

## *Psychromonas ingrahamii* sp. nov., a novel gas vacuolate, psychrophilic bacterium isolated from Arctic polar sea ice

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A gas vacuolate bacterium, designated strain 37<sup>T</sup>, was isolated from a sea ice core collected from Point Barrow, Alaska, USA. Cells of strain 37<sup>T</sup> were large (6–14 µm in length), rod-shaped, contained gas vacuoles of two distinct morphologies, and grew well at NaCl concentrations of 1–10% and at temperatures of –12 to 10 °C. The DNA G + C content was 40 mol%. Whole-cell fatty acid analysis showed that 16 : 1ω7c comprised 67% of the total fatty acid content. Phylogenetic analysis of 16S rRNA gene sequences indicated that this bacterium was closely related to members of the genus *Psychromonas*, with highest sequence similarity (96.8%) to *Psychromonas antarctica*. Phenotypic analysis differentiated strain 37<sup>T</sup> from *P. antarctica* on the basis of several characteristics, including cell morphology, growth temperature range and the ability to hydrolyse polymers. DNA–DNA hybridization experiments revealed a level of relatedness of 37% between strain 37<sup>T</sup> and *P. antarctica*, providing further support that it represents a distinct species. The name *Psychromonas ingrahamii* sp. nov. is proposed for this novel species. The type strain is 37<sup>T</sup> (=CCUG 51855<sup>T</sup> = CIP 108865<sup>T</sup>).

Most of the Earth's biosphere never reaches temperatures above 5 °C and is home to a diverse group of microorganisms termed psychrophiles, having minimum, optimum and maximum growth temperatures at or below 0, 15 and 20 °C, respectively (Morita, 1975). One psychrophilic ecosystem, polar sea ice, comprises 7–13% of the Earth's surface at its maximum (Maykut, 1985; Parkinson & Gloersen, 1993; Weeks & Ackley, 1982). Polar sea ice is seasonably variable and its formation begins during polar winter as the ocean surface waters freeze, forming a surface slush termed 'frazil ice'. This ice consolidates into circular sheets of 'pancake ice', which become colonized by microbes that eventually establish the sea ice microbial community (SIMCO) (Nichol & Allison, 1997; Staley & Gosink, 1999; Garrison *et al.*, 1983). Polar sea ice is semisolid, containing channels of brine formed during ice crystallization.

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Abbreviations: PUFA, polyunsaturated fatty acid; SIMCO, sea ice microbial community.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 37<sup>T</sup> is U73721.

Brine pockets may reach salinity levels of 150% (Maykut, 1985), providing a liquid-phase environment at subzero temperatures.

Sea ice is an active environment with large gradients in light, temperature, nutrient availability and salinity, all of which change seasonally (Eicken, 1992). The SIMCOs are typically concentrated in the lower 10–20 cm of a sea ice column, at the ice–water interface, where both sufficient nutrients from the water column and sufficient surface light are present (Staley & Gosink, 1999). The SIMCOs are stratified, containing large varieties of both eukaryotes and prokaryotes. Recent attempts to characterize the bacterial component of SIMCOs have revealed great diversity. To our knowledge, six new genera of the phylum *Bacteroidetes* (Gosink *et al.*, 1998; Bowman *et al.*, 1998a, 1997, 2003; Bowman & Nichols, 2002) and three new genera of *Proteobacteria* (Gosink *et al.*, 1997; Irgens *et al.*, 1996; Bowman *et al.*, 1998b) have been identified within or near the SIMCO, along with known Gram-positive genera (Junge *et al.*, 1998).

Among the SIMCOs, gas vacuolate heterotrophs have been discovered in high numbers from both the Arctic and the Antarctic (Gosink *et al.*, 1993; Staley *et al.*, 1989), located either in the water column below or in the ice above the

nutrient-rich SIMCO band (Gosink *et al.*, 1993). Gas vacuoles contain small, rigid, proteinaceous subunit vesicles that are gas-permeable, reducing cell density as compared with the cytoplasm, and thereby providing buoyancy (Walsby, 1972). Gas vesicles act as organelles of motility, regulating the vertical movement of cells via their synthesis and degradation (Staley, 1980). Although gas vacuolate bacteria in polar sea ice are prevalent and phylogenetically diverse, belonging to the *Alpha*-, *Beta*- and *Gammaproteobacteria* and to the *Bacteroidetes* (Gosink & Staley, 1995), the function of gas vacuoles in this environment remains unknown.

