NOTE***Pseudoalteromonas prydzensis* sp. nov., a psychrotrophic, halotolerant bacterium from Antarctic sea ice**

John P. Bowman

Tel: +61 03 6226 2776. Fax: +61 03 6226 2642. e-mail: john.bowman@utas.edu.au

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

Species of the genus *Pseudoalteromonas* are frequently isolated from marine ecosystems and appear to be particularly abundant in Antarctic coastal waters. Most *Pseudoalteromonas* strains isolated from sea ice and underlying seawater samples are phenotypically similar to the species *Pseudoalteromonas antarctica* and *Pseudoalteromonas nigrifaciens*. However, a minority of isolates were recognized by phenotypic, DNA–DNA hybridization and 16S rRNA-based phylogenetic studies to represent a distinct genospecies clustering at the periphery of the non-pigmented *Pseudoalteromonas* species clade. These strains are non-pigmented, halotolerant psychrotrophs that are capable of hydrolysing starch and chitin, and possess a DNA G+C content of 38–39 mol%. It is proposed that this group represents a novel species, *Pseudoalteromonas prydzensis* sp. nov., for which the type strain is ACAM 620^T.

Keywords: *Pseudoalteromonas*, sea ice, seawater, Antarctica, most probable number counting

The majority of species originally residing in the genus *Alteromonas* were recently transferred to *Pseudoalteromonas* (Gauthier *et al.*, 1995) with *Alteromonas macleodii* remaining as the sole species of *Alteromonas*. Though *Alteromonas* and *Pseudoalteromonas* share considerable phenotypic (Baumann *et al.*, 1984) and chemotaxonomic similarities (Svetashev *et al.*, 1995), 16S rRNA-based sequence analysis indicated that these genera were distinct from one another and shared 86–88% sequence similarity. Currently there are 13 species in *Pseudoalteromonas* including: *Pseudoalteromonas antarctica*, *Pseudoalteromonas atlantica*, *Pseudoalteromonas aurantia*, *Pseudoalteromonas carrageenovora*, *Pseudoalteromonas citrea*, *Pseudoalteromonas denitrificans*, *Pseudoalteromonas espejiana*, *Pseudoalteromonas haloplanktis* subsp. *haloplanktis*, *Pseudoalteromonas haloplanktis* subsp. *tetradonidis*, *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas nigrifaciens*, *Pseudoalteromonas piscicida*, *Pseudoalteromonas rubra* and *Pseudoalteromonas undina* (Bozal *et al.*, 1997; Gauthier *et al.*, 1995).

Pseudoalteromonas species are characteristically

Gram-negative rod-shaped bacteria which are motile by means of one or two polar flagella. Strains also require sodium ions for growth, have an oxidative metabolism and are able to produce a range of exoenzymes, including lipases, proteases, amylases and chitinases. A number of species have the capacity to form high molecular mass compounds with antibiotic properties (Baumann *et al.*, 1984). Though in general mesophilic, several *Pseudoalteromonas* species are psychrotrophic, able to grow at 4 °C and with growth temperature optima of approximately 30 °C.

The sea pack ice and coastal attached (fast) ice around Antarctica has been found to contain abundant populations of bacteria. Bacteria concentrate in diatom assemblages which occur either as surface populations, internal band assemblages, or at the sea ice/seawater interface (Palmisano & Garrison, 1993). The majority of the bacterial populations in sea ice assemblages are psychrophilic (Bowman *et al.*, 1997c; DeLille, 1996). In seawater underlying sea ice the presumable lack of nutrients, lack of surfaces or stable matrices for colonization prevents the establishment of psychrophilic populations even though the temperature is comparable to that of the lower sections of sea ice (about –2 °C). Psychrotrophic bacteria predominate in under-ice seawater, however compared to popu-

The GenBank/EMBL/DBJ accession number for the 16S rRNA sequence reported in this paper is U85855.

