***Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats**

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A group of pigmented, psychrophilic, strictly aerobic chemoheterotrophs isolated from sea-ice cores collected from coastal areas of eastern Antarctica was found to represent a novel 16S rRNA lineage within the gamma subclass of the *Proteobacteria*, adjacent to the genus *Alteromonas*. The isolates are motile, Gram-negative, rod-shaped cells, which are psychrophilic and slightly halophilic, and possess an absolute requirement for seawater. Differences in phenotypic characteristics and DNA–DNA hybridization indicated the isolates formed two distinct taxa which have DNA G+C contents of 44–46 mol% and 40 mol%, respectively. Whole-cell fatty acid profiles of the isolates were however very similar and included 16:1 ω 7c, 18:1 ω 7c, 16:0 and 17:1 ω 8c as the major fatty acid components. Overall, sufficient differences exist to distinguish the sea-ice strains from currently recognized bacterial genera. It is proposed the sea-ice strains represent a new genus, *Glaciecola*, which contains two species, *Glaciecola punicea* gen. nov., sp. nov. (ACAM 611^T) and *Glaciecola pallidula* gen. nov., sp. nov. (ACAM 615^T).

Keywords: Antarctica, sea ice, psychrophilic bacteria, *Glaciecola* gen. nov., *Alteromonas*

INTRODUCTION

Sea-ice microbial communities have a major influence on various trophic levels of the polar oceanic food webs (Palmisano & Garrison, 1993) and occur as surface layer populations, within ice floes, and concentrated near the sea ice/seawater interface. Sea ice represents a permanently cold but temporally and spatially highly variable habitat, rarely lasting longer than a few seasons, with internal temperatures typically ranging from –1 to –15 °C. The salinity of internal sea-ice brine inclusions correlates to the local *in situ* ice temperature and ranges from <10‰ to >150‰ (Eicken & Lange, 1989). The ecological structure and biological rates in sea ice are essentially driven by the prevailing temperature and salinity (Nichols *et al.*, 1995). Nevertheless, sea ice is a biologically dynamic habitat and is in general more productive than the underlying pelagic zone (Dieckmann *et al.*, 1992). Bacterial populations are tightly coupled to primary productivity levels in sea-ice microbial communities (Grossi *et al.*, 1984) and have

been observed heavily colonizing the surfaces of several ice diatom species, particularly those in the process of senescence. Phenotypic and 16S rRNA phylogenetic-based surveys of sea-ice bacteria (Bowman *et al.*, 1997a, b; Delille, 1996; Gosink & Staley, 1995; Irgens *et al.*, 1989) indicate sea ice has a diverse bacterial population dominated by psychrophilic bacteria which appear to be absent from the underlying pelagic zone. In this study, two groups of psychrophilic sea-ice isolates, one pigmented and another coloured pale pink, forming a novel 16S rRNA lineage adjacent to the genus *Alteromonas* (*Proteobacteria*, gamma subclass) were investigated. On the basis of polyphasic taxonomic characterization, the two groups of strains represent two distinct species in a novel genus, *Glaciecola* gen. nov.

METHODS

Isolation and cultivation. The isolation and initial phenotypic analysis of strains investigated in this study has been previously described (Bowman *et al.*, 1997a). In summary,

sea-ice cores were melted in an equal volume of seawater at 2–4 °C to avoid hypotonic shock of halophilic bacteria, diluted to an approximately 1:10 ratio in marine 2216 liquid media at 2 °C and incubated for 1–2 d before being serially diluted onto marine 2216 agar. Plates were then incubated at 2–4 °C for up to 2 months before colonies were subcultured for purification. Colonies were then restreaked on fresh plates for purification of individual strains. The sea-ice isolates were routinely cultivated on marine 2216 agar at 5–10 °C.

