

29 The Family *Psychromonadaceae*

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Abstract

The family *Psychromonadaceae*, within the order *Alteromonadales*, includes only the genus *Psychromonas*; it is closely related to the three families *Pseudoalteromonadaceae*, *Colwelliaceae*, and *Idiomarinaceae*. The main habitats of the family *Psychromonadaceae* appear to be marine, sea ice, or saline aquatic environments, from which original cultures are isolated. Cells can be Gram-negative rods or oval cells, non-spore, and motile or nonmotile. The flagellum may be single or subpolar, sheathed or unsheathed. The cells grow at temperatures less than 37 °C. They are facultative anaerobes, but some species may grow to be aerotolerant anaerobes or aerobes. They use carbohydrates for energy for growth. Oxidase is positive and catalase is variable. The major isoprenoid quinone is Q-8. The predominant cellular fatty acids are C_{16:0} and C_{16:1}. The abundance ratio of unsaturated fatty acids was ≥50 %. *P. ingrahamii* 37^T, which can grow at –12 °C, underwent genomic analysis. Some species produced C_{20:5}Ω3 and/or C_{22:6}. The G+C DNA is 38–43.8 mol%. This chapter is a modified and updated version of previously published family descriptions (Ivanova et al. *Int J Syst Evol Microbiol* 54:1773–1788, 2004).

Taxonomy, Historical and Current

Short Description of the Families and their Genera

Psychromonadaceae (Psy.chro.mo.na.da'ce.ae. N.L. fem. n. *Psychromonas* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Psychromonadaceae* the *Psychromonas* family Ivanova et al. 2004; Hosoya et al. 2009).

The family *Psychromonadaceae* is phylogenetically a member of the order *Alteromonadales* (Bowman and McMeekin 2005) and class *Gammaproteobacteria*. The family contains only the type genus *Psychromonas* (Mountfort et al. 1998; emended by Nogi et al. 2002). They are Gram-negative, rod- or oval-shaped bacteria and may be motile or nonmotile. They are chemo-organotrophs that do not form endospores or microcysts. They are facultative anaerobes, but some species may grow to be aerotolerant anaerobes or aerobes. Some species do not require Na⁺ ions for growth. In most species, the major isoprenoid quinone is Q-8. The major fatty acids are C_{16:0} and C_{16:1}Ω7. Members of the family have been isolated from coastal, open, and deep-sea waters, as well as sea ice, sediment, and marine environments. The family has the following nucleotide sequence characteristics: 811 (A), 842 (A), 845 (T), 1336 (T). The G+C contents of DNA range between 38 and 43.8 mol%.

Phylogenetic Structure of the Family and its Genera

The phylogenetic tree in Fig. 29.1 shows the family *Psychromonadaceae*. The family *Psychromonadaceae* includes only the genus *Psychromonas* (Mountfort et al. 1998), and it is closely related to the three families *Pseudoalteromonadaceae*, *Colwelliaceae*, and *Idiomarinaceae*. The species *Psychromonas antarctica* was first reported to be closely related to *Moritella marina* (similarity was 91.0 %), belonging to *Gammaproteobacteria* (Mountfort et al. 1998). This family was created by Ivanova et al. (2004) based on phylogenetic position and the presence of a unique set of 16S rRNA gene sequence signature nucleotides. The phylogenetic dendrogram of *Psychromonas*-type strains indicates the presence of two clades. One clade is composed of *P. antarctica*, *P. boydii*, *P. japonica*, *P. macrocephali*, *P. ossibalaenae*, and *P. aquimarina*, which share 96.5–99.2 % 16S rRNA gene sequence similarity with *P. arctica*. The second clade group of *P. marina*, *P. kaikoeae*, *P. profunda*, *P. heitensis*, and *P. hadalis* share a 96.0–98.6 % similarity with *P. agarivorans*. The two clades share ~96.3 % similarity.