Table 1. Source and phylogenetic comparison of *Pseudoalteromonas* strains with *Pseudoalteromonas prydzensis*

Strain*	Isolation site	Accession no.	ACAM 620 ^T 16S rRNA (% similarity)
<i>P. prydzensis</i> ACAM 620 ^T	Sea ice, Prdyz Bay, Antarctica	U85855	100
MB6-23	Sea ice, Prdyz Bay, Antarctica	—	—
MB8-01	Sea ice, Prdyz Bay, Antarctica	—	—
<i>P. atlantica</i> ACAM 583 ^T (= ATCC 19262 ^T)	Seaweed, Nova Scotia, Canada	X82134	97.5
<i>P. carrageenovora</i> ACAM 579 ^T (= ATCC 43555 ^T)	Marine algae/seawater, Nova Scotia, Canada	X82136	97.3
MB6-03	Sea ice, Long Fjord, Antarctica	U85857	97.3
IC013	Sea ice, Ellis Fjord, Antarctica	U85859	97.3
<i>P. espejiana</i> ACAM 548 ^T (= ATCC 29659 ^T)	Seawater, California, USA	X82143	97.2
MB6-05	Sea ice, Long Fjord, Antarctica	U85860	97.2
<i>P. nigrifaciens</i> ACAM 545 ^T (= ATCC 19375 ^T)	Salted butter	X82146	97.1
SW08	Seawater, Long Fjord, Antarctica	U85861	97.1
<i>P. haloplanktis</i> subsp. <i>haloplanktis</i> ACAM 547 ^T (= ATCC 14393 ^T)	Unknown	X67024	97.0
IC006	Sea ice, Ellis Fjord, Antarctica	U85856	96.9
MB8-02	Sea ice, Prydz Bay, Antarctica	U85858	96.9
SW29	Seawater, Ellis Fjord, Antarctica	U85862	96.8
<i>P. undina</i> ACAM 546 ^T (= ATCC 29660 ^T)	Seawater, California, USA	X82140	96.7
<i>P. antarctica</i> CECT 4664 ^T	Seawater, Antarctica	X98336	96.6
<i>P. haloplanktis</i> subsp. <i>tetraodonis</i> ATCC 51193 ^T	Skin slime of <i>Fugu poecilonotus</i>	X82139	96.5
<i>P. aurantia</i> ATCC 33046 ^T	Seawater, France	X82135	96.3
<i>P. citrea</i> ATCC 29719 ^T	Seawater, France	X82137	96.3
<i>P. denitrificans</i> ATCC 43337 ^T	Seawater, Norway	X82138	94.8
<i>P. luteoviolacea</i> ATCC 33492 ^T	Seawater, France	X82144	94.5
<i>P. piscicida</i> ATCC 15057 ^T	Area of dead fish, Florida, USA	X82141	94.3
<i>P. rubra</i> ATCC 29570 ^T	Seawater, France	X82147	93.8

* ACAM, Australian Collection of Antarctic Microorganisms, Antarctic CRC, University of Tasmania, Hobart, Tasmania, Australia; ATCC, American Type Culture Collection, Rockville, MD, USA; CECT, Spanish Type Culture Collection, Valencia, Spain.

lations in sea ice assemblages their activity and productivity is low. Psychrotrophic bacterial species appear to be as common in sea ice as in the underlying seawater (Bowman *et al.*, 1997c; DeLille, 1996; Helmke & Weyland, 1995) with *Pseudoalteromonas* strains being the most frequently isolated psychrophilic (Bowman *et al.*, 1997a, c).

Most probable number (MPN) counting was used to determine the viable heterotrophic bacterial count for sea ice and underlying seawater samples at incubation temperatures of 2 and 25 °C (Bowman *et al.*, 1997b). Colonies of *Pseudoalteromonas*-like strains were frequently isolated from the highest positive dilution incubated at 25 °C (77 % of all samples) of both sea ice and seawater samples. *Pseudoalteromonas*-like colonies were non-pigmented, mucoid, translucent, and with slightly spreading or entire edges. Identification of isolates as *Pseudoalteromonas* was further confirmed by a number of simple phenotypic criteria as follows: (1) the strains were Gram-negative, generally rod-shaped and motile; (2) they could grow at 4 °C and 30 °C but usually not at 37 °C; (3) growth occurred on

media prepared with 1–4 × strength seawater, however no growth occurred on media with no added seawater or NaCl; and (4) the strains possess an oxidative metabolism. On this basis, *Pseudoalteromonas*-like strains were estimated to be 1.8×10^6 – 7.8×10^8 cells l⁻¹ and 1.3×10^6 – 8.1×10^7 cells l⁻¹ in seawater and sea ice samples, respectively. Overall, these values represented 2–38 % of the total MPN viable count.