Phenotypic characterization. Gram-stain, oxidase and catalase tests were performed as described by Smibert & Krieg (1994). Motility was tested by microscopically examining cell wet mounts. Growth at different temperatures was tested in marine 2216 liquid media in 10 ml screw-cap tubes and incubated in a series of controlled temperature waterbaths set at different temperatures. Seawater requirement and tolerance of various NaCl concentrations (0–12% NaCl) was tested in R2A agar (Oxoid). Hydrolysis of Tween 80, starch, egg-yolk, aesculin and casein was tested as described by Smibert & Krieg (1994) using marine 2216 agar as the basal medium. DNA hydrolysis was tested using DNase test agar (Oxoid) supplemented with artificial seawater. Chitin and alginate hydrolysis was tested on media prepared with artificial seawater (West & Colwell, 1984). Additional biochemical tests were performed using the API 20E and API 32 ID test kits (bioMérieux) which were prepared according to the manufacturer's specifications except that bacterial strains were suspended in chilled artificial sea water. The oxidation/fermentation (O/F) medium of Leifson (1963) was used for testing acid production from carbohydrates. Dissimilatory iron reduction was tested in a medium adapted from Widdel & Bak (1992) and prepared using the Hungate technique (Hungate, 1968). The medium prepared with artificial seawater contained 10 mM amorphous ferric oxide/neutralized ferric chloride or 10 mM soluble ferric oxide/ferric pyrophosphate (Lovely & Phillips, 1986) as the electron acceptors and 10 mM sodium lactate as the electron donor and carbon source. Uninoculated controls lacking the iron electron donor were also prepared. Iron reduction was confirmed by the appearance of black precipitate (magnetite) and by use of the Merckoquant Iron Test (Merck). Anaerobic growth with 10 mM trimethylamine N-oxide (TMAO) was tested in mineral salts medium containing (per litre artificial sea water): 2 g ammonium chloride, 2 mM potassium phosphate buffer pH 7.0, 2 ml SL-10 trace element solution (Overmann & Pfennig, 1989), 1 g yeast extract and 10 ml vitamin solution no. 6 (Staley *et al.*, 1992). The medium pH was adjusted to 7.0 using 1 M KOH and was solidified with 1.3% (w/v) purified agar (Oxoid). Sodium acetate (20 mM) was used as the electron donor and carbon source. Plates were incubated in an anaerobic jar using Anaerogen gaspaks (Oxoid). Growth on the plates was compared with control plates lacking TMAO. Carbon and energy substrate screening utilized 0.1% (w/v) concentrations of test compounds, except carbohydrates, which were tested at a concentration of 0.2% (w/v). The basal medium used was the same as that utilized for the TMAO reduction test shown above. Media lacking a carbon source were prepared as negative controls to take account of background growth.

Numerical taxonomy. Phenotypic data were recorded in a compressed binomial format and analysed using computer software. A matrix of Jaccard similarity values was calculated by using the software package TAXON version 1 (Ross & Shields, 1993). Cluster analysis used unweighted pair-group

averaging, which was calculated using the NEIGHBOR program from the PHYLIP version 3.57c software package (Felsenstein, 1993).

Genotypic analysis. Genomic DNA was extracted and purified from cells grown on marine 2216 agar for 3–7 d at 10 °C (Marmur & Doty, 1962). The DNA G + C content was then determined from thermal denaturation (T_m) profiles (Sly *et al.*, 1986).

The spectrophotometric renaturation rate kinetic procedure as 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: $T_{OR} = 48.5 + (0.41 \times \text{mol \% G + C})$. The decline in absorbance of DNA mixtures and control DNA samples over a 40 min interval was used to calculate DNA hybridization values from the following equation (Huss *et al.*, 1983):

% DNA hybridization =

$$[4AB - A - B/2\sqrt{(A \times B)}] \times 100\%$$

A and B each represent the change in absorbance for two different 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 significant (Huss *et al.*, 1983).

Fatty acid analysis. Representative strains and *Alteromonas macleodii* subsp. *macleodii* NCIMB 1963^T were grown in marine 2216 broth at 15 °C for 1–4 d, 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 & Russell, 1996). The geometry and position of double bonds in monounsaturated fatty acids was confirmed using dimethyldisulfide 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 sea isolates (ACAM 610, ACAM 611^T, ACAM 613 and ACAM 615^T) were obtained in an earlier study of sea-ice bacteria (Bowman *et al.*, 1997b) and have been deposited under GenBank accession nos U85851–U85854. These sequences were compared to the compilation of 16S rRNA genes available in the GenBank nucleotide library using BLAST searching (Altschul *et al.*, 1990) through the US National Institutes of Health internet site and then aligned with related sequences of the gamma subclass (*Proteobacteria*). Subsequent phylogenetic analyses of the sequence data sets 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 Fitch–Margoliash method by using the program FITCH. Bootstrap analysis using 1000 replicates utilized the programs SEQBOOT and CONSENSE. For construction of the phylogenetic tree, the sequence of *Psychrobacter immobilis* was used as the out-group. Sequences used for comparison included (GenBank accession no. in parentheses): *Alteromonas macleodii* subsp. *macleodii* IAM 12920^T (X82145), *Alteromonas macleodii*