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) studies have been performed on several *Psychromonas*-type strains. The phylogenetic neighbors *P. aquimarina*, *P. macrocephali*, and *P. ossibalaenae*, sharing 98.8–99.2 % 16S rRNA gene sequence similarity, exhibit 36–47 % DDH similarity, whereas *P. antarctica* and *P. ingrahamii*

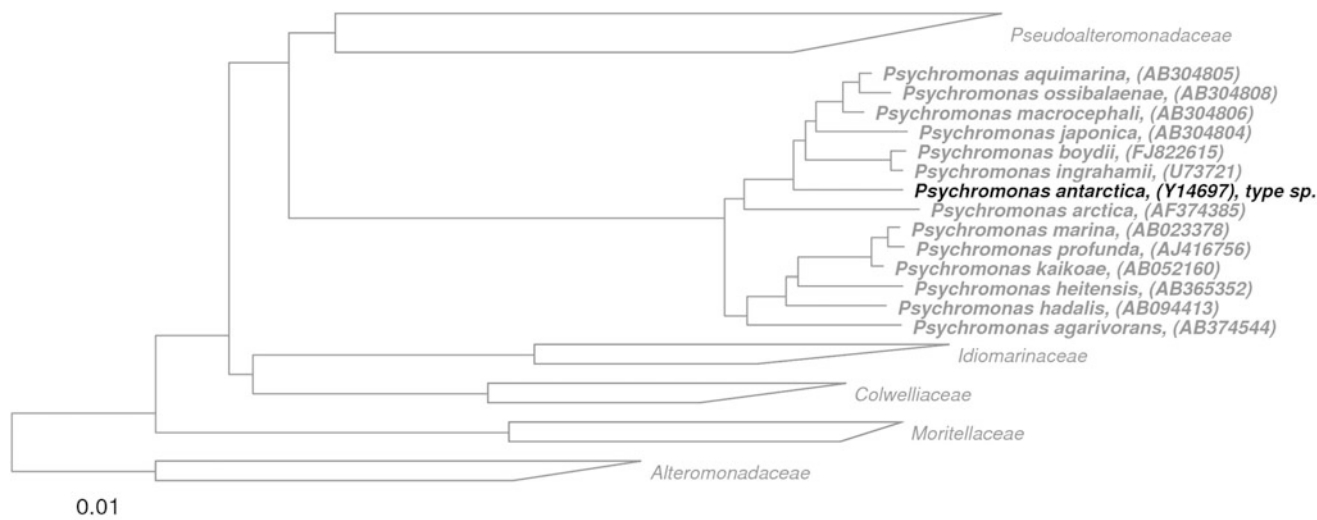


Fig. 29.1

Phylogenetic reconstruction of the family *Psychromonadaceae* based on the neighbor-joining algorithm with the Jukes-Cantor correction. Sequence dataset and alignments are according to the All-Species Living Tree Project (LTP) online database (LTPs108, July 2012) (Yarza et al., 2008). The tree topology was stabilized with the use of a representative set of 767 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

show DDH values less than <20 % (Miyazaki et al. 2008). Additional DDH values are available for the neighboring species *P. boydii* and *P. ingrahamii*, sharing 98.9 % 16S rRNA gene sequence similarity (<63 % DDH similarity; Auman et al. 2010), as well as for *P. marina*, *P. kaikoa* and *P. profunda*, which share 98.3–98.6 % 16S rRNA gene sequence similarity (<38 % DDH similarity) (Xu et al. 2003).

Genome Analyses

Psychromonas ingrahamii 37^T is the only strain of the family for which the full genome sequence has been generated (INSDC ID CP000510) (Riley et al. 2008). The single replicon genome is 4,559,598 bp long, with 40.1 % of extrachromosomal elements absent. There are 10 ribosomal RNA clusters containing 5S, 16S, 23S RNAs. Altogether 3,708 genes were identified, 3,545 of which were proteins of 83 residues or longer. Correspondence analysis of the composition of all *P. ingrahamii* proteins showed the following: (1) there are six classes of proteins, at least one more than other bacteria; (2) integral inner membrane proteins are not sharply separated from bulk proteins, suggesting that, overall, they may have a lower hydrophobic character; (3) there is strong opposition between asparagine and the oxygen-sensitive amino acids methionine, arginine, cysteine, and histidine; and (4) one of the previously unseen clusters of proteins has a high proportion of “orphan” hypothetical proteins, raising the possibility that these are cold-specific proteins. Based on annotation of proteins by sequence similarity, the following can be concluded: (1) *P. ingrahamii* has a large number (61) of regulators of cyclic GDP, suggesting that this bacterium produces an extracellular polysaccharide that may help sequester

water or lower the freezing point in the vicinity of the cell; (2) *P. ingrahamii* has genes for production of the osmolyte betaine choline, which may balance the osmotic pressure as sea ice freezes; (3) *P. ingrahamii* has a large number (11) of three-subunit TRAP systems, which may play an important role in the transport of nutrients into the cell at low temperatures; (4) chaperones and stress proteins may play a critical role in transforming nascent polypeptides into three-dimensional configurations that permit low temperature growth; and (5) the metabolic properties of *P. ingrahamii* were deduced. Finally, a few small sets of proteins of unknown function, which may play a role in psychrophily, have been singled out as worthy of future study.

