franzmann *et al.*, 1990). By comparison [*Flavobacterium*] *gondwanense* is psychrotrophic, moderately halophilic, non-motile and has a non-exacting nutrient requirement (Dobson *et al.*, 1993; this study). [*Flavobacterium*] *gondwanense* has been isolated and observed by indirect immunofluorescence in the upper layers of a number of Antarctic hypersaline lakes (James *et al.*, 1994) but so far has not been isolated from or detected in Antarctic marine salinity sites (Bowman *et al.*, 1997a, b).

Both the sea ice strains and [*Flavobacterium*] *gondwanense* have similar fatty acid profiles, differing only in that [*Flavobacterium*] *gondwanense* lacks PUFAs and has a more even iso-to anteiso-branched fatty acid ratio. The fatty acid profiles of the two taxa differ considerably from other phylogenetically related flavobacteria (Table 3). The synthesis of PUFAs and high levels of anteiso-branched fatty acids in the sea ice strains represents a higher degree of cold adaptation owing to the continually low sympagic *in situ* temperatures, typically between -1 and -15 °C. By comparison the oxic layer temperatures of hypersaline lakes which [*Flavobacterium*] *gondwanense* is known to inhabit are often higher, ranging from -2 to 12 °C (summer temperature range; James *et al.*, 1994). It is known that both PUFAs and branched fatty acids tend to reduce lipid bilayer viscosity (Kaneda, 1991). The sea ice strains are the first bacterial strains found conclusively to contain arachidonic acid, possibly as a by-product of eicosapentaenoic acid synthesis. Studies using ^{14}C -labelling have revealed arachidonic acid as a transient precursor in the synthesis of eicosapentaenoic acid in *Vibrio*-like strains (Iwanami *et al.*, 1995). Amongst

the flavobacteria, PUFA synthesis has only been observed previously in [*Flexibacter*] *polymorphus* (Johns & Perry, 1977) which contains eicosapentaenoic acid which in turn was believed to have a role in promoting gliding motility. [*Flexibacter*] *polymorphus* is phylogenetically remote from the sea ice strain groups and has a very different fatty acid pattern (Table 3). Bacterial $\omega 3$ (but not $\omega 6$) PUFA synthesis has also been found to occur in species of the genera *Shewanella* and *Colwellia* and in the species [*Vibrio*] *marinus* (DeLong *et al.*, 1997). No information exists on how bacteria have obtained the genes for PUFA synthesis but it is known that phytoplankton able to form $\omega 3$ PUFA use a variety of different synthetic pathways (Sprecher *et al.*, 1995). Studies currently examining the specific synthetic pathways of PUFA formation in bacteria may provide further insight into the development of this unusual characteristic.

Overall, the similar fatty acid profile and close phylogenetic association provide evidence that the sea ice strains and [*Flavobacterium*] *gondwanense* belong to a group distinct from other members of the family *Flavobacteriaceae*. The closest relatives, [*Flavobacterium*] *salegens* and [*Cytophaga*] *latercula*, each possess fatty acid profiles quite different from each other and distinct from [*Flavobacterium*] *gondwanense* and the sea ice strains, thus suggesting that these species each may represent a distinct genus. On the basis of polyphasic taxonomic data presented in this study, the sea ice strains clearly represent a novel taxonomic group equivalent to a new genus, *Psychroflexus* gen. nov. The closely related [*Flavobacterium*] *gondwanense* also belongs to this new group but represents a distinct species. Thus, the sea ice strains constitute a new species, *Psychroflexus torquis* sp. nov., while [*Flavobacterium*] *gondwanense* becomes *Psychroflexus gondwanense* gen. nov., comb. nov.

Description of *Psychroflexus* gen. nov.

Psychroflexus (Psy.chro'flex.us. Gr. adj. *psychros* cold; L. masc. n. *flexus* bend, curve; M. L. masc. n. *Psychroflexus* cold bend).

Gram-negative. Cellular morphology ranges from rod-like cells to coiled filaments and filaments of indeterminate length. Coccoid cells are present in older cultures. Ring-shaped cells and gas vesicles are not formed. Resting stages are absent. Either non-motile or motile via slow gliding motility. Orange carotenoid-like pigments are formed. Flexirubin pigments are absent. Strictly aerobic chemoheterotroph. Psychrophilic or psychrotrophic. Slightly or moderately halophilic. Major cellular fatty acids are a15:0, a15:10 ω 10c, i16:0, 3-OH i16:0 and 3-OH a17:0. Major respiratory quinone is menaquinone-6 (determined for *Psychroflexus gondwanense* only). G+C content 32–36 mol% (determined by thermal denaturation). Member of the family *Flavobacteriaceae*. Genus contains two species, *Psychroflexus gondwanense* and *Psychroflexus torquis*. Type species is *Psychroflexus torquis*.