subsp. *fijiensis* CNCM I-1627^T (X85174), '*Alteromonas infernus*' CNCM I-1628^T (X85175), '*Arhodomonas oleifer-hydrans*' ATCC 49307^T (M26631), *Chromohalobacter marismortui* ATCC 17056^T (X87210), barotolerant strain CNPT3 (U91588), *Colwellia psychrerythraea* ATCC 27634^T (AF001375), *Ferrimonas balearica* DSM 9799^T (X93021), *Halomonas elongata* ATCC 33173^T (M93355), *Marinobacter hydrocarbonoclasticus* ATCC 49840^T (X67022), *Marinobacterium georgiense* ATCC 700074^T (U58339), *Marinomonas vaga* ATCC 27119^T (X67025), *Microbulbifer hydrolyticus* ATCC 700072^T (U58338), *Oceanospirillum linum* ATCC 11336^T (M22365), *Pseudoalteromonas haloplanktis* ATCC 14393^T (X67024), *Psychrobacter immobilis* ATCC 43116^T (U39399), *Shewanella putrefaciens* ATCC 8071^T (X82133), *Vibrio cholerae* ATCC 14035^T (X74695), and [*Vibrio*] *marinus* NCIMB 1144^T (X82142).

RESULTS

Numerical taxonomic analysis

Polyphasic taxonomic data from this and earlier studies was used to assess the phenotypic diversity and systematics of a group of pink-red- and pale-pink-pigmented, seawater-requiring psychrophilic bacterial strains isolated from sea ice (Table 1). The group was identified as a novel and coherent phylogenetic group in the gamma subclass of the *Proteobacteria* (Bowman *et al.*, 1997b). Numerical taxonomic analysis calcu-

lated on the basis of simple matching coefficients indicated the strains formed a single group sharing a similarity of approximately 80%. In this study, Jaccard similarity coefficients (S_j) were used to better discriminate between strains and thus provide a clearer picture of phenotypic variations amongst the novel strains. Applying this procedure, two distinct phenetic clusters (Fig. 1) with an S_j value of 67% were formed. The first cluster included 15 strains all possessing a bright red-pink pigmentation. The remaining strains possessed a pale pink pigmentation and form a second cluster. By comparison the *A. macleodii* reference strain possessed S_j values of only 45% to the sea-ice strains (Fig. 1). In this study, it is demonstrated that each of these clusters constitutes a species in a novel genus, with the first cluster designated *Glaciecola punicea* and the second cluster called *Glaciecola pallidula*.

Cellular and colony morphology

G. punicea strains possessed colonies which were pigmented pink-red, circular and convex in shape, with lobate edges, a butyrous consistency and 3–5 mm in diameter after 14 d incubation. The colonies of *G. pallidula* were pale pink, circular and convex in shape, entire-edged with a butyrous-mucoid consistency and

Table 1. Antarctic sea-ice isolates investigated in this study

Strains*	Antarctic sea-ice sampling sites
<i>G. punicea</i> gen. nov., sp. nov.	
IC036 (= ACAM 612)	Shirokaya Bay, Long Fjord
IC037	Ellis Fjord
IC056 (= ACAM 609)	Ellis Fjord
IC059 (= ACAM 610)	Taynaya Bay
IC067 ^T (= ACAM 611 ^T)	O'Gorman Rocks, Prydz Bay
IC070	Ellis Fjord
IC074	O'Gorman Rocks, Prydz Bay
IC078	O'Gorman Rocks, Prydz Bay
IC083	Ellis Fjord
IC085 (= ACAM 613)	Shirokaya Bay, Long Fjord
IC173	Ellis Fjord
IC181	Ellis Fjord
IC185	Ellis Fjord
IC186	Ellis Fjord
IC187 (= ACAM 614)	Ellis Fjord
<i>G. pallidula</i> gen. nov., sp. nov.	
IC079 ^T (= ACAM 615 ^T)	Taynaya Bay
IC178	Ellis Fjord
IC182 (= ACM 616)	Ellis Fjord
<i>A. macleodii</i> subsp. <i>macleodii</i>	
NCIMB 1963 ^T	Seawater