Recently, we reported that a bacterial isolate, designated strain 37<sup>T</sup>, isolated from a sea ice core from Point Barrow, Alaska, USA, could grow at subfreezing temperatures, with a generation time of 240 h at  $-12^{\circ}\text{C}$ , the lowest recorded growth temperature of any organism verified by a growth curve (Breezee *et al.*, 2004). Strain 37<sup>T</sup> was considered to represent a novel species, which was provisionally named *Psychromonas ingrahamii* sp. nov. It is most similar to *Psychromonas antarctica* and other members of the genus *Psychromonas*, a group of psychrophiles having a wide variety of physiological characteristics including piezophily, facultative psychrophily and halophily (Breezee *et al.*, 2004; Mountfort *et al.*, 1998; Kawasaki *et al.*, 2002; Nogi *et al.*, 2002; Xu *et al.*, 2003; Groudieva *et al.*, 2003; Ivanova *et al.*, 2004). Here we report additional information for this sea ice isolate to justify recognition of this novel species.

Strain 37<sup>T</sup> was isolated in May 1991 from Elson Lagoon (Point Barrow, Alaska) about 130 cm from the ice–water interface of a 1.8 m ice core (Gosink *et al.*, 1993). Ordal's sea water cytophaga medium (SWC<sub>m</sub>) prepared in full-strength artificial sea water (ASW) was used for the isolation and routine growth of strain 37<sup>T</sup> (Irgens *et al.*, 1989). Colonies on plates were white, circular, smooth and convex, with an entire margin. Phenotypic characteristics of strain 37<sup>T</sup> are summarized in Table 1.

Cell size, shape and the presence of gas vacuoles were determined for cells grown in Difco marine broth 2216 (Becton Dickinson) via phase contrast microscopy using a Zeiss Photomicroscope I. Electron micrographs were obtained of unstained whole cells by using a Zeiss EM900 transmission electron microscope at 50 kV. Cells of strain 37<sup>T</sup> were unusually large, ranging from 6 to 14  $\mu\text{m}$  long by 1.25 to 1.5  $\mu\text{m}$  wide (Fig. 1; Breezee *et al.*, 2004), and were arranged singly, in pairs or in short chains. Motility was examined by incubation of strain 37<sup>T</sup> in liquid SWC<sub>m</sub> for 12 days with periodic examination by phase contrast microscopy, and cells were found to be non-motile. Gas vacuoles were also visible, appearing as bright, refractive areas inside cells (Fig. 1). Electron microscopy revealed two distinct morphologies of gas vacuoles: numerous short, wide cylinders with conical tips; and rare, longer but narrower cylinders with conical ends (Fig. 1b). The presence of two gas vacuole types is unusual, having been reported before only in the halophilic archaeon *Halobacterium halobium* (Walsby, 1994).

The range of temperatures allowing growth of strain 37<sup>T</sup> was determined by observation of growth on SWC<sub>m</sub> plates at 5, 10, 15 and  $20^{\circ}\text{C}$  for 8 days. Growth at subzero temperatures was also tested using liquid SWC<sub>m</sub>. Strain 37<sup>T</sup> was psychrophilic, growing at temperatures from  $-12$  to  $10^{\circ}\text{C}$  with a generation time of 240 h at  $-12^{\circ}\text{C}$  (Breezee *et al.*, 2004). No growth was observed at  $15^{\circ}\text{C}$ . Attempts to grow strain 37<sup>T</sup> at  $-15^{\circ}\text{C}$  were unsuccessful because the culture medium routinely froze. The true minimum growth temperature may in fact be lower than  $-12^{\circ}\text{C}$ .

The pH range for growth was tested using SWC<sub>m</sub> buffered to various pH values with 25 mM solutions of the following buffers: MES, pH 5.7; ACES, pH 6.6; TAPSO, pH 7.4; TAPS, pH 8.3; CHES, pH 9.0 (Dyksterhouse *et al.*, 1995). Growth at each pH was determined turbidometrically using a Bausch and Lomb 20-D spectrophotometer at 600 nm. Growth was observed at near neutral pH values (pH 6.5, 6.8 and 7.4), but not at moderately acidic (pH 5.0) or basic (pH 8.3, 9.0) values.