Table 3. Whole-cell fatty acid profiles of PUFA-containing sea ice isolates compared with other members of the *Flavobacteriaceae* and [*Flexibacter*] *polymorphus*All values are means $\pm$ SD (in parentheses); TR, trace amount. Bold type indicates predominating fatty acids.

Fatty acid	<i>Psychroflexus</i> <i>torquis</i> No. of strains: 4	[<i>Flavobacterium</i>] <i>gondwanense</i> 3	[<i>Flavobacterium</i>] <i>salegens</i> 1	[<i>Cytophaga</i>] <i>latercula</i> 1	<i>Psychroserpens</i> <i>burtonensis</i> * 3	<i>Gelidibacter</i> <i>algens</i> * 3	<i>Polaribacter</i> spp.† 3	[<i>Flexibacter</i>] <i>maritimus</i> 1	[<i>Cytophaga</i>] <i>lytica</i> group‡ 4	[<i>Flexibacter</i>] <i>polymorphus</i> § 1
14:0	0.1 (0.1)	0.4 (0.3)	0.3	1.1	0.2 (0.1)	0.3 (0.1)	0.8 (0.5)	0.5	0.7 (0.3)	2.1
15:0	4.2 (0.6)	1.9 (0.8)	4.5	5.4	10.1 (2.8)	8.8 (2.5)	5.3 (2.5)	5.1	13.2 (4.5)	0.9
16:0	0.6 (0.3)	1.1 (0.5)	3.7	0.8	0.4 (0.1)	1.4 (0.1)	1.6 (0.4)	3.3	2.7 (1.7)	31.5
17:0	—	0.2 (0.2)	—	0.3	—	—	—	—	0.1 (0.1)	0.6
18:0	0.1 (0.1)	0.2 (0.2)	—	0.6	—	0.2 (0.1)	0.4 (0.2)	0.3	—	4.1
19:0	—	—	—	—	—	—	0.4 (0.4)	—	—	—
i13:0	0.7 (0.2)	1.4 (0.4)	—	—	TR	—	1.0 (0.9)	—	0.1 (0.1)	0.3
i14:1 ω 9c	—	2.8 (0.3)	—	—	—	—	—	0.2	—	—
i14:0	1.0 (0.2)	4.8 (0.8)	—	0.2	0.4 (0.1)	0.5 (0.2)	2.1 (1.9)	0.5	0.2 (0.2)	0.5
i15:1 ω 10c	0.4 (0.4)	2.2 (1.0)	3.8	6.7	14.1 (3.4)	11.3 (3.8)	12.0 (3.1)	15.0	11.0 (5.1)	—
a15:1 ω 10c	16.9 (1.8) [A1]	18.4 (2.5)	—	—	8.4 (2.3)	8.8 (2.9)	0.1 (0.1)	1.1	0.9 (0.8)	—
i15:0	1.1 (0.3)	2.1 (0.5)	23.7	18.2	10.0 (1.3)	7.9 (4.3)	38.7 (12.4)	23.4	18.5 (9.7)	8.2
a15:0	35.2 (4.4)	23.0 (2.7)	11.6	0.2	10.4 (1.0)	13.2 (2.8)	1.0 (0.3)	3.0	2.9 (1.5)	0.9
i16:1 ω 11c	0.1 (0.1)	0.7 (0.3)	—	—	9.1 (2.7)	—	—	—	—	—
i16:1 ω 6c	—	—	1.5	—	—	7.7 (2.6)	6.0 (1.5)	7.5	1.4 (1.3)	—