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

Fig. 1. Unweighted pair group mean dendrogram based on Jaccard similarity (S_j) values obtained from phenotypic data (Table 2) for *Glaciecola* strains and *A. macleodii* subsp. *macleodii*.

Fig. 2. Phase-contrast photomicrographs of *G. punicea* ACAM 611^T grown in liquid marine 2216 media at 10 °C. Bar, 10 µm.

3–5 mm in diameter after 14 d incubation. All strains were similar in terms of cellular morphology, occurring as motile, slender, slightly curved to curved rod-shaped cells, 1–5 µm in length and 0.2–0.4 µm in width (Fig.

2). Strains of both phenetic groups may also possess a spiral-like morphology or occur as short filaments. These morphological variants occur frequently in stationary-phase cultures.

Table 2. Phenotypic characteristics differentiating *G. punicea* and *G. pallidula*

+, Test positive for all strains; v+, variable results between strains, type strain positive; v-, variable results between strains, type strain negative; -, all strains negative for test; (+), weak and delayed reaction. All strains were positive for the following tests: growth at 0–10 °C; growth on marine 2216 agar, R2A seawater agar and Zobell's agar; production of catalase, cytochrome-*c* oxidase, α -galactosidase and β -galactosidase; acid production (weak and/or delayed) from D-glucose, D-galactose, D-melibiose and glycerol; and utilization of oxaloacetate as a sole carbon and energy source. All strains were negative for the following tests: growth at 25 °C or higher; growth with 3 × and 4 × strength seawater, tolerance to 5% ox bile salts; nitrate as a nitrogen source; nitrate reduction, denitrification and nitrogen fixation; hydrolysis of urea, xanthine, egg yolk, casein, gelatin, chitin, dextran and DNA; production of L-phenylalanine deaminase, L-tryptophan deaminase, arginine dihydrolase, glutamate decarboxylase, lysine decarboxylase, ornithine decarboxylase, α -glucosidase, β -glucosidase, α -fucosidase, α -arabinosidase, β -glucuronidase, β -N-acetylglucosaminidase; indole from L-tryptophan; hydrogen sulfide from thiosulfate; acid production from L-arabinose, D-mannose, D-fructose, L-rhamnose, D-xylose, D-mannitol, N-acetylglucosamine, sucrose, lactose, cellobiose, trehalose, D-raffinose, dextran, adonitol, L-arabitol, D-sorbitol and *m*-inositol; utilization of N-acetylglucosamine, L-arabinose, cellobiose, D-xylose, L-rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, lactose, maltose, D-melibiose, D-raffinose, sucrose, trehalose, starch, chitin, adonitol, L-arabitol, i-erythritol, *m*-inositol, D-mannitol, D-sorbitol, D-gluconate, D-glucuronate, saccharate, propionate, isobutyrate, valerate, isovalerate, caproate, heptanoate, caprylate, nonanoate, malonate, adipate, glutarate, pimelate, azelate, *trans*-aconitate, citrate, 3-hydroxybutyrate, 2-oxoglutarate, L-alanine, L-asparagine, L-aspartate, L-histidine, L-leucine, L-phenylalanine, hydroxy- L-proline, L-ornithine, L-serine, L-threonine, putrescine, γ -aminobutyrate and urate.

Characteristic	<i>G. punicea</i> (n = 15)	<i>G. pallidula</i> (n = 3)
Pigmentation	Pink-red	Pale pink
Yeast extract requirement	v+	—
β -Galactosidase-6-phosphate	+	—
Hydrolysis of:		
Urate	v—	—
Aesculin	v+	—
Starch	—	v+
Tween 80	v—	+
Acid from maltose	—	(+)
Utilization of:		
α -Glycerophosphate	v+	+
Succinate, L-malate, fumarate, L-proline	+	—
Glycerol, glycogen, acetate, pyruvate, DL-lactate, L-glutamate	—	+
L-Tyrosine	v+	—
Butyrate	—	v—
G+C content by T_m (mol%)	44–46	40

Physiology and metabolism

The physiological, biochemical and nutritional properties of the sea-ice strain taxa are shown in Table 2. The sea-ice isolates were strictly aerobic, seawater-requiring chemoheterotrophs. The strains have an absolute requirement for seawater, growing between 0.5 × and 1.5 × seawater. Very poor or no growth occurred when the media was supplemented with only NaCl. The strains were unable to grow anaerobically either by fermentation or anaerobic respiration. Only oxidative acid production from carbohydrates was observed and these reactions were weak and slow,

taking up to 10 d to form on Leifson's O/F medium. In marine 2216 broth, all strains grew between –2 °C and 20 °C, with most *G. punicea* strains also able to grow slowly at 25 °C. None of the strains exhibited growth at 25 °C and at best scanty growth at 20 °C on marine 2216 agar plates.

Enzyme production and nutrition

The hydrolytic capacity of the strains was restricted, with a few strains of *G. punicea* able to slowly hydrolyse aesculin and uric acid whilst some *G. pallidula* strains

Table 3. DNA–DNA hybridization levels between representative *Glaciecola* strains

Species/strain	G + C content by T_m (mol %)	DNA–DNA hybridization (%)*	
		<i>G. punicea</i> ACAM 611 ^T	<i>G. pallidula</i> ACAM 615 ^T
<i>G. punicea</i> ACAM 609	44.8	95	NT
<i>G. punicea</i> ACAM 610	45.6	NT	NT
<i>G. punicea</i> ACAM 611 ^T	43.9	100	18
<i>G. punicea</i> ACAM 612	45.1	85	NT
<i>G. punicea</i> ACAM 613	46.3	82	NT
<i>G. punicea</i> ACAM 614	46.0	91	NT
<i>G. pallidula</i> ACAM 615 ^T	40.3	18	100
<i>G. pallidula</i> ACAM 616	39.5	10	97
<i>A. macleodii</i> subsp. <i>macleodii</i> NCIMB 1973 ^T	42.0	13	10

NT, Not tested.

* Values based on the mean of 3–5 experimental replicates.

Table 4. Whole-cell fatty acid profiles of *Glaciecola* species and related genera

Standard deviations are given in parentheses. ND, Not tested.

Fatty acid	Fatty acid composition (%)		
	<i>G. punicea</i> (n = 4)	<i>G. pallidula</i> (n = 2)	<i>A. macleodii</i> (n = 1)
13:0	ND	0.1 (0.1)	0.4
14:0	1.2 (0.4)	3.6 (0.2)	3.0
15:0	1.0 (0.3)	1.6 (0.1)	3.7
16:0	10.0 (2.2)	9.4 (0.4)	25.0
17:0	0.7 (0.2)	1.3 (0.1)	5.6
18:0	0.4 (0.1)	0.4 (0.1)	1.0
14:1 ω 7c	0.5 (0.2)	3.7 (0.2)	1.1
14:1 ω 5c	ND	0.2 (0.1)	ND
15:1 ω 8c	0.8 (0.1)	3.4 (0.1)	1.3
15:1 ω 6c	0.4 (0.1)	0.5 (0.1)	0.4
16:1 ω 7c	60.7 (0.7)	54.2 (1.9)	34.6
16:1 ω 5c	0.1 (0.1)	ND	ND
17:1 ω 8c	4.6 (0.5)	5.0 (0.3)	8.0
17:1 ω 6c	0.4 (0.1)	0.6 (0.1)	0.3
18:1 ω 11c	ND	ND	0.6
18:1 ω 9c	0.2 (0.1)	0.2 (0.1)	0.3
18:1 ω 7c	16.3 (4.3)	12.6 (0.4)	9.4
19:1	ND	ND	0.2
i13:0	ND	0.3 (0.1)	0.4
i14:0	ND	0.4 (0.1)	0.2
i15:0	0.2 (0.1)	ND	0.1
a15:0	ND	ND	0.5
i16:0	1.8 (0.8)	2.5 (0.3)	2.0
i17:0	0.5 (0.1)	0.1 (0.1)	0.4
a17:0	ND	ND	0.6
i17:1 ω 7c	0.3 (0.1)	ND	ND
Mean	5.3 (0.3)	4.1 (0.4)	1.3
monounsaturated:saturated fatty acid ratio

Fig. 3. Phylogenetic tree constructed on the basis of 16S rRNA sequences showing the relationship of *G. punicea* and *G. pallidula* to other marine chemoheterotrophs of the gamma subclass. The bar corresponds to a 5% difference in nucleotide sequences, as determined by measuring the length of horizontal lines connecting any two organisms. The numbers shown at branch points indicate percentage bootstrap values from 1000 data sets. Only bootstrap values greater than 60% are shown.

were able to hydrolyse starch. However, the strains were strongly catalase- and oxidase-positive, degraded Tween 80 and were able to form the following enzymes, as indicated by API 32 ID test kit results: alkaline phosphatase, α -galactosidase and β -galactosidase. *G. punicea* strains could also form β -galactosidase-6-phosphate. Though most of the strains could use ammonium chloride or ammonium sulfate as a nitrogen source, some strains of *G. punicea* were only able to grow with an organic nitrogen source, which could be supplied in the form of peptone or yeast extract. Vitamin growth factors were not required by any of the strains, but appeared to stimulate growth. The strains were able to utilize a limited number of substrates as sole carbon and energy sources, which were mostly organic acids, especially those related to the tricarboxylic acid cycle. Carbohydrates and alcohols were in general not utilized by the sea-ice strains (Table 2).

DNA base composition and DNA–DNA hybridization

G. punicea strains possessed a DNA G+C content ranging from 44 to 46 mol% (mean 45.1 ± 1.0 , $n = 9$), comparatively higher than *G. pallidula* which possessed a value of about 40 mol% (mean 39.8 ± 0.5 , $n = 3$) (Table 3). *G. punicea* strains ACAM 610, ACAM 611^T,

ACAM 612, ACAM 613 and ACAM 614 shared high DNA–DNA reassociation levels, ranging from 82 to 95% whilst exhibiting only low hybridization levels with *G. pallidula* strains ACAM 615^T and ACAM 616 (levels of 10–19%) (Table 3). *G. pallidula* strains were very similar, showing 97% DNA–DNA hybridization. Neither ACAM 611^T or ACAM 615^T exhibited any significant hybridization to *A. macleodii* subsp. *macleodii* (Table 3).

Fatty acid profiles

Representative strains possessed very similar whole-cell fatty acid profiles and the major constituents included 16:1 ω 7c (52–62%), 18:1 ω 7c (12–21%), 16:0 (9–12%) and 17:1 ω 8c (4–5%) (Table 4). The strains also contained a number of iso-branched-chain fatty acids as minor components; however these are too low in relative concentration to be useful in distinguishing the two species (Table 4). Polyunsaturated fatty acids and hydroxylated fatty acids were either not present or present at only trace levels. The fatty acid profiles, though sharing many of the same fatty acid constituents as *A. macleodii*, were substantially more enriched in monounsaturated fatty acid, as reflected by the high ratio of monounsaturated fatty acid to saturated fatty acid content (Table 4).

Phylogeny

The phylogenetic relationships of four sea-ice strains were examined by distance and maximum-likelihood methods. Strains of *G. punicea* (ACAM 610, ACAM 611^T and ACAM 613) grouped closely together with a similarity ranging from 99.0 to 99.7%. *G. pallidula* ACAM 615^T grouped more distantly at a similarity of 93.4–94.0%; however all four strains grouped monophyletically, supported by the high bootstrap value (92%) at the branch node (Fig. 3). *A. macleodii* subsp. *macleodii*, *A. macleodii* subsp. *fijiensis* and '*A. infernus*' were the closest relatives of the sea-ice strains, with a sequence similarity of 91.9–92.1% with ACAM 611^T and 89.7–91.1% with the *G. punicea* strains. As a result, the sea-ice strains form a distinct branch adjacent to *A. macleodii* within the *Colwellia* assemblage radiation of the gamma subclass (Fig. 3). The *Colwellia* assemblage contains several other genera also isolated from sea ice including species of *Colwellia*, *Pseudoalteromonas* and *Shewanella* (Bowman *et al.*, 1997b).

DISCUSSION

Recent phenotypic and phylogenetic surveys of Antarctic sea-ice bacterial diversity detected the presence of several novel phylogenetic lineages within the gamma subclass of the *Proteobacteria* and within the *Bacteroides-Flavobacterium-Flexibacter* phylum (Bowman *et al.*, 1997b). A lineage adjacent to *Alteromonas* included Gram-negative, motile, slightly halophilic psychrophiles with a rod-like morphology forming distinctive pink-red- or pale-pink-pigmented colonies on marine 2216 agar. The pigmentation made these isolates readily identifiable on sea-ice bacterial isolation media and the isolates were particularly evident from sea-ice samples which possessed well-developed sea-ice diatom assemblages. Preliminary evidence suggests these isolates are restricted entirely to sea-ice habitats as they so far have not been recovered from extensive sampling of under-ice seawater (Bowman *et al.*, 1997a, b). Likewise, they appear to be absent from the water column of Burton Lake, a marine salinity, meromictic water body which has been noted for a substantial enrichment of psychrophilic bacteria within its oxic zone (Franzmann *et al.*, 1990). The sea-ice strains making up this novel group were examined in this study to determine their taxonomic relationships. The novel taxa form two groups as shown by numerical taxonomic analysis (Fig. 1) and 16S rRNA sequence analysis (Fig. 3). Both groups have a relatively restricted biochemical and nutritional versatility (Table 2). *Alteromonas* species, the closest relatives, by comparison have a mesophilic growth temperature range, are significantly more halotolerant, and have a much greater nutritional versatility in terms of carbon substrates utilized and in the production of extracellular enzymes (Baumann *et al.*, 1984; Raguene *et al.*, 1997). The phylogenetic relatedness of the sea-ice strains and *A. macleodii* is supported by the

similar types of components making up their fatty acid profiles, including the presence of branched-chain fatty acids and general lack of hydroxylated fatty acids and polyunsaturated fatty acids. However, the differences in ecophysiology are indicated by the presence of a three- to fourfold higher monounsaturated fatty acid to saturated fatty acid ratio in the sea-ice strains. The capacity of the sea-ice strains to synthesize higher levels of monounsaturated fatty acid may be connected to cold adaptation processes, particularly associated with maintenance of cytoplasmic membrane homeoviscosity (Nichols *et al.*, 1995).

On the basis of ecophysiological, phenotypic and phylogenetic differences, the sea-ice strains are proposed to form a taxonomic entity distinct from the genus *Alteromonas*. The sea-ice strain group can also be distinguished from other marine chemoheterotrophs belonging to the gamma subclass, including species of *Shewanella*, *Colwellia* and *Marinobacter*, which occupy the same sea-ice habitats (Table 5). From these conclusions, we propose that the sea-ice strains constitute a new genus, *Glaciecola* gen. nov. The genus *Glaciecola* contains two distinct species, including *Glaciecola punicea* gen. nov., sp. nov., which includes strains that have the characteristic pink-red pigmentation. The second group is designated *Glaciecola pallidula* gen. nov., sp. nov. and includes the pale-pink-pigmented strains.

Description of *Glaciecola* gen. nov.

Glaciecola (Gla.ci.e'co.la. L. fem. n. *glacies* ice; L. gen. n. *incola* an inhabitant; M.L. fem. n. *Glaciecola* inhabitant of ice).

Cells are slender with a rod-like shape. Cells may be slightly curved, curved or spiral. Gram-negative, motile, non-sporulating, strictly aerobic chemoheterotroph. Psychrophilic and requiring seawater for growth. Slightly halophilic. Oxidase- and catalase-positive. Major fatty acids are hexadecaenoic acid (16:1 ω 7c), hexadecanoic acid (16:0) and octadecaenoic acid (18:1 ω 7c). DNA G+C content is 40–46 mol% (determined from thermal denaturation curves, T_m). Member of the gamma subclass of the *Proteobacteria*. Antarctic sea-ice diatom assemblages are the only known habitat. Type species is *Glaciecola punicea*.

Description of *Glaciecola punicea* sp. nov.

Glaciecola punicea (pu.nice'a. L. fem. adj. *punicea* pink-red, referring to the species pigmentation).

Same as genus description plus the following. Temperature optimum is about 15 °C and temperature maximum is 20–25 °C. Some strains require yeast extract for growth and growth is stimulated by vitamin growth factors. Oxidative acid production from carbohydrates is weak and delayed and may be formed from D-glucose, D-galactose, D-melibiose and glycerol. Some

Table 5. Phenotypic traits differentiating the genus *Glaciecola* from other phylogenetically related marine chemoheterotrophic bacteria of the gamma subclass

+, Trait present; V, varies between species of genus; —, trait absent.

Genus	Known habitats*	Pigmentation	Cellular morphology	Psychrophilic growth	Growth with 10% NaCl	Facultatively anaerobic	PUFA production†	G + C content by T_m (mol%)
<i>Glaciecola</i>	I	Pink-red or pale pink	Curved-straight rods, spiral	+	—	—	—	40–46
<i>Alteromonas</i>	SW	None	Straight rods	—	+	—	—	44–47
<i>Pseudalteromonas</i>	I, SW	None, yellow, orange, red or violet	Straight or curved rods	—	V	—	—	37–45
<i>Ferrimonas</i>	B	Pink-tan	Straight rods	—	—	+	—	50–52
<i>Shewanella</i>	I, SW, B, D, W, F, C	None or pink-tan	Straight/curved rods	V	V	+	V(EPA)	40–54
<i>Colwellia</i>	I, SW, B, D	None	Curved rods‡	+	—	+	+(DHA)	35–46
[<i>Vibrio</i>] <i>marinus</i>	SW, B, D	None	Straight/curved rods	+	—	+	+(DHA)	42

* I, sea ice; SW, seawater (includes marine salinity lakes, decaying seaweed and fish etc.); B, benthic sediments; D, deep-sea sites (including the intestines of deep sea fish); W, freshwater; C, clinical samples; F, food samples.

† PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid (20:5 ω 3); DHA, docosahexaenoic acid (22:6).

‡ May form gas vesicles.

strains slowly degrade uric acid, aesculin or L-tyrosine. The enzymes α -galactosidase, β -galactosidase, β -galactosidase-6-phosphate and alkaline phosphatase are formed. Utilizes the following substrates as carbon and energy sources: succinate, L-malate, fumarate, oxaloacetate and L-proline. Some strains can utilize α -glycerophosphate. Other phenotypic and biochemical test results are shown in Table 2. Colonies have a bright pink-red pigment, are circular and convex in shape, with an entire edge and possessing a butyrous consistency. DNA G + C content is 44–46 mol% (T_m). Isolated from Antarctic sea-ice diatom assemblages. Type strain is ACAM 611^T isolated from a sea-ice core collected from the O'Gorman Rocks area of Prydz Bay, Antarctica.

Description of *Glaciecola pallidula* sp. nov.

Glaciecola pallidula (pal.lid'u.la. L. adj. *pallidula* somewhat pale, referring to the weak pigmentation of the species).

Same as genus description plus the following. The temperature optimum is 10–15 °C and the temperature maximum is 18–20 °C. Nutritionally non-exacting but growth is stimulated by the addition of vitamin growth factors. Oxidative acid production from carbohydrates is weak and delayed and may be formed from D-glucose, D-galactose, maltose, D-melibiose and glycerol. Tween 80 is hydrolysed and the enzymes α -galactosidase, β -galactosidase and alkaline phosphatase are formed. Strains may also slowly degrade starch. Utilizes the following substrates as carbon and energy sources: glycogen, glycerol, α -glycerophosphate, acetate, pyruvate, DL-lactate, oxaloacetate and L-glutamate. Some strains can also utilize butyrate. Other phenotypic and biochemical test results

are shown in Table 2. Colonies are pale pink, circular, raised convex, with entire edges and a butyrous-mucoid consistency. DNA G + C content is 40 mol% (T_m). Isolated from Antarctic sea-ice diatom assemblages. Type strain is ACAM 615^T isolated from sea-ice cores collected from Taynaya Bay, Antarctica.

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