Phenotypic Analyses

Psychromonas (Mountfort et al. 1998, emended by Nogi et al. 2002) Psy.chro.mo'nas. Gr. adj. psychros cold; Gr. fem. n. monas a unit; N.L. n. *Psychromonas* a cold monad.

Psychromonas cells may be Gram-negative rods or oval cells, nonspore, and motile or nonmotile. The flagellum is single or subpolar, sheathed or unsheathed. The cells grow at temperatures less than 37 °C. They are facultative anaerobes, but some species may grow to be aerotolerant anaerobes or aerobes. They use carbohydrates for energy for growth. Oxidase is positive and catalase is variable. The major isoprenoid quinone is Q-8. The predominant cellular fatty acids are C_{16:0} and C_{16:1}. The G+C DNA is 38–43.8 mol%.

The type species is *Psychromonas antarctica*, and the type strain is star-1^T. Type strains of the other species are indicated in Table 29.1.

■ Table 29.1

Differential characteristics of *Psychromonas* species

Characteristic	<i>P. antarctica</i> ^a	<i>P. agarivorans</i> ^b	<i>P. aquamarina</i> ^c	<i>P. arctica</i> ^d	<i>P. boydii</i> ^e	<i>P. hadalis</i> ^f	<i>P. heitensis</i> ^g
Morphology							
Cells form	Ovoid rods	Rods	Rods	Rods	Large rods, chains, single cells	Rods	Rods
Size (length)	1.3 × 2.5–6	0.5–0.8 × 0.8–1.2	0.9–1.1 × 1.6–3.2	0.7–1.7 × 1.3–2.6	8–18	0.8–1.0 × 1.5–2	0.5–1.0 × 1.0–1.5
Flagellum	Single polar	Single subpolar	Single polar sheathed	ND	No flagella	Single subpolar	Single subpolar
Production of gas vesicles	–	ND	–	–	+	–	–
Colony color	White	ND	Cream	White	White	ND	ND
Motility	+	+	+	+	–	+	+
Growth(°C)							
Optimum	12	20–25	20–25	20	ND	6, 60 MPa	20–25
Maximum	17	30	27	25	10	12	30
Minimum	2	2	5	0	0	2	2
NaCl(%)							
Optimum	3	ND	3	2	3.5	3	ND
Maximum	4	4	5	7	18	ND	4
Minimum	0	1	2	1	2	>0	3
pH							
Optimum	6.5	ND	8.5	8.5–8.8	ND	ND	ND
Maximum	ND	9.0	9.0	9.8	7.4	ND	9.0
Minimum	ND	6.0	6.0	6.5	6.5	ND	6.0
O ₂ requirement for growth ^h	ATAN	FAN	FAN	A	FAN	FAN	FAN
Growth at atmospheric pressure	+	+	+	+	+	–	+
Enzyme							
Catalase	+	–	–	+	+	+	+
Oxidase	+	+	+	+	+	+	+
Lipase (tri-n-butyrin)	ND	ND	–	ND	ND	ND	ND
Hydrolysis of							
Gelatin	+	–	+	–	–	–	+
Casein	ND	+	w	ND	ND	ND	ND
Starch	+	v	+	+	–	–	+
DNA	ND	+	+	ND	ND	ND	+
Tween 20	ND	–	+	ND	–	ND	+
Tween 40	ND	–	+	ND	–	ND	+
Tween 60	ND	–	ND	ND	–	ND	+
Tween 80	ND	–	+	ND	–	ND	+
Agar	–	+	–	–	ND	–	–
Production of							
H ₂ S	–	–	–	ND	–	–	–
Indole	–	–	–	ND	–	–	–

Table 29.1 (continued)

Characteristic	<i>P. antarctica</i> ^a	<i>P. agarivorans</i> ^b	<i>P. aquamarina</i> ^c	<i>P. arctica</i> ^d	<i>P. boydii</i> ^e	<i>P. hadalis</i> ^f	<i>P. heitensis</i> ^g
Reduction of							
Nitrate	–	–	+	–	+	+	–
Nitrite	–	ND	–	ND	ND	+	ND
Utilizes or acid production from							
L-Arabinose	–	–	–	ND	–	–	–
Cellobiose	+	+	–	ND	+	–	+
D-Fructose	+	–	+	+	+	+	+
D-Galactose	+	+	+	ND	+	+	–
D-Glucose	+	+	+	+	+	+	+
Glycerol	–	–	+	+	–	+	–
myo-inositol	–	–	–	ND	+	+	–
D-Lactose ^m	–	ND	ONPG+	+	–	–	ONPG+
Maltose	+	+	+	+	+	+	+
D-Mannitol	+	–	+	+	+	+	+
D-Mannose	w	–	+	+	+	+	–
D-Raffinose	–	–	–	ND	ND	–	–
L-Rhamnose	–	–	–	ND	ND	–	–
D-Sorbitol	–	–	–	ND	–	–	ND
Sucrose	+	–	+	+	+	–	+
D-Trehalose	+	–	+	ND	+	+	+
D-Xylose	–	–	–	–	+	–	–
Chemotaxonomic properties							
Predominant fatty acids	C _{16:1} ω7c and C _{16:0}	C _{16:1} ω7c and C _{16:0}	C _{16:1} and C _{16:0}	C _{16:0} , C _{16:1} ω7c, C _{16:1} ω7t and C _{18:1} ω7	C _{16:1} ω7c and C _{16:0}	C _{16:1} , C _{16:0} and C _{14:1}	C _{16:1} ω7c and C _{16:0}
GC (mol%)	42.8	41–42	42.2	40.1	40	39.1	38
Characteristic	<i>P. ingrahamii</i> ^h	<i>P. japonicus</i> ^c	<i>P. kaikoei</i> ⁱ	<i>P. macrocephalus</i> ^c	<i>P. marina</i> ^j	<i>P. ossibalaenae</i> ^c	<i>P. profunda</i> ^k
Morphology							
Cells form	Large rods	Rods	Rods	Rods	Rods	Rods	Rods
Size (length)	1.25–1.5 × 6–14	0.5–0.8 × 1.6–2.2	0.8–1.0 × 2.0–4.0	0.6–0.8 × 2.2–2.9	0.8–1.2 × 1.5–2.0	0.7–1.1 × 1.9–3.7	0.9–1.2 × 2.0–5.5
Flagellum	ND	Single polar	Single polar	Single polar sheathed	Single polar	Single polar	Single polar
Production of gas vesicles	+	–	–	–	–	–	–
Colony color	White	Translucence cream	ND	Cream	Colorless	Cream	Colorless
Motility	–	+	+	+	+	+	+
Growth (°C)							
Optimum	ND	20–22	10, 50 MPa	20	ND	20	ND
Maximum	10	25	15	25	25	25	14
Minimum	–12	5	4	0	0	0	2
NaCl (%)							
Optimum	ND	3	3	3	3–5	3	ND
Maximum	12	5	ND	6	7	5	ND
Minimum	1	2	>0	2	>0	2	>0

■ Table 29.1 (continued)

Characteristic	<i>P. ingrahamii</i> ^h	<i>P. japonicus</i> ^c	<i>P. kaikoeae</i> ^l	<i>P. macrocephalus</i> ^c	<i>P. marina</i> ^l	<i>P. ossibalaenae</i> ^c	<i>P. profunda</i> ^k
pH							
Optimum	ND	9.0	ND	8.5	ND	9.0	ND
Maximum	7.4	9.5	ND	9.0	ND	9.5	ND
Minimum	6.5	6.5	ND	6.0	ND	6.5	ND
O ₂ requirement for growth ^l	FAN	FAN	FAN	FAN	FAN	FAN	FAN
Growth at atmospheric pressure	+	+	–	+	+	+	+
Enzyme							
Catalase	+	+	+	–	+	–	+
Oxidase	+	+	+	+	+	+	+
Lipase (tri-n-butyrin)	ND	–	ND	–	+	+	–
Hydrolysis of							
Gelatin	–	w	+	+	–	–	–
Casein	ND	–	ND	+	–	–	ND
Starch	–	–	–	–	+	–	w
DNA	ND	+	ND	+	+	+	+
Tween 20	ND	+	ND	+	+	–	ND
Tween 40	ND	–	ND	+	+	–	ND
Tween 60	ND	ND	ND	ND	+	ND	ND
Tween 80	ND	–	ND	+	+	–	ND
Agar	–	–	–	–	–	–	–
Production of							
H ₂ S	ND	–	–	–	+	–	+
Indole	–	–	–	–	–	–	+
Reduction of							
Nitrate	+	+	+	+	+	+	+
Nitrite	ND	–	–	–	ND	–	ND
Utilizes or acid production from							
L-Arabinose	–	–	–	–	–	–	–
Cellobiose	+	+	+	–	+	+	+
D-Fructose	+	+	+	+	+	+	+
D-Galactose	+	–	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+
Glycerol	+	–	–	+	+	+	+
myo-inositol	–	–	–	–	–	–	+
D-Lactose ^m	–	ONPG–	–	ONPG+	+	ONPG+	+
Maltose	ND	–	+	+	+	+	+
D-Mannitol	+	–	+	–	+	–	+
D-Mannose	–	+	+	+	–	+	+
D-Raffinose	ND	–	–	–	–	w	–
L-Rhamnose	ND	–	–	–	–	–	+
D-Sorbitol	–	–	–	–	–	–	–
Sucrose	+	–	+	–	+	+	+

Table 29.1 (continued)

Characteristic	<i>P. ingrahamii</i> ^h	<i>P. japonicus</i> ^c	<i>P. kaikoei</i> ⁱ	<i>P. macrocephalus</i> ^c	<i>P. marina</i> ^j	<i>P. ossibalaenae</i> ^c	<i>P. profunda</i> ^k
D-Trehalose	+	–	+	+	–	+	+
D-Xylose	–	–	–	–	+	+	+
Chemotaxonomic properties							
Predominant fatty acids	C _{16:1} ω7c and C _{16:0}	C _{16:1} and C _{16:0}	C _{16:1} ω7c, C _{16:0} and C _{14:1} ω7t	C _{16:1} and C _{16:0}	C _{16:1} and C _{16:0}	C _{16:1} and C _{16:0}	C _{16:1} , C _{16:0} and C _{14:1}
GC(mol%)	40	38.8	43.8	41.5	43.5	41.3	38.1

All strains showed identical biochemical characteristics, except those indicated here. + Positive, –, negative, v variable reaction, w week positive, ND no data from

^aMountfort et al. 1998

^bHosoya et al. 2009

^cMiyazaki et al. 2008

^dGroudieva et al. 2003

^eAuman et al. 2010

^fNogi et al. 2007

^gHosoya et al. 2008

^hAuman et al. 2006

ⁱNogi et al. 2002

^jKawasaki et al. 2002

^kXu et al. 2003

^lA Aerobe, ATAN aerotolerant anaerobe, FAN facultative anaerobe

^mResults prefixed with ONPG indicate that strains were not tested for lactose utilization directly but, rather, for ONPG hydrolysis/b-galactosidase activity

Some species produced C_{20:5}ω3 and/or C_{22:6}. In addition, *P. ingrahamii* (Auman et al. 2006) and *P. boydii* (Auman et al. 2010) have the following properties. They contain two gas vesicle morphologies. Both strains use the following as sole carbon sources: DL-aspartate, DL-lactate, L-aspartate, L-cysteine, L-glutamate, acetate, pyruvate, and N-acetyl-glucosamine; they do not use D-gluconate, D-glucuronate, DL-malate, L-leucine, L-proline, α-ketoglutarate, benzoate, caproate, glycolate, or methanol. *P. ingrahamii* uses D-glucuronate, fumarate, propionate, salicin, succinate, and glucosamine as sole carbon sources. *P. boydii* uses only citrate as a sole carbon source. *P. agarivorans* (Hosoya et al. 2009) and *P. heitensis* (Hosoya et al. 2008) use API 20NE, API 50CH, and API ZYM (bioMérieux). The major fatty acids are C_{16:0} and C_{16:1}. The abundance ratio of unsaturated fatty acids was ≥50 %, with the exception of *P. marina*.

Isolation, Enrichment and Maintenance Procedures

Isolation and Enrichment

P. agarivorans J42-3A^T (Hosoya et al. 2009) was isolated from marine sediments collected at Kori, off Nomozaki, Nagasaki in Japan, on solidified 1/10 strength marine broth [900 ml filtered seawater and 100 ml marine broth 2216 (Difco)] with 1.5 % (w/v) agar and supplemented with 0.5 % Rose bengal.

P. antarcticus star