i16:0	6.0 (1.2)	10.9 (0.3)	7.6	2.3	0.6 (0.5)	8.0 (2.0)	0.1 (0.1)	1.6	1.2 (1.0)	1.5
i17:0	0.3 (0.3)	0.3 (0.3)	2.4	4.9	—	—	0.2 (0.2)	—	0.4 (0.3)	5.0
a17:0	—	—	2.1	—	—	1.5 (0.1)	0.2 (0.2)	—	—	1.4
i17:1 ω 7c	—	—	13.2	11.6	1.5 (0.5)	4.9 (1.6)	0.6 (0.6)	1.7	8.3 (4.6)	—
a17:1 ω 7c	—	—	7.3	0.3	TR	5.2 (0.5)	—	1.9	0.9 (0.6)	—
i17:1 ω 5c	—	—	—	1.0	—	—	0.5 (0.5)	TR	—	4.5
i18:0	—	—	—	—	—	—	0.2 (0.2)	—	0.1 (0.1)	—
14:1 ω 7c	—	—	—	—	—	—	0.1 (0.1)	0.2	—	0.1
14:1 ω 5c	—	—	—	—	—	—	—	—	—	0.2
15:1 ω 11c	—	—	—	—	6.4 (0.6)	3.5 (0.1)	—	—	—	—
15:1 ω 6c	—	—	0.6	0.2	3.5 (0.8)	—	9.0 (3.8)	8.0	2.0 (0.6)	—
15:1 ω 4c	—	—	—	—	7.6 (0.8)	1.3 (0.1)	—	1.1	—	—
16:1 ω 9c	—	—	—	—	0.7 (0.6)	—	—	0.5	—	—
16:1 ω 7c	—	—	9.4	0.1	0.4 (0.3)	5.9 (0.8)	1.9 (1.0)	5.7	12.3 (4.9)	1.8
16:1 ω 5c	—	—	—	—	6.9 (1.9)	1.5 (0.8)	0.5 (0.5)	2.3	—	6.5
17:1 ω 8c	—	—	—	—	1.2 (0.4)	—	—	—	0.2 (0.1)	—
17:1 ω 6c	—	—	0.8	1.1	1.5 (0.4)	3.7 (0.8)	—	0.4	0.1 (0.1)	TR
18:1	—	—	—	—	—	—	0.1 (0.1)	0.3	0.2 (0.2)	4.6
3-OH 14:0	—	0.1 (0.1)	—	1.2	—	—	—	—	—	—
3-OH i15:0	0.3 (0.2)	0.9 (0.7)	—	7.1	—	0.5 (0.3)	12.4 (5.8)	8.0	4.6 (3.9)	—
3-OH a15:0	0.2 (0.2)	0.1 (0.1)	0.8	—	—	—	—	—	1.1 (0.3)	—
3-OH 15:0	2.5 (0.7)	0.9 (0.5)	—	—	—	—	—	0.7	0.4 (0.2)	—
3-OH i16:0	15.4 (1.0)	18.5 (1.6)	0.3	0.1	—	0.8 (0.6)	0.1 (0.1)	4.0	6.6 (4.9)	—
3-OH 16:0	1.2 (1.2)	0.4 (0.2)	0.4	—	—	0.3 (0.1)	4.1 (3.4)	0.6	1.6 (0.7)	—
3-OH i17:0	0.2 (0.1)	0.9 (0.4)	3.0	34.7	—	0.4 (0.4)	0.2 (0.2)	1.9	10.4 (3.9)	—
3-OH a17:0	10.9 (3.4)	6.6 (0.8)	2.0	1.1	—	0.4 (0.4)	2.1 (2.0)	0.3	2.3 (2.1)	—
3-OH 17:0	0.4 (0.2)	0.7 (0.1)	—	0.8	—	—	—	—	0.1 (0.1)	—
3-OH i18:0	—	0.1 (0.1)	—	—	—	—	—	—	—	—
16:2	—	—	—	—	—	—	—	1.1	0.1 (0.1)	—
18:3 ω 3	—	—	—	—	—	—	—	—	—	1.4
18:4 ω 3	—	—	—	—	—	—	—	—	—	0.6
20:4 ω 3	—	—	—	—	—	—	—	—	—	2.0
20:4 ω 6	2.1 (1.2)	—	—	—	—	—	—	—	—	—
20:5 ω 3	4.9 (3.0)	—	—	—	—	—	—	—	—	17.8