Requirement for and tolerance to NaCl were determined by observing growth on CLED agar (Difco) supplemented with 0–22 % NaCl. Strain 37<sup>T</sup> required NaCl for growth, showing no growth at 0 % NaCl. It grew well at 1–12 % NaCl, and weak growth was observed at NaCl concentrations as high as 20 %.

The ability of strain 37<sup>T</sup> to use a particular substrate as its sole carbon source was tested at substrate concentrations of 0.2 % in SWC<sub>m</sub> in microtitre plate wells. Strain 37<sup>T</sup> was inoculated in triplicate and incubated for 21 days at  $5^{\circ}\text{C}$ . Growth was determined by measuring the absorbance at 600 nm using a DeltaSoft II microplate reader. Strain 37<sup>T</sup> was able to use a wide variety of carbon sources, as detailed in the species description later. Sugar fermentation was tested using the Hugh–Leifson method (Gerhardt *et al.*, 1981). BBL brand OF basal medium was dissolved in ONR-7a salt solution (Dyksterhouse *et al.*, 1995). Each carbon source was diluted to a concentration of 1 %. *Vibrio splendidus* and inoculated medium without added carbon source were used as positive and negative controls, respectively. Gas production from glucose metabolism was detected by growing strain 37<sup>T</sup> in liquid SWC<sub>m</sub> supplemented with glucose into which Durham tubes were placed for gas detection. Strain 37<sup>T</sup> was facultatively anaerobic and fermented several carbon sources, including lactose, sucrose, D-mannitol, salicin, maltose, trehalose, cellobiose, D-galactose, melibiose and D-glucose (without gas production), but not dulcitol, *myo*-inositol, D-sorbitol, L-arabinose or D-xylose.

Biochemical tests were performed using standard methodology (Gerhardt *et al.*, 1981). For these tests, cultures of strain 37<sup>T</sup> were grown in SWC<sub>m</sub> supplemented with the appropriate substrates. For nitrate reduction, strains were supplemented with 0.1 or 0.01 %  $\text{NaNO}_3$  and 0.17 % agar. Cells of strain 37<sup>T</sup> were Gram-negative, oxidase-positive, weakly catalase-positive and positive for nitrate reduction, all traits characteristic of members of the genus *Psychromonas*.

**Table 1.** Comparison of characteristics of *P. ingrahamii* sp. nov. and other members of the genus *Psychromonas*

Taxa: 1, *P. ingrahamii* 37<sup>T</sup>; 2, *P. antarctica* DSM 10704<sup>T</sup>; 3, *P. arctica* Pull 5.3<sup>T</sup>; 4, *P. kaikoa* JT7304<sup>T</sup>; 5, *P. marina* 4-22<sup>T</sup>; 6, *P. profunda* 2825<sup>T</sup>. All were Gram-negative, oxidase-positive, catalase-positive, and able to use D-glucose and D-fructose as sole carbon sources. Characteristics are scored as: +, positive; -, negative; w, weakly positive after 3 weeks; (w), weakly positive after 6 weeks. NR, Not reported; ND, not determined. Data for other *Psychromonas* species were taken from Breezee *et al.* (2004), Mountfort *et al.* (1998), Kawasaki *et al.* (2002), Nogi *et al.* (2002), Groudieva *et al.* (2003), Xu *et al.* (2003) and Brenner *et al.* (2005).

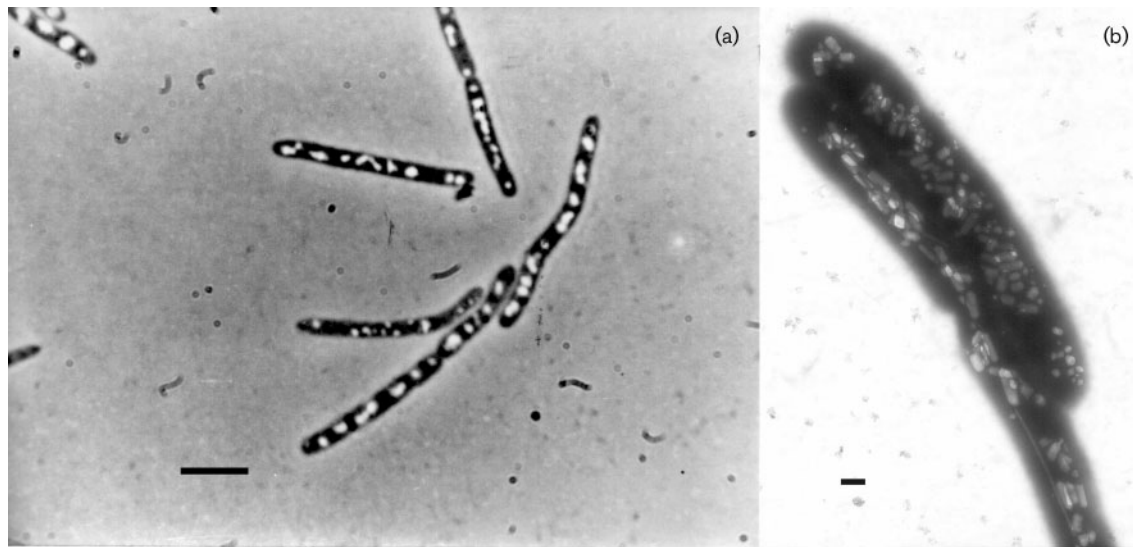
Characteristic	1	2	3	4	5	6
Cell morphology and arrangement	Large rods; singles, pairs	Ovoid rods; singles, pairs	Rods; singles, pairs	Ovoid rods	Rods	Rods
Cell length (µm)	6–14	2.5–6	1.3–2.6	2–4	1.5–2.0	2.0–5.5
Production of gas vesicles	+	–	–	–	–	–
Colony colour	White	White	White	NR	Colourless	Colourless
Motility	–	+	+	+	+	+
Carbon sources utilized:						
D-Galactose	+	+	ND	+	+	+
D-Mannose	–	–	+	+	–	–
D-Mannitol	+	+	+	+	+	w
D-Sorbitol	–	–	ND	–	–	–
N-Acetylglucosamine	+	+	ND	ND	+	ND
Arabinose	–	ND	ND	–	–	–
D-Xylose	–	–	–	–	+	+
Cellobiose	+	–	ND	+	+	+
Lactose	–	–	+	–	+	+
Maltose	ND	+	+	+	+	+
Sucrose	+	+	+	+	+	w
Fumarate	+	–	+	ND	ND	+
DL-Lactate	+	–	–	ND	ND	(w)
DL-Malate	–	–	–	ND	ND	ND
Glycerol	+	–	+	–	+	(w)
Carbon sources fermented:						
D-Glucose (Gas)	+	+(G)	+(G)	+	+	+
myo-Inositol	–	–	ND	–	–	+
Lactose	+	–	ND	–	+	+
Trehalose	+	+	ND	+	–	+
D-Xylose	–	–	–	–	+	+
Polymers hydrolysed:						
Starch	–	+	+	–	+	w
Gelatin	–	+	–	+	–	–
NaCl concentration allowing growth (%)	1–12*	0–4	1–7	>0	0–7%	>0
Growth temperature range (°C)	–12 to 10†	2–17	0–25	4–15	0–25	2–14
pH range (optimum)	6.5–7.4	(6.5)	6.5–9.8 (8.8)	ND	ND	ND
O <sub>2</sub> requirement for growth	Facultative anaerobe	Aerotolerant anaerobe	Aerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe
Growth at atmospheric pressure	+	+	+	–	+	+
Indole	–	–	ND	–	–	+
Nitrate reduction	+	–	–	+	+	+
DNA G+C content (mol%)	40	42.8	40.1	43.8	43.5	38.1

\*Weak growth was seen at concentrations up to 20%.

†Attempts to grow *P. ingrahamii* at temperatures below –12 °C were inconclusive because the culture medium froze.

However, strain 37<sup>T</sup> could not hydrolyse starch or gelatin. For determination of indole production, cultures were grown in SWC<sub>m</sub> lacking succinate and were tested at three different stages of growth; indole production was not observed.

The whole-cell fatty acid composition was determined using fatty acid methyl ester analysis of cells grown on SWC<sub>m</sub> plates at 4 °C. Cells were scraped into 13 × 100 mm Teflon-lined tubes, frozen at –80 °C and lysed. The fatty acids were



**Fig. 1.** Phase contrast (a) and transmission electron (b) micrographs of cells of strain 37<sup>T</sup>. Bars, 5 and 0.6 µm, respectively. Bright areas within the cells observed by phase contrast microscopy are gas vacuoles. The characteristic morphology of the subunit gas vesicles, i.e. their cylindrical shape with conical polar caps, is shown in (b).

saponified with methanolic base, then converted to fatty acid methyl esters with HCl using the MIDI protocol as previously described (MIDI, 1993). A Hewlett Packard model 5890 Series II gas chromatograph was used to identify and quantify the fatty acid methyl esters. This analysis revealed the principal constituents to be 16-carbon unsaturated and saturated fatty acids 16:1 $\omega$ 7c and 16:0, making up 67 and 18.7%, respectively, of the whole-cell fatty acid content. Other *Psychromonas* species also contain high concentrations of 16:1, ranging between 39% in *Psychromonas marina* 4-22<sup>T</sup> and 58% in *P. antarctica* DSM 10704<sup>T</sup> (Table 2). Other fatty acids found in measurable quantities in strain

37<sup>T</sup> included 18:1 (3.6%) and 12:0 (2.5%). Our analysis was unable to distinguish between the fatty acids 12:0 alde, 16:1 ISO and 14:0 3-OH, and 4.5% of the fatty acids from strain 37<sup>T</sup> were among this group. The fatty acid composition of strain 37<sup>T</sup> is summarized in Table 2.

Genomic DNA from strain 37<sup>T</sup> was isolated using a hexadecyltrimethylammonium bromide miniprep protocol (Ausubel *et al.*, 1989). The DNA G + C content of strain 37<sup>T</sup> was determined by HPLC according to the method of Mesbah *et al.* (1989) and found to be 40 mol%, within the range of 38.1–43.8 mol% reported for other members of the genus

**Table 2.** Fatty acid content of *P. ingrahamii* sp. nov. and other members of the genus *Psychromonas*

Taxa: 1, *P. ingrahamii* 37<sup>T</sup>; 2, *P. antarctica* DSM 10704<sup>T</sup>; 3, *P. arctica* Pull 5.3<sup>T</sup>; 4, *P. kaikoeae* JT7304<sup>T</sup>; 5, *P. marina* 4-22<sup>T</sup>; 6, *P. profunda* 2825<sup>T</sup>. Values are percentages of total fatty acids. Isomers are shown in parentheses if known. Results below 1% are not shown. Data for other *Psychromonas* species were taken from Kawasaki *et al.* (2002), Nogi *et al.* (2002), Groudieva *et al.* (2003) and Xu *et al.* (2003).

Fatty acid	1	2	3	4	5	6
12:0	2.5	1	2.7–5.2	1		
14:0				6		
15:0				1		
16:0	18.7	24	7.0–16.2	15	43.6	31
14:1		8 ( $\omega$ 7t)	2.7–5.2 ( $\omega$ 5t)	10 ( $\omega$ 7t)	3.2	15
16:1	67 ( $\omega$ 7c)	58 ( $\omega$ 7c)	~50 ( $\omega$ 7c), 7.0–16.2 ( $\omega$ 7t)	52 ( $\omega$ 7c), 2 ( $\omega$ 9c)	39.4	44
18:1	3.6	3 ( $\omega$ 7c)	7.0–16.2 ( $\omega$ 7)	2 ( $\omega$ 7c)	3.1	
20:5 $\omega$ 3				2		
22:6				2	1.6	
12:0 3-OH				2		
12:0 alde, 16:1 ISO or 14:0 3-OH	4.5	6 (14:0 3-OH)		4 (14:0 3-OH)	2.7 (16:1 ISO)	

*Psychromonas* (Mountfort *et al.*, 1998; Kawasaki *et al.*, 2002; Nogi *et al.*, 2002; Groudieva *et al.*, 2003; Xu *et al.*, 2003).

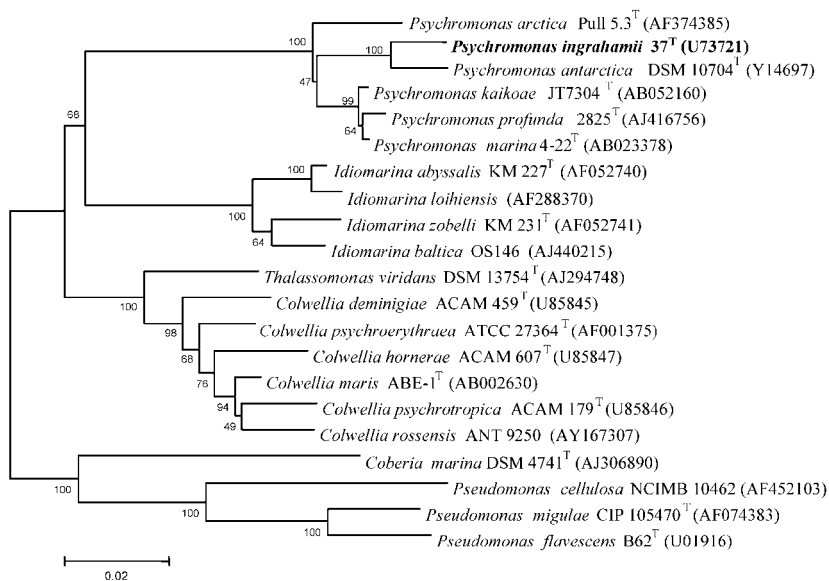
The 16S rRNA gene from strain 37<sup>T</sup> was sequenced as described by Gosink & Staley (1995). The EMBL accession numbers for additional 16S rRNA gene sequences used for analysis are given in parentheses in Fig. 2. These sequences were aligned using CLUSTAL\_X (Thompson *et al.*, 1997). Phylogenetic trees were constructed by determining distances (according to the Kimura two-parameter model) and clustering (with the neighbour-joining method) by using the MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 software package (Kumar *et al.*, 2001). Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain 37<sup>T</sup> was a member of the *Gammaproteobacteria*, was related most closely to *P. antarctica* DSM 10704<sup>T</sup>, showing 96.8% similarity at the nucleotide level, and clustered with other members of the genus *Psychromonas* (Fig. 2). Strain 37<sup>T</sup> was also related closely (>98% sequence similarity) to two other polar sea ice taxa, strain 174 (EMBL accession no. U73722) and strain 90Pgv1 (EMBL accession no. U14582), isolated from the Arctic and Antarctic, respectively, that have not yet been fully characterized (Staley & Gosink, 1999).

Although strain 37<sup>T</sup> differed significantly at the phenotypic level from *P. antarctica* DSM 10704<sup>T</sup> and other members of the genus *Psychromonas* (see Table 1), the high degree of 16S rRNA gene sequence similarity warranted further examination at the molecular level. DNA–DNA hybridization experiments were performed using the method described by Ziemke *et al.* (1998), except that for nick translation, 2 µg DNA was labelled during 3 h incubation at 15 °C using genomic DNA isolated from strain 37<sup>T</sup> and *P. antarctica* DSM 10704<sup>T</sup>. The reassociation value between these two strains was 37.1% (reciprocal 38.8%), confirming that strain 37<sup>T</sup> represents a novel species, according to accepted criteria (Wayne *et al.*, 1987).

Members of the genus *Psychromonas* have been isolated from a variety of low-temperature environments, including a high-salinity pond on the McMurdo ice-shelf (Mountfort *et al.*, 1998), deep-sea cold-seep sediments near Japan (Xu *et al.*, 2003; Nogi *et al.*, 2002), Japanese cold-current coastal sea water (Kawasaki *et al.*, 2002), and northern European Arctic sea water and sea ice (Groudieva *et al.*, 2003). Members of this genus display great phenotypic diversity, ranging in degrees of piezophily and temperature range of growth. Strain 37<sup>T</sup>, isolated from a sea ice core, represents a novel species within this genus and is unique among this group in its unusually large cell size, its ability to grow at subfreezing temperatures, its tolerance to high salt concentrations and its ability to produce gas vacuoles (see Table 1). Unlike other *Psychromonas* strains, strain 37<sup>T</sup> cannot hydrolyse the polymers starch or gelatin and appears to be non-flagellated.

The abilities of strain 37<sup>T</sup> to withstand both high salt concentrations and subfreezing temperatures are consistent with the polar sea ice environment from which it was isolated. The semisolid matrix of polar sea ice consists of ice crystals around which extruded brine accumulates to high concentrations. The high salt concentrations within these brine pockets allow the water to remain liquid at temperatures well below freezing. It is within these high-salt, low-temperature microenvironments that members of the SIMCOs persist.

Although the formation of gas vacuoles by strain 37<sup>T</sup> is unique among members of the *Psychromonas* genus, it is not unusual for a polar sea ice bacterium. Within the SIMCOs, gas vacuolate bacteria are abundant and phylogenetically diverse, with representatives in the *Alpha*-, *Beta*- and *Gammaproteobacteria*, and within the phylum *Bacteroidetes* (Gosink *et al.*, 1993; Gosink & Staley, 1995; Irgens *et al.*, 1989). Strain 37<sup>T</sup> is unusual, however, in its ability to produce two distinct gas vacuole morphotypes within a single



**Fig. 2.** Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers are given in parentheses), constructed after multiple alignment of data by using CLUSTAL\_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were determined by using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001). Bootstrap values, based on 1000 replications, are given as percentages at branch points. Bar, 0.02 substitutions per mean nucleotide position.

cell, previously reported only in a halophilic archaeon (Walsby, 1994).

Unlike *Psychromonas kaihoae* JT7304<sup>T</sup> and *P. marina* 4-22<sup>T</sup>, strain 37<sup>T</sup> cell membranes contain no measurable amounts of polyunsaturated fatty acids (PUFAs) such as 20:5 (eicosapentaenoic acid) or 22:6 (docosahexaenoic acid). As the concentration of PUFAs has been suggested to be inversely proportional to optimum growth temperature, the lack of PUFAs in strain 37<sup>T</sup>, which grows at subfreezing temperatures lower than those of other *Psychromonas* species, is inconsistent with this hypothesis (Bowman *et al.*, 1998c).

Phylogenetic analysis of 16S rRNA gene sequences indicated that strain 37<sup>T</sup>, isolated from sea ice from Point Barrow, Alaska, was most closely related to *P. antarctica* DSM 10704<sup>T</sup>, isolated from a high-salinity pond sediment (96.8% sequence similarity). Strain 37<sup>T</sup> was also closely related to two polar sea ice taxa, 174 and 90Pgv1, isolated from Arctic and Antarctic sea ice, respectively (Staley & Gosink, 1999). It is interesting that such closely related organisms have been isolated from opposite polar regions. This phenomenon has been shown previously for other sea ice genera, including *Polaribacter* and *Octadecabacter* (Gosink *et al.*, 1997, 1998), and is supported by studies of Arctic and Antarctic sea ice communities using culture-independent molecular techniques (Brown & Bowman, 2001; Brinkmeyer *et al.*, 2003). This suggests that organismal dispersal was followed by acquisition of traits required for adaptation to particular microenvironments.

### Description of *Psychromonas ingrahamii* sp. nov.

*Psychromonas ingrahamii* (in.gra.ham'.i.i. N.L. gen. n. *ingrahamii* of Inghram, in honour of John L. Inghram for his extensive research on psychrophilic bacteria).

Cells are Gram-negative, non-motile large rods, 6–14 µm long and 1.25–1.5 µm wide, found either singly or in pairs, and containing two gas vesicle morphotypes. On SWC<sub>m</sub>, colonies are white, circular, smooth and convex, with an entire margin. Moderately halophilic (growth at NaCl concentrations of 1–12%, with weak growth up to 20%, but no growth without NaCl), and strictly psychrophilic. Temperature range for growth is –12 °C (with a generation time of 240 h) or lower (not tested) to 10 °C or higher (not tested between 10 and 15 °C, but no growth is observed at 15 °C). The pH range for growth is 6.5–7.4. Grows at atmospheric pressure. Facultative anaerobe, capable of both respiratory and fermentative metabolism. Catalase- and cytochrome oxidase-positive. Reduces inorganic nitrate. Indole test is negative. Predominant cellular fatty acids are 16:1ω7c and 16:0. Utilizes as sole carbon sources D-glucose, D-ribose, D-fructose, sucrose, L-glutamate, L-cysteine, DL-aspartate, fumarate, succinate, pyruvate, propionate, acetate, glycerol, N-acetylglucosamine, glucosamine, cellobiose, DL-lactate, D-mannitol, salicin, trehalose and D-glucuronate, but not

lactose, L-leucine, L-proline, α-ketoglutarate, citrate, benzoate, glycolate, methanol, arabinose, caproate, D-gluconate, myo-inositol, DL-malate, D-mannose, D-sorbitol or D-xylose. Can ferment lactose, sucrose, D-mannitol, salicin, maltose, trehalose, cellobiose, D-galactose, melibiose and D-glucose (without gas production), but not dulcitol, myo-inositol, D-sorbitol, L-arabinose or D-xylose. No starch or gelatin hydrolysis. The DNA G + C content is 40 mol%.

The type and only strain, 37<sup>T</sup> (=CCUG 51855<sup>T</sup>=CIP 108865<sup>T</sup>), was isolated from Elson Lagoon (Point Barrow, Alaska, USA) about 130 cm from the ice–water interface from a 1.8 m ice core.

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