An extensive array of phenotypic characteristics for the Antarctic *Pseudoalteromonas*-like isolates were available from previous studies (Bowman *et al.*, 1997a, c). The binomially coded data were subsequently analysed by numerical taxonomy (Bowman *et al.*, 1997c). Two distinct phenotypic clusters were derived after cluster analysis. The first cluster (phenon A) included the vast majority of strains (47 out of 50 isolates) which were phenotypically similar to *P. antarctica* and to *P. nigrifaciens* (Table 1). A second cluster (phenon B) included three strains (ACAM 620^T, MB6-23 and MB8-01; ACAM, Australian Antarctic Collection of Microorganisms, Antarctic CRC, University of Tasmania, Hobart, Tasmania Australia)

Fig. 1. Unrooted phylogenetic tree based on 16S rRNA sequences of species of *Pseudoalteromonas prydzensis* and other *Pseudoalteromonas* species (γ subclass of the *Proteobacteria*). The topology was obtained by using the maximum-likelihood method.

which phenotypically differed from the first cluster and from other non-pigmented *Pseudoalteromonas* species as shown in Table 1. The three isolates making up cluster B were isolated from sea ice collected at three separate locations within Prydz Bay, Antarctica (68°S, 76°E). All ice samples from which these strains were obtained lacked any visible diatom assemblage.

DNA G+C contents for the isolates were determined by the thermal denaturation procedure as adapted by Sly *et al.* (1986). Strains from cluster A possessed values of 41–43 mol % ($n = 26$, 42.1 ± 0.6), whereas those of cluster B possessed lower values, in the range 38–39 mol % ($n = 3$, 38.4 ± 0.5).

DNA–DNA hybridization was performed to determine if strains of phenon B were similar to each other and distinct from other *Pseudoalteromonas* species. The spectrophotometric renaturation rate kinetics procedure of Huss *et al.* (1983) was employed utilizing a GBC 916 spectrophotometer. The DNA at a concentration of $100 \mu\text{g ml}^{-1}$ was sheared by sonication to a mean size of 1 kb, filtered using disposable $0.22 \mu\text{m}$ pore-size filter cartridge, and then dialysed at 4 °C overnight in $0.1 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M NaCl , 0.015 M sodium citrate, pH 7.0). Mixtures of DNA samples and control samples were denatured at 95 °C, and the SSC concentration was increased to $2 \times \text{SSC}$ by addition of a small volume of a concentrated SSC stock solution. An optimal renaturation rate temperature of 66 °C (based on a DNA G+C content of 40 mol %) was used, and the renaturation was followed by measuring the decline in absorbance over a 40–50 min period. ACAM 620^T was found to have only background hybridization levels (10–28 %) with the following *Pseudoalteromonas* strains: *P. haloplanktis* subsp. *haloplanktis* ACAM 547^T, *P. atlantica* ACAM

583^T, *P. espejiana* ACAM 548^T, *P. undina* ACAM 546^T, *P. nigrifaciens* ACAM 545^T, *P. carrageenovora* ACAM 579^T and sea ice/seawater isolates IC013, MB8-02, MB6-05, SW08 and SW29. Hybridization levels of 96 % were recorded between ACAM 620^T and MB6-23, and 89 % between ACAM 620^T and MB8-01. This result indicated that the ACAM strain group was a distinct genospecies.

Whole-cell fatty acid analysis was also performed to determine if the sea ice isolates formed a homogeneous group. The profiles were generated using previously described GC-MS procedures with double-bond positions and lipid geometry confirmed by GC-MS analysis of dimethyldisulfide derivatized fatty acids (Nichols & Russell, 1996). No significant difference was evident between the fatty acid profiles of the Antarctic isolates from both clusters A and B. Predominant fatty acids included 16:1 ω 7c (40–45 %), 16:0 (28–33 %), 18:1 ω 7c (16–21 %) and 17:1 ω 8c (5–8 %). Low levels of saturated iso-branched fatty acids and 3-OH fatty acids were also detected, but these were at levels too low to be reliable for differentiation of the groups. Overall, the lipid profiles were very similar to those found for other *Pseudoalteromonas* species and *Alteromonas macleodii* (Svetashev *et al.*, 1995).

16S rRNA sequence analysis was used to ascertain the relationship of the Antarctic isolates to other *Pseudoalteromonas* species. Sequences for the Antarctic *Pseudoalteromonas* strains were previously determined (Bowman *et al.*, 1997c). 16S rRNA sequences available for *Pseudoalteromonas* species (Table 1) were aligned and analysed using programs from PHYLIP (Felsenstein, 1993). Near complete 16S rRNA sequences, using a total of 1518 nucleotide positions,

Table 2. Phenotypic differentiation of *Pseudoalteromonas prydzensis* from other non-pigmented *Pseudoalteromonas* species

Phenotypic data are from references Akagawa-Matsushita *et al.* (1993), Baumann *et al.* (1984, 1997a, b), Bozal *et al.* (1997) and Bowman *et al.* (1997c). Abbreviations: +, positive for 90 % or more strains; v, positive for 11–89 % of strains; –, trait positive for 0–10 % of strains; ND, no data.

Characteristic	<i>P. prydzensis</i> (cluster B)	Other Antarctic isolates (cluster A)	<i>P. antarctica</i>	<i>P. nigrifaciens</i>	<i>P. haloplanktis</i> subsp. <i>haloplanktis</i>	<i>P. haloplanktis</i> subsp. <i>tetraodonis</i>	<i>P. espejiana</i>	<i>P. undina</i>	<i>P. atlantica</i>	<i>P. carrageenovora</i>
Melanin formation	–	–	–	v	v	–	–	–	+	+
Growth at 4 °C	+	+	+	+	–	+	–	v	+	+
Growth at 35 °C	–	v	–	–	v	–	v	v	+	+
Amylase production	+	–	v	–	v	–	+	v	+	–
Chitinase production	+	–	ND	–	v	–	–	+	–	–
Utilization of:										
L-Arabinose	+	–	–	–	–	–	–	–	–	–
D-Mannose	+	v	v	+	+	–	v	–	+	–
D-Fructose	–	–	–	+	v	–	v	–	+	+
Lactose	–	v	–	+	–	–	+	–	+	+
Sucrose	+	–	–	–	+	+	+	+	+	+
N-Acetylglucosamine	+	v	v	–	+	+	–	+	–	–
Mannitol	+	+	+	–	v	–	+	–	+	+
Glycerol	+	+	ND	+	–	–	–	–	+	+
Succinate	+	+	+	+	+	+	–	+	+	+
Fumarate	+	+	ND	+	+	+	–	+	+	+
Citrate	+	+	+	+	+	–	+	–	+	+
DL-Lactate	–	v	–	+	–	–	–	–	–	–
L-Malate	+	v	–	–	–	+	–	–	–	+
DL-3-Hydroxybutyrate	+	–	–	–	v	+	v	+	–	–
G + C content (mol %)	38–39	41–43	41–42	42–44	41–45	42	43–44	43–44	41–42	39

were compared in the analysis. DNADIST (maximum-likelihood option) and NEIGHBOR (neighbourliness option) were utilized to construct an unrooted phylogenetic tree (Fig. 1). Antarctic isolates falling into cluster A (IC006, IC013, MB6-03, MB6-05, MB8-02, SW08, SW29) grouped within a shallow clade which included seven non-pigmented *Pseudoalteromonas* species (Fig. 1). Overall sequence similarities between these strains was 97.9–99.9 %, thus most 16S rRNA sequences of the non-pigmented *Pseudoalteromonas* species are so closely related that other procedures are necessary to determine interspecies relationships, such as phenotypic analyses and DNA–DNA hybridization. For instance, strains of *P. haloplanktis* subsp. *haloplanktis* and *P. haloplanktis* subsp. *tetraodonis* (sequence similarity of 98.5 %) do not cluster together even though they share high levels of DNA hybridization (Akagawa-Matsushita *et al.*, 1993). ACAM 620^T (phenon B) clustered at the periphery of this clade, sharing 96.5–97.5 % similarity with other non-pigmented *Pseudoalteromonas* species (Table 1). The lower degree of 16S rRNA sequence similarity that ACAM 620^T has with other non-pigmented species corresponds well with the low levels of DNA–DNA hybridization.

Overall ACAM 620^T, MB6-23 and MB8-01 represents a distinct taxa within the genus *Pseudoalteromonas* as defined by differences in phenotype (Table 2), DNA–DNA hybridization and 16S rRNA sequence results (Table 1, Fig. 1). This group of Antarctic strains is proposed as a new species, designated *Pseudoalteromonas prydzensis* sp. nov.

Description of *Pseudoalteromonas prydzensis* sp. nov.

Pseudoalteromonas prydzensis (prydz.en.sis M.L. neut. adj. *prydzensis* pertaining to Prydz Bay, Antarctica, the site of sea ice samples from which the species was derived).

Gram-negative, rod-shaped, non-spore-forming, motile organism (0.5–0.7 µm in width and 1.0–2.5 µm in length). Colonies are non-pigmented, translucent, convex, irregular to circular in shape with a lobate to circular edge, 3–7 mm in diameter and mucoid consistency. Psychrotrophic, growing from 0 °C to 30 °C (optimum growth is 22–25 °C), no growth occurs at 35 °C or higher. Requires sodium ions for growth, growing on media with a salinity ranging from 0.5 to 15 % NaCl. Tolerates 5 % ox bile salts, 100 µg vibriostatic agent 0/129 ml^{–1} and 20 µg ampicillin ml^{–1}. Catalase- and oxidase-positive. Produces alkaline phosphatase. Hydrolyses Tween 20, Tween 40, Tween 80, starch, chitin, casein and gelatin. Strains may also hydrolyse aesculin and urea. Agar, alginate, dextran, DNA, urate and xanthine are not hydrolysed. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, L-tryptophan and L-phenylalanine deaminase, indole from L-tryptophan, hydrogen sulfide from L-cysteine, ONPG (β-galactosidase), nitrate reduction and denitrification tests are negative. Strictly oxidative. No growth occurs anaerobically by fermentation or by respiration with the following electron acceptors: ferric oxide, ferric pyrophosphate, nitrate and trimethylamine N-oxide (with acetate and

DL-lactate as electron donors). Acid is formed oxidatively from L-arabinose, D-glucose, D-mannose, maltose, *N*-acetylglucosamine, sucrose and trehalose. Acid may also be formed from cellobiose. Acid is not formed from dextran, D-fructose, D-galactose, lactose, D-melibiose, D-raffinose, D-xylose, L-rhamnose, adonitol, glycerol, D-mannitol, m-inositol, or D-sorbitol. Utilizes the following substrates for carbon and energy: glycogen, *N*-acetylglucosamine, L-arabinose, D-glucose, maltose, D-mannose, sucrose, trehalose, D-mannitol, glycerol, D-gluconate, acetate, propionate, butyrate, isobutyrate, succinate, citrate, aconitate, DL-3-hydroxybutyrate, L-malate, fumarate, pyruvate, oxaloacetate, L-glutamate, L-proline, hydroxy-L-proline, L-serine and γ -aminobutyrate. Some strains can also utilize cellobiose, D-galactose, *m*-inositol, α -glycerophosphate, valerate, isovalerate, octanoate, malonate, azelate, L-phenylalanine and L-tyrosine. The following substrates are not utilized: D-xylose, D-fructose, L-rhamnose, lactose, D-melibiose, D-raffinose, D-arabitol, D-sorbitol, D-glucuronate, saccharate, hexanoate, heptanoate, nonanoate, adipate, glutarate, pimelate, 2-oxoglutarate, DL-lactate, L-alanine, L-asparagine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-threonine, putrescine and urate. The G+C content of the DNA is 38–39 mol%. The major fatty acids are 16:1 ω 7c, 16:0, 18:1 ω 7c and 17:1 ω 8c. Isolated from sea ice. The type strain is ACAM 620^T (isolated from sea ice, Prydz Bay, Antarctica).

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