* Data from Bowman *et al.* (1997c).† Strains of *Polaribacter* spp. include [*Flectobacillus*] *glomeratus*, gas vacuolate strain 23-P and sea ice strains IC054 and IC066.‡ Strains in the [*Cytophaga*] *lytica* group include [*Cytophaga*] *lytica*, [*Cytophaga*] *marinoflava*, [*Cytophaga*] *uliginosa* and 'Melosira-colonizing' strain IC166.

§ Data from Johns & Perry (1977).

|| Double bond positions in 18:1 fatty acids were either ω 7c or ω 9c, while double bond positions in 16:2 fatty acids were not determined.**Description of *Psychroflexus torquis* gen. nov., sp. nov.***Psychroflexus torquis* (tor'quis. L. masc. n. *torquis* something twisted, referring to coiling of filaments).

On solid media cells range from filaments of indeterminate length in very young cultures, which slowly glide, to coccoid bodies in older cultures. On solid media

and when in liquid media rod-like to filamentous cells (5–50 μ m in length, 0.4–1.5 μ m in width) are formed. Filamentous cells often appear coiled. The incidence of coccoid bodies increases in older cultures. Stenohaline. Requires seawater for growth. Psychrophilic, with growth occurring at 0 °C and optimal growth occurring at 10–15 °C in liquid media. No growth occurs at temperatures at or above 20 °C in liquid media. Requires

Fig. 3. Phylogenetic tree showing the relationship of *Psychroflexus torquis* strains to other members of the family *Flavobacteriaceae*. The topology of the tree is based on maximum-likelihood distances. GenBank accession numbers are shown in parentheses. Bar, sequence dissimilarity value of 5%.

Table 4. Phenotypic characteristics differentiating *Psychroflexus* species from other members of the *Flavobacteriaceae*

Abbreviations: —, negative; +, positive; V, variable; O, orange; Y, yellow; R, red; SH, slightly halophilic; MH, moderately halophilic.

Species	Gliding motility	Pigment	Growth at 25 °C	Salinity requirement	Yeast extract requirement	Carbohydrate utilization	Hydrolysis		β -Galactosidase activity	Nitrate reduction	G + C (mol %)
							Starch	Agar			
<i>Psychroflexus torquis</i>	+	O	—	SH	+	+	+	—	—	—	32–33
<i>Psychroflexus gondwanense</i>	—	O	+	MH	—	+	+	—	—	—	36
[<i>Flavobacterium</i>] <i>salegens</i>	—	Y	+	MH	—	+	+	—	+	+	37–38
[<i>Cytophaga</i>] <i>latercula</i>	—	O–R	+	SH	—	+	—	+	+	—	32
<i>Psychroserpens burtonensis</i>	—	Y	—	SH	+	—	—	—	V	—	27–29
<i>Gelidibacter algens</i>	+	Y	V	SH	—	+	+	—	—	—	36–38
<i>Polaribacter</i> [<i>Flectobacillus</i>] <i>glomeratus</i> group	—	O	—	SH	+	+	V	—	V	—	31–34
[<i>Flexibacter</i>] <i>maritimus</i> group*	+	Y	+	SH	+	—	—	—	—	+	29–32
[<i>Cytophaga</i>] <i>lytica</i> group†	+	O–Y	+	SH	—	+	+	V	V	V	32–42

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

† Includes [*Cytophaga*] *lytica*, [*Cytophaga*] *marinoflava*, [*Cytophaga*] *uliginosa* and ‘*Melosira*-colonizing’ strain IC166. [*Cytophaga*] *uliginosa* forms flexirubin.

yeast extract for growth in chemically defined media. Catalase- and oxidase-positive. Alkaline phosphatase, α -glucosidase and β -glucosidase activity present. Gelatin, Tween 80, starch and DNA degraded. Acid formation from carbohydrates is slow and delayed in Leifson's O/F medium. Acid is formed by most strains from D-glucose, D-mannose and maltose. Some strains can also form acid from glycerol. Other traits are shown in Table 2. Contains polyunsaturated fatty acids 20:5 ω 3 and 20:4 ω 6. G + C content 32–33 mol % (determined by thermal denaturation). Colonies on seawater medium are circular, raised convex with slightly spreading edges,

an elastic-gummy consistency, 5–10 mm diameter after 21 d incubation and pigmented bright orange. Type strain is ACAM 623^T isolated from sea ice collected from Prydz Bay, Vestfold Hills, Antarctica.

Description of *Psychroflexus gondwanense* (Dobson et al. 1993) gen. nov., comb. nov.

The description is identical to that given for *Flavobacterium gondwanense* by Dobson et al. (1993). Type strain is ACAM 48^T, isolated from Organic Lake, Vestfold Hills, Antarctica.

ACKNOWLEDGEMENTS

This research was supported by grants from the Australian Research Council (grant no. A19602517) and Antarctic Science Advisory Committee (grant no. 1012). We would like to thank Professor Jim Staley (University of Washington, Seattle) for strain 23-P and Professor Hans Trüper (Bonn University, Germany) for advice with the Latin names.

