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Biodiversity and ecophysiology of bacteria associated with Antarctic sea ice

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Abstract: A total of 135 bacterial strains were isolated from congelation (land fast) sea ice samples and ice algae biomass samples obtained from the coastal areas of the Vestfold Hills in East Antarctica (68°S, 78°E) during the summers of 1992–95. The sea ice isolates, along with reference strains, were analysed by numerical taxonomy and for DNA base composition in order to determine the biodiversity of sea ice bacteria. From these analyses 22 clusters of strains (phena) were obtained with most phena apparently representing novel bacterial taxa. The sea ice isolates could be categorized into three groups based on their ecophysiology: 1) slightly halophilic, psychrophilic bacteria often possessing fastidious growth requirements and which were predominantly isolated from sea ice algae biomass or from algae-rich ice samples; 2) halotolerant and psychrotolerant bacteria; and 3) non-halophilic bacteria isolated primarily from upper sections of congelation ice and other ice samples with low levels of algal biomass.

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Introduction

Sea ice provides an extensive but transient habitat for a wide variety of organisms, including diatoms, flagellates, protozoa, and bacteria. Bacteria have been found throughout annual sea ice, including the brine pockets and channels located within sea ice as well as at the sea ice–sea water interface in association with phytoplankton blooms. The activity and productivity of sea ice bacteria and their relationship to sea ice diatoms has been investigated in several studies (Palmisano & Garrison 1993, Grossmann & Dieckmann 1994, Helmke & Weyland 1995). Sea ice bacterial communties have been shown to be strongly vertically stratified and the highest populations correspond with high algal-derived chlorophyll *a* levels and are enriched in sea ice relative to the underlying seawater (Kottmeier *et al.* 1987, Kottmeier & Sullivan 1990). Bacteria forming holdfasts of polysaccharide either directly from cell surfaces or via prostheca and stalks have been demonstrated colonizing diatom surfaces (McConville & Wetherbee 1983, Sullivan & Palmisano 1984). Recent studies have shown a close correlation between bacterial colonization patterns with the genetic classes of sea ice (Grossmann & Dieckmann 1994, Helmke & Weyland 1995). Bacterial populations have been found to be highest in thick annual pack ice with psychrophilic bacteria being particularly common in samples of brown ice and ice porewaters (Delille 1992) versus psychrotolerant bacteria which predominate in grease and pancake sea ice samples and in the underlying sea water. Psychrophilic bacteria have optimal growth temperatures of less than 20°C and will not grow above 25°C (Morita 1975). Psychrotolerant bacteria are able to grow at 5°C and less; however they have optimal growth temperatures above 20°C. Delille (1993) has suggested that there appears to be little exchange of bacteria from sea ice to the underlying seawater which in turn suggests older consolidated sea ice contains bacterial populations which may specifically form in sea ice and nowhere else.

Annual and multi-year ice offers an environment which is selective for psychrophilic bacteria, but exactly why this occurs is still unknown. With better knowledge of the physiology and nutritional requirements of sea ice bacteria, their ecology can be better understood. This study uses extensive phenotypic characterization of bacteria isolated from congelated, platelet, and grease ice samples to determine which types of bacteria are found colonizing sea ice and what ecophysiological strategies they employ to adapt to the sea ice environment.

Materials and methods

Sea ice sampling

Sea ice cores were collected from sites along the coastal areas of the Vestfold Hills ice free zone in Prydz Bay, Eastern Antarctica (Fig. 1) during the summers of 1992–95. Ice samples were also collected from Ace Lake, Burton Lake and an anonymous lake (all of which have salinities close to seawater). A SIPRE corer (7 cm diameter) was used to collect 15–80 cm long ice cores from mid-way through the ice sheet or ice cover, which averaged 2 m in thickness at most sites, to the ice–water interface (Table I). Particular care was taken not to disturb the platelet ice layer present at many sites. A number of ice cores (ice core samples 1, 2, 4, and 10) were

divided into upper and lower sections based on a discontinuity in the temperature profile of the ice. Algal biomass was assessed by visually examining the discoloration of sea ice cores giving only qualitative estimates.

Isolation of sea ice bacteria

Sea ice cores were melted at 4°C on the day of collection, in 2 l of filtered, sterile sea water. Melted sea ice was then serially diluted in seawater and plated onto marine 2216 medium (Difco Laboratories, Detroit, MI, USA) containing 5 g bacto-peptone, 2 g yeast extract and 10 mg ferric phosphate in 1 l of artifical seawater and solidified with 1.5% (w/v) agar. Duplicate plates were incubated at 25°C and 4°C for up to 2 months. Individual colonies with differing morphologies were selected from the isolation plates in order to obtain maximal bacterial biodiversity.

Phenotypic characterization

All isolates including reference strains (Table II) were routinely cultivated on marine agar at 10°C. Gram stain, oxidase, and catalase tests were performed as described by Smibert & Krieg (1994). Motility was tested by microscopically examining wet mounts of strains prepared from 3–7 day old cultures. Tolerance to various seawater concentrations (from 0–600%), 5% (w/v) bile salts, vibriostat agent 0/129 (100 μg ml^{-1}), and ampicillin (20 μg ml^{-1}) was tested

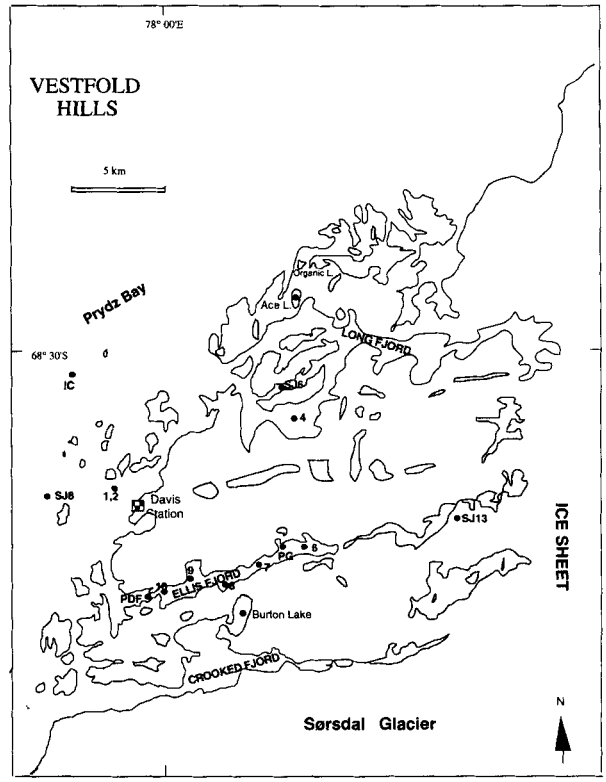

Fig. 1. Diagrammatic map of the Vestholds Hills ice free zone showing location of sea ice core sampling sites.

Table I. Description of sea ice samples utilized in this study.

Ice core no.	Location	Depth[a]	Characteristics of ice cores	No. of taxa[b] isolated per ice sample psychrophilic	psychrotrophic
1B	O'Gorman Rocks	0–23 cm	platelet layer 0–4 cm, algae 1.5–4 cm	3	1
1T	O'Gorman Rocks	23–44 cm	algae not visible	0	2
2B	O'Gorman Rocks	0–25 cm	platelet layer 0–1.5 cm, algae 0–2 cm	4	2
2T	O'Gorman Rocks	25–50 cm	algae not visible	1	1
4B	Shirokaya Bay	0–31 cm	algae 0–1.5 cm	5	2
4T	Shirokaya Bay	31–46 cm	algae not visible	1	1
6	upper Ellis Fjord	0–34.5 cm	platelet layer 0–2.5 cm, algae 0–3.5 cm	1	1
7	mid Ellis Fjord	0–15 cm	platelet and algae layer 0–3.5 cm	2	1
8	mid Ellis Fjord	0–42 cm	platelet and algae layer 0–2.0 cm	5	2
9	lower Ellis Fjord	0–43.5 cm	platelet and algae layer 0–2.5 cm	3	0
10B[c]	lower Ellis Fjord	0–25 cm	platelet and algae layer 0–2.5 cm	2	0
PG	lower Ellis Fjord	nd[d]	platelet and algae layer present	1	1
12[e]	Burton Lake	40–80 cm	algae not visible	4	2
LF	Long Fjord	nd	algae not visible	0	1
SJ6[e]	Long Fjord	30–60 cm	limited presence of algae	1	9
SJ13[e]	anonymous lake	40–70 cm	algae not visible	0	3
SJ8	ice edge	0–70 cm	algae not visible	0	6
IC	ice edge	nd	algae not visible	0	1
PDF	upper Ellis Fjord	–	grease ice; algae not visible	0	3
	Amery Ice Shelf	–	anchor ice layer of 350 m deep core	1	1

[a]Distance from ice/water interface.
[b]Taxa represent phena defined by the numerical taxonomic analysis (Fig. 2) and do not represent individual strains.
[c]Bottom section of 47 cm ice core.
[d]nd, no data.
[e]Mid sections of sea ice cores.

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in marine agar. Hydrolysis of uric acid and xanthine was tested by observations of clearing zones on media supplemented with 1% (w/v) concentrations of these compounds. Starch hydrolysis test plates were prepared similarly with hydrolysis activity detected by flooding plates with a 1% iodine solution. Tween 40, tween 60, tween 80, egg-yolk, esculin, and casein hydrolysis and alkaline phosphatase activity was tested as described by Smibert & Krieg (1994). DNA hydrolysis was tested using DNase test agar. Chitin hydrolysis was tested according to the procedure of West & Colwell (1984). Acid production from carbohydrates followed the procedure of Leifson (1963). Additional biochemical tests were performed using the API 20E test kit (BioMerieux, Lyon, France) which was prepared according to the manufacturer's specifications except bacterial strains were suspended in chilled artificial sea water. Fermentation of carbohydrates was tested in Leifson's oxidation/fermentation medium supplemented with 0.2% (w/v) cysteine hydrochloride in serum vials. The media were prepared in a semi-solid form with 0.3% (w/v) agar, sparged with nitrogen and sealed with gas-tight butyl rubber seals and aluminium crimps prior to sterilization. Inoculation of media was via syringe. Controls lacking carbohydrates were also prepared.

Carbon and energy source screening utilized 0.1% (w/v) concentrations of the test compounds (except carbohydrates which were tested at a concentration of 0.2%), in a mineral salts medium containing (per litre of artificial sea water): 2 g ammonium chloride, 2 mM phosphate buffer (pH 7.0), 0.1 g of yeast extract, 2 ml of SL-10 trace element solution and 10 ml of vitamin solution no. 6 (Staley *et al.* 1991). The media were adjusted to pH 7.0 using 1 M KOH and solidified with 1.3% (w/v) purified agar (Oxoid Ltd., Basingstoke, UK). Inoculated media lacking carbon source were used as negative controls to account for background growth.

Numerical taxonomic analysis

Phenotypic data were converted into a binary format and analysed using the Taxon v. 1.0 program (Ross & Shields 1993). Simple matching similarity coefficients were utilized to generate the similarity matrix. The dendrogram was generated from the similarity matrix with the unweighted pair group mean averaging (UPGMA) procedure using the program NEIGHBOR from the software package PHYLIP v. 3.57c (phylogenetic inference program) (Felsenstein 1993).

Table II. Reference strains used in this study.

Phenon[a]	Name	Strain[b]	Mol% G+C	Source
2	*"Flectobacillus glomeratus"*	ACAM 171 ^{T}	32.0	Burton L. pycnocline
4	*Micrococcus*-like	ACAM 306	58.1	ornithogenic soil
6	marine *Cytophaga* sp.	ACAM 123	32.4	Burton L. pycnocline
6	marine *Cytophaga* sp.	ACAM 140	35.9	Burton L. pycnocline
6	marine *Cytophaga* sp.	ACAM 167	29.5	Burton L. pycnocline
6	marine *Cytophaga* sp.	ACAM 181	30.5	Burton L. pycnocline
6	marine *Cytophaga* sp.	ACAM 188	29.0	Burton L. pycnocline
6	marine *Cytophaga* sp.	ACAM 210	35.4	Burton L. pycnocline
8	*Psychrobacter* sp.	ACAM 483	43.6	Amery Ice Shelf
10	*Psychrobacter immobilis*	ACAM 521 ^{T}	44.7	poultry carcass
11	*Shewanella* sp.	ACAM 122	ND	Burton L. pycnocline
14	*Micrococcus*-like	ACAM 303	66.5	ornithogenic soil
15	*Micrococcus*-like	ACAM 307	66.5	ornithogenic soil
22	*Pseudomonas* sp.	ACAM 120	ND	Burton L. pycnocline
22	*Pseudomonas* sp.	ACAM 147	ND	Burton L. pycnocline
22	*Pseudomonas* sp.	ACAM 213	63.5	Burton L. pycnocline
–	*Cytophaga lytica*	ACAM 57 ^{T}	33.1	marine mud
–	*Cytophaga marinoflava*	ACAM 59 ^{T}	36.5	sea water
–	*"Cytophaga xantha"*	ACAM 75 ^{T}	35.9	pond mud
–	*"Flavobacterium gondwanense"*	ACAM 48 ^{T}	35.7	Organic L.
–	*"Flavobacterium salegens"*	ACAM 44 ^{T}	37.4	Organic L.
–	*Halomonas subglaciescola*	ACAM 12 ^{T}	61.7	Organic L.
–	*Halomonas meridiana*	ACAM 246 ^{T}	59.0	Organic L.
–	*"Vibrio marinus"*	ATCC 33508 ^{T}	49.3	sea water
–	*Pseudoalteromonas nigrefaciens*	ACAM 545 ^{T}	42.9	butter
–	*Pseudoalteromonas undina*	ACAM 546 ^{T}	43.6	seawater
–	*Psychrobacter urativorans*	ACAM 534 ^{T}	43.4	pork sausage
–	*Psychrobacter frigidicola*	ACAM 304 ^{T}	41.2	ornithogenic soil
–	*Shewanella hanedai*	ACAM 585 ^{T}	45.8	Arctic Ocean sediment
–	*Shewanella putrefaciens*	ATCC 8071 ^{T}	46.0	butter

[a]Refer to Table III for phenotypic characteristics for phena; –, unclustered in dendrogram.

[b]Abbreviations: ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart; ATCC, American Type Culture Collection, Rockville, Maryland;

^{T}, type strain of species.

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DNA base composition analysis

DNA was extracted from sea isolates and reference strains using a modification of the Marmur procedure (Sly *et al.* 1986). Gram positive strains were pretreated with lysozyme at 1 mg ml^{-1} for 24 h at 25°C. DNA base composition (mol% G+C values) were determined from thermal denaturation curves using spectrophotometry (Sly *et al.* 1986).

Results

Isolation

A total of 135 strains isolated from sea ice samples (Table I) were examined in this study. In most cases direct spread plates of sea ice samples were rapidly overgrown by non-pigmented strains which grew particularly rapidly at 2–10°C. Pigmented colonies usually appeared only after several weeks of incubation. A higher diversity of colonies was found to be present in most ice samples containing significant algal biomass. The diversity of colony morphology was correspondingly reduced for sections of ice with low or negligible algal biomass. A similar result was found for the grease ice from Ellis Fjord and for the anchor ice from the Amery Ice Shelf. Psychrophilic bacteria were predominantly isolated from sea ice containing significant levels of algal biomass associated with the platelet ice layer, with psychrophilic taxa outnumbering psychrotolerant taxa by about 3:1. Further from the water/ice interface the probability of isolating psychrotolerant taxa increased with only a small proportion of bacterial taxa isolated found to be psychrophilic (Table I).

Phenotypic characterization

Of the 135 sea ice and algae-derived isolates, 52 strains possessed optimal growth temperatures at 15°C or below. Most of the remaining strains grew optimally between 20–30°C while no strains were able to grow at 37°C. Gram negative bacteria accounted for 79% of the strains. All of the psychrophiles isolated were gram-negative except for two gram positive strains. About 23% of the gram negative strains were able to grow fermentatively. Gram positive strains were for the most part brightly pigmented, psychrotolerant, and possessed an oxidative metabolism. Numerical taxonomic analysis grouped the sea ice strains and reference strains into 22 phena at a similarity level of 90% through the usage of simple matching similarity coefficients and the UPGMA procedure (Fig. 2). A total of 15 sea ice isolates were unclustered in this analysis (data not shown). Summarized phenotypic data is shown in Table III. Detailed results and strain data can be obtained directly from the authors.

Phenetic groups and identification

Phena 1 through to 7, except phenon 4, included psychrophilic gram negative bacteria which have an absolute requirement for seawater for growth.

Phenon 1 and 7 include strains with growth optima of 10–15°C and which were non-pigmented, rod-like or spiral in morphology, motile, and facultatively anaerobic. The presence of a strong chitinolytic activity, expressed aerobically and anaerobically, was a striking characteristic of phenon 1 and most strains did not utilize any other carbohydrate tested except for N-acetylglucosamine. Phenon 7 strains were similar to phenon 1 strains except they were somewhat more versatile in their metabolic range. No reference strains grouped with either phenon. The DNA base composition values of the strains in phenon 1 range from 32–38% except for two rod-shaped strains (ACAM 456 and ACAM 459) which have a G+C content of 47–48%. The latter strains appear most similar to the genus *Shewanella*. The remaining spiral-shaped strains of phenon 1 form a coherent cluster with a G+C content below the range found for other psychrophilic and psychrotolerant, marine, facultatively anaerobic bacteria such as *Vibrio* and *Colwellia* (mol% G+C range of 39–52). They thus appear to represent a novel bacterial taxon. Phenon 7 strains have a G+C content of 40–41 and appear similar to either the genus *Vibrio* or *Colwellia*.

Phenon 2 and 3 included strains with growth optima of 5–10°C, with a rod-like to filamentous morphology with some strains forming filaments over 50μm long. Isolates were also coiled, were non-motile and possessed a strictly oxidative metabolism. The strains have a very restricted metabolic versatility and required vitamins and yeast extract for growth. The strains in phenon 2 were pigmented bright orange while phenon 3 strains possessed a characteristic pink to purple pigment. Phenon 2 contained the reference strain, "*Flectobacillus glomeratus*" ACAM 171^T (T = type strain), originally isolated from Burton Lake, at a similarity of 92–93% (SSM) but no reference cultures grouped into phenon 3. The strains in phenon 2 most resemble orange-pigmented, gas-vacuolated, filamentous bacteria isolated from sea ice collected from McMurdo Sound (Irgens *et al.* 1989, Gosink & Staley 1995). The mol% G+C content range of phenon 3 was from 39–46%, with two strains possessing values of 39–40% versus 45–46% for the remaining isolates suggesting the phenon contains at least two distinct species.

The two strains in phenon 5 possessed gliding motility, were yellow, grew optimally at about 15°C, were moderately saccharolytic with the unusual capacity to produce dextranase, and were strictly oxidative. No reference strains were clustered into this phenon. Phenotypic comparison and DNA base composition suggests these strains are most similar to the genus *Flexibacter*, though unlike any of the recognized species of this genus as they currently stand.

Phenon 6 contained 16 strains which grew best at around

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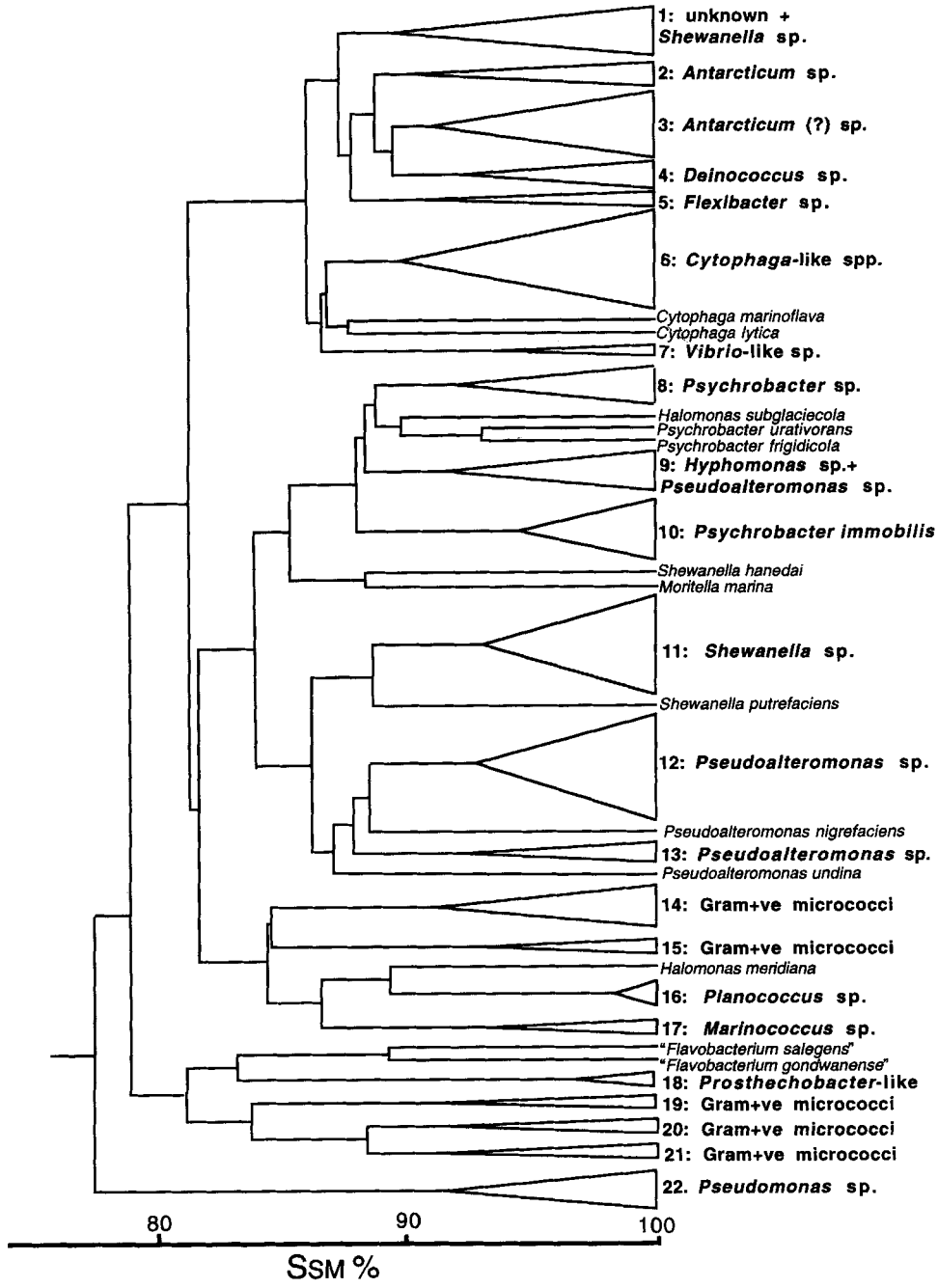

Fig. 2. Simplified dendrogram of sea ice and other isolates and reference strains clustered by the UPGMA method of association from simple matching coefficients. Numbers designate phena as shown in Table II & III.

10°C, possessed yellow or orange pigments. The strains were either non-motile or able to move by gliding and appeared as rods to filaments which did not coil. The strains in general had a relatively restricted metabolic range, possessed an oxidative metabolism, and required vitamins and/or yeast extract for growth. Several reference strains were clustered in this phenon and included strains previously isolated from seawater taken at the pycnocline of Burton Lake at depths of 10–12 m (ACAM 123, ACAM 140, ACAM 167, ACAM 181, ACAM 188, ACAM 210). Though they lack the ability to glide these strains appear most similar to marine species of the genus *Cytophaga*.

Phena 8 to 13 included a diverse set of psychrotolerant, gram-negative strains many of which were strictly oxidative and halotolerant.

Phena 8 and 10 contained non-pigmented, coccoidal, non-motile strains with a strictly oxidative metabolism. The strains also exhibited moderate halotolerance and in most cases strains could grow well on triple and quadruple-strength seawater amended media. The growth of strains belonging to phenon 8 were stimulated by seawater and grew optimally at *c.* 15°C. Strains belonging to phenon 10 were not stimulated by sodium ions, could produce acid from carbohydrates and grew optimally at 27–30°C. The reference strain *Psychrobacter* sp. ACAM 483, isolated from anchor

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Table III. Summarized phenotypic data for sea ice bacterial phenotypic groups.

Phenon No.	1	2	3	4	5	6	7	8	9	10	11
no. of strains	8	4	11	4	2	16	2	5	6	11	16
Gram Stain[a]	–	–	–	+	–	–	–	–	–	–	–
Cell shape[b]	S,R	R,F	R,F	C,R	R,F	R,F	R,V	C	R	C	R,V
Budding /appendages	–	–	–	–	–	–	–	–	±	–	–
Motility[c]	F	–	–	–	G	–/G	F	–	F	–	F
Colony pigmentation[d]	–	O	P–R	R	Y	Y,O	–	–	–	–	P
Requires for growth[e]	s	vys	vs	–	vsy	vsy	s	s	s	–	–
Psychrophilic	+	+	+	–	+	+	+	+	–	–	–
Psychrotolerant	–	–	–	+	–	–	–	–	+	+	+
Slight halophile	+	+	+	–	+	+	+	–	+	–	–
Halotolerant	–	–	–	+	–	–	–	+	–	+	–
Non-halophilic	–	–	–	–	–	–	–	–	–	–	+
Oxidative	+	+	–	–	+	±	+	–	–	+	+
Fermentative	+	–	–	–	–	–	+	–	–	–	+
% of substrates utilized	11	9	9	8	26	29	29	38	34	31	34
mol% G+C range	32–48	32–35	39–46	58–60	38–39	28–38	40–41	42–44	46–58	43–47	39–43

Phenon No.	12	13	14	15	16	17	18	19	20	21	22
no. of strains	17	3	7	3	4	2	2	2	2	2	6
Gram Stain	–	–	+	+	+	+	–	+	+	+	–
Cell shape	R	R	C	C	C	R	R,V	C	C	C	R
Budding/appendages	–	–	–	–	–	–	+	–	–	–	–
Motility	F	F	–	–	–	F	F	–	–	–	F
Colony pigmentation	–	–	Y	O	O	O	P	Y	Y	–	(F)
Requires for growth	s	s	–	s	s	ys	ys	–	–	–	–
Psychrophilic	–	–	–	–	–	+	+	–	–	–	–
Psychrotolerant	+	+	+	+	+	–	–	–	+	–	–
Slight halophile	–	–	–	+	–	–	+	–	–	–	–
Halotolerant	+	+	–	–	+	+	–	–	+	–	–
Non-halophilic	–	–	+	–	–	–	–	+	–	+	+
Oxidative	+	+	+	+	+	+	+	+	+	+	+
Fermentative	–	–	–	–	–	–	–	–	–	–	–
% of substrates utilized	49	54	54	42	68	55	52	40	89	69	91
mol% G+C range	39–42	38–39	44–69	66–67	47–48	47	35–36	75	63–68	56–65	63–65

[a]+ all strains positive, – all strains negative.
[b]Cell shape (abbreviations): R, rod; S, spiral; F, filamentous; C, cocci/spherical; V, vibrioid/curved.
[c]Motility (abbreviations): F, motile via flagella; G, motile via gliding.
[d]Colonial pigmentation: –, non-pigmented; O, orange; P, pink; R, red; Y, yellow; (F), fluorescent pigments formed on some media.
[e]Growth requirements (abbreviations): v, vitamins; y, yeast extract, s, seawater.

ice of the Amery Ice Shelf, grouped with phenon 8 while *Psychrobacter immobilis* ACAM 521^T grouped with phenon 10.

Phena 9, 12, and 13 contained strains requiring seawater for growth and were in general quite halotolerant. The strains were non-pigmented, rod-shaped, motile, and strictly oxidative. The optimal temperature for growth was27–30°C. The differences between the phena were primarily in their carbon source utilization spectra, with all strains being saccharolytic and relatively versatile. No reference strains grouped with these phena except *Pseudoalteromonas nigrefaciens* which clustered at the periphery of phenon 12 (Fig. 2). The strains in these phena appear to be most similar to the genus *Pseudoalteromonas* and for a few strains this has been confirmed by 16S rDNA sequence analysis (Brown 1995). The high halotolerance and carbon source-utilization spectra of strains in these phena are different from currently recognized *Pseudoalteromonas* species. Two strains in phenon 9 were found by microscopy to form hyphae and divide by budding division. The characteristic morphology of these strains and DNA base composition values of 56–58% suggest a relationship to the marine genus *Hyphomonas*.

Phenon 11 included motile, rod-shaped strains which grew optimally at *c.* 22–25°C and possessed a pink to tan pigmentation. The strains were fermentative, but not chitinolytic, were nutritionally versatile and were strongly lipolytic and proteolytic. The strains were able to produce hydrogen sulphide from cysteine and were in most cases able to denitrify. All could grow anaerobically with nitrate, trimethylamine N-oxide, ferric pyrophospate and ferric citrate as electron acceptors. The reference strain *Shewanella* sp. ACAM 122, isolated from Burton Lake grouped within this phenon.

Phenon 18 included two strains which produced a salmon to pink pigment, stalk-like and hyphal structures, and reproduced by budding division. The prosthecate forms were non-motile

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and no motile daughter cells were observed. These moderately versatile strains required seawater and yeast extract for growth and grew optimally at 15–20°C. No reference strains grouped with this phenon. The morphology of these strains most resembled members of the genus *Prosthecobacter*, however their G+C values were found to be 35–36% compared to values of 55–70% found for appendaged bacteria (including the genus *Prosthecobacter*) so far known (Staley & Fuerst 1989).

Phenon 22 included six non-halophilic strains which grew optimally between 20–25°C, formed green fluorescent pigments on certain defined media and which were not halotolerant. The strains were motile, rod-shaped, formed arginine dihydrolase, and could denitrify. The strains were also the most versatile of all the bacterial strains isolated. The phenon included three reference strains (ACAM 120, ACAM 147, ACAM 213) which were isolated from the Burton Lake pycnocline. The strains appeared most similar to the genus *Pseudomonas* confirmed by the presence of fluorescent pigments and G+C contents of 63–65%.

Phenon 4, phena 14–17 and phena 19–21 included gram positive strains which are psychrotolerant (except phenon 17), non-sporulating, and strictly oxidative.

Phenon 4 comprised four red-pigmented, gram positive strains, with optimal growth temperatures of 22–25°C. The strains were moderately halotolerant but grew in the absence of seawater. The lack of metabolic versatility was the primary reason why these strains grouped in the vicinity of the halophilic, psychrophilic gram negative strains (phena 1–8). The strains in this phenon grouped with reference strain ACAM 306, isolated from ornithogenic soil. The strains in these phena show the greatest similarity to the radiation-resistant genus *Deinococcus.*

Phena 14 and 19–21 included yellow, coccoidal, gram positive strains were non-halophilic showing only limited salt tolerance and capable of growing in the absence of NaCl. The strains had a fairly broad nutritional versatility but overall were quite variable resulting in the formation of four phena with only limited similarity. A reference strain, ACAM 303, isolated from ornithogenic soil clustered with phenon 14, but no reference strains clustered with phena 19, 20, or 21. The strains in these phena showed the greatest similarity to gram-positive genera *Arthrobacter, Micrococcus*, and *Luteococcus.*

Phena 15 and 16 included orange-pigmented, coccoidal, gram positive strains which required seawater for growth and which also had considerable halotolerance but otherwise possessed a fairly broad nutritional versatility. A reference strain, ACAM 307, isolated from ornithogenic soil clustered with phenon 15, but no reference strains clustered with phenon 16. The strains in these phena showed greatest resemblance to the genera *Marinococcus* or *Planococcus.*

Phenon 17 included motile, rod-shaped, pale orange-pigmented strains which required seawater and yeast extract for growth and had a growth optima of *c.* 15°C. This phenon appeared most similar to the genus *Marinococcus.*

Discussion

In this study sea ice isolates could be grouped on the basis of their ecophysiology as psychrophilic halophiles, pychrotolerant and halotolerant bacteria, and non-halophilic, psychrotolerant bacteria. All groups included gram negative and gram positive members as well as strains which were either oxidative or facultatively anaerobic.

Psychrophilic sea ice strains

Most of the psychrophilic strains except for the *Psychrobacter* strains of phenon 8, were slight halophiles with an obligate requirement for seawater. The sea ice psychrophiles were generally nutritionally fastidious (phena 1 and 7 being exceptions) and were found to grow over a relatively narrow range of salinities. They required a number of divalent cations for growth, e.g. magnesium and calcium, in addition to sodium ions. The isolates utilized only organic compounds as sources of nitrogen. Perhaps not coincidentally most of the strains were isolated from sea ice containing high algal biomass and in some cases directly from ice algae. Reference strains falling in with these strains were also often isolated directly from algal material. Moreover the diversity of psychrophilic taxa was considerably higher in the algae rich ice samples compared with ice samples essentially lacking algal biomass (Table I). Delille (1992) demonstrated earlier that psychrophiles made up more than 90% of the bacterial population in brown ice and ice porewater samples collected from the Wedell Sea ice edge and that a high proportion of the isolates were phenotypically different to what was found in underlying seawater. In addition other studies have shown sea ice bacteria were to a greater extent particle associated and had a larger biovolume compared to those found in open seawater (Grossi *et al.* 1984, Sullivan & Palmisano 1984, Kottmeier *et al.* 1987, Palmisano & Garrison 1993). The high level of algal activity and lysed algae material would probably supply these bacteria with the factors required for cell division. Epiphytic bacteria associated with sea ice algae have been shown to have enhanced growth rates compared with the growth rate typical of free-living bacteria in sea ice (Grossi *et al.* 1984). The *Prosthecobacter*-like strains of phenon 18 have a close resemblance to some of the unusual morphological forms observed colonizing the surface of the diatom *Entomoneis kjellmanii* (Sullivan & Palmisano 1984). The ability of strains belonging to phena 1 and 7 to degrade chitin suggests they may have a significant role in mineralization processes within the ice with infiltration of sea ice by ephausiids, amphipods, and copepods supplying the source of chitin to these bacteria. Helmke & Weyland (1995) observed higher viable bacterial counts from sea ice samples on chitin agar compared with standard marine agar. This indicates chitinolytic bacteria may actually dominate the sea ice bacterial community in some areas and thus chitin levels may represent a contributory factor leading to

enrichment of psychrophilic bacteria in annual sea ice.

Sea ice derived psychrophilic strains including the several unclustered strains represent up to 18 novel bacterial taxa. There is evidence of a higher degree of diversity in phena 1, 2, 3, and 6 on the basis of whole cell fatty acid profiles (Brown 1996) and DNA base composition values. The oxidative psychrophilic bacteria appear to be related mostly to members of the *Flavobacterium-Cytophaga-Bacteroides* group and appear to be novel members of *Cytophaga* and *Flexibacter*. The systematic status of the latter two genera is highly questionable at the moment, but it is clear the sea ice strains are distinct from any known taxa in these genera including invalid species such as "*Cytophaga xantha*" isolated from an Antarctic mud pool and the apparently misclassified "*Flectobacillus glomeratus*". Phenon 8 represents a novel *Psychrobacter* species distinct from *Psychrobacter immobilis* and two other recently described *Psychrobacter* species from ornithogenic soils (Bowman *et al.* 1996). Fermentative psychrophilic bacteria isolated from sea ice (phena 1 and 7, and some unclustered strains) are *Vibrio*-like with some strains showing relationship to the genera *Colwellia* and *Shewanella*. Gosink & Staley (1995) recently reported gas vacuolated strains related to *Colwellia psychroerythrus* from sea ice environments. The *Vibrio*-like strains found to make up a large proportion of the psychrophiles observed in brown ice by Delille (1992) could be similar to strains in phena 1 and 7. Some members of phena 1 and 2 have been found to have significant levels of polyunsaturated fatty acids (Nichols *et al.* 1995 and unpublished data).

Halotolerant strains

A high proportion of halotolerant or "haloversatile" strains reported from saline Vestfold Hill lakes (James *et al.* 1994) have been shown to belong to the genera *Halomonas* and *Halorubrum* (an archaean), as well as the *Cytophaga*-like bacteria, "*Flavobacterium salegens*" and "*Flavobacterium gondwanense*". These bacterial groups were absent from sea ice samples examined in this study. Instead halotolerant strains included gram-positive cocci, *Psychrobacter* spp. and *Pseudoalteromonas* spp. Most of these groups are likely to be novel on the bases of phenotypic comparisons with known taxa. *Pseudoalteromonas* species are extremely common in seawater globally with most species being psychrotolerant. They probably make up the bulk of the non-fermentative, motile, gram negative, *Pseudomonas*-like bacteria observed in sea water underlying sea ice (Franzmann *et al.* 1990, Delille 1993, Zdanowsky & Donachie 1993). *Psychrobacter* (often confused with the genera *Moraxella* or *Acinetobacter*) have been found globally in both marine and terrestrial environments and have been found to predominate in Antarctic ornithogenic soils (Bowman *et al.* 1996). Gram positive bacteria, which for the most part are halotolerant regardless of isolation site, seem to be ubiquitous in Antarctic environments.

Non-halophilic strains

Several strains were isolated from sea ice which were able to grow well on marine agar but did not require sea water for growth and which had only limited halotolerance, e.g. phena 12, 14 and 22. All of these strains were psychrotolerant. The major taxa represented by this ecophysiological group included *Shewanella*, fluorescent pseudomonads, and a number of gram-positive cocci related phenotypically to the genus *Micrococcus* and its relatives *Kocuria* and *Luteococcus*. Many of the non-halophilic strains, possessed lipolytic and proteolytic enzymes suggesting a role in heterotrophic cycling of decaying organic material in the sea ice. The gram positive cocci isolated in this study have been previously frequently isolated from ornithogenic soils and from the water column, reinforcing the ubiquitous distribution of these bacteria.

Conclusions

A variety of taxa were observed in sea ice samples which have not been generally observed in open sea water samples (Delille 1993, Zdanowski & Donachie 1993). Psychrophilic taxa were highly represented in this group including those from phena 1 to 3 and 5 to 8. In some cases algae and marine snow may provide alternative habitats for some of these taxa as seen for representatives of phenon 6 in which 11 strains were isolated directly from algal material or from the pycnocline of Burton Lake. More than one-third of taxa isolated from Burton Lake were found to be psychrophilic in an earlier study by Franzmann *et al.* (1990), however the limited data used for identification in their study makes it difficult to draw comparisons with taxa described in this study. *Psychrobacter immobilis* (phenon 10), *Pseudoalteromonas* (phenon 9, 12 and 13), *Pseudomonas* sp. (phenon 22), and most of the gram positive taxa have been isolated from Antarctic marine and terrestrial sites (Vishniac 1993). It will remain difficult to prove a bacterial species specificially colonizes sea ice until a comprehensive biodiversity survey of seawater underlying sea ice is performed. Further studies are also necessary to pinpoint specialized bacterial groups, especially chemoautotrophs, which may have roles in geochemical and mineralization processes in sea ice. Additionally the determination of which particular bacterial species numerically dominates a sea ice microbial community may provide further understanding of adaptation of bacteria to sympagic environments. Overall sea ice is a rich source of novel bacterial biodiversity, in particular psychrophilic bacteria which are most populous and diverse in sea ice algae assemblages.

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