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., Brown, M. V. & Nichols, D. S. (1997a). Biodiversity and ecophysiology of bacteria associated with Antarctic sea ice. *Antarct Sci* **9**, 134–142.
- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997b). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997c). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria from Antarctic lacustrine and sea ice habitats. *Int J Syst Microbiol* **47**, 670–677.
- Christian, J. R. & Karl, D. M. (1995). Bacterial ectoenzymes in marine waters: activity ratios and temperature responses in three oceanographic provinces. *Limnol Oceanogr* **40**, 1042–1049.
- Delille, D. (1996). Biodiversity and function of bacteria in the Southern Ocean. *Biodivers Conserv* **5**, 1505–1523.
- DeLong, E. F., Franks, D. G. & Yayanos, A. A. (1997). Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. *Appl Environ Microbiol* **63**, 2105–2108.
- Dobson, S. J., Colwell, R. R., McMeekin, T. A. & Franzmann, P. D. (1993). Direct sequencing of the polymerase chain reaction-amplified 16S rRNA gene of *Flavobacterium gondwanense* sp. nov. and *Flavobacterium salegens* sp. nov., two new species from a hypersaline Antarctic lake. *Int J Syst Bacteriol* **43**, 77–83.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.57c. University of Washington, Seattle.
- Franzmann, P. D., Deprez, P. P., McGuire, A. J., McMeekin, T. A. & Burton, H. R. (1990). The heterotrophic bacterial microbiota of Burton Lake, Antarctica. *Polar Biol* **10**, 261–264.
- Gosink, J. J. & Staley, J. T. (1995). Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. *Appl Environ Microbiol* **61**, 3486–3489.
- Gosink, J. J., Woese, C. R. & Staley, J. T. (1998). *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of '*Flectobacillus glomeratus*' as *Polaribacter glomeratus* comb. nov. *Int J Syst Bacteriol* **48**, 223–235.
- Helmke, E. & Weyland, H. (1995). Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. *Mar Ecol Prog Ser* **117**, 269–287.
- Huss, V. A. R., Festl, H. & Schleifer, K.-H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Irgens, R., Gosink, J. J. & Staley, J. T. (1996). *Polaromonas vacuolata* gen. nov., sp. nov., a psychrophilic, marine, gas vacuolate bacterium from Antarctica. *Int J Syst Bacteriol* **46**, 822–826.
- Iwanami, H., Yamaguchi, T. & Takeuchi, M. (1995). Fatty acid metabolism in bacteria that produce eicosapentaenoic acid isolated from sea urchin *Strongylocentrotus nudus*. *Nippon Suisan Gakkaishi* **61**, 205–210.
- James, S. R., Burton, H. R., McMeekin, T. A. & Mancuso, C. A. (1994). Seasonal abundance of *Halomonas meridiana*, *Halomonas subglaciescola*, *Flavobacterium gondwanense* and *Flavobacterium salegens*. *Antarct Sci* **6**, 325–332.
- Johns, R. B. & Perry, G. J. (1977). Lipids of the marine bacterium *Flexibacter polymorphus*. *Arch Microbiol* **114**, 267–271.
- Kaneda, T. (1991). Iso- and anteiso-fatty acids in bacteria: biosynthesis, function and taxonomic significance. *Microbiol Rev* **55**, 288–302.
- Malinsky-Rushansky, N. Z. & Legrand, C. (1996). Excretion of dissolved organic carbon by phytoplankton of different sizes and subsequent bacterial uptake. *Mar Ecol Prog Ser* **132**, 249–255.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Nichols, D. S., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997). Production of eicosapentaenoic acid and arachidonic acids by an Antarctic bacterium: response to growth temperature. *FEMS Microbiol Lett* **152**, 349–354.
- 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.
- Palmisano, A. C. & Garrison, D. L. (1993). Microorganisms in Antarctic sea ice. In *Antarctic Microbiology*, pp. 167–218. Edited by E. I. Friedmann. New York: Wiley-Liss.
- Priddle, J., Leakey, R. J. G., Archer, S. D. & Murphy, E. J. (1996). Eukaryotic microbiota in the surface waters and sea ice of the Southern Ocean: aspects of physiology, ecology and biodiversity in a 'two-phase' ecosystem. *Biodivers Conserv* **5**, 1473–1504.
- Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N. & Chandler, R. E. (1983). Model for bacterial growth throughout the entire biokinetic range. *J Bacteriol* **154**, 1222–1226.
- 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.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Microbiology*, pp. 611–654. Edited by P. Gerhardt, R. G. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Sprecher, H., Luthria, D. L., Mohammed, B. S. & Baykousheva, S. P. (1995). Re-evaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *J Lipid Res* **36**, 2471–2477.
- Sullivan, C. W. & Palmisano, A. C. (1984). Sea ice microbial communities: distribution, abundance and diversity of ice bacteria in McMurdo Sound, Antarctica, in 1980. *Appl Environ Microbiol* **47**, 788–795.

Received 1 September 1997; revised 19 January 1998; accepted 25 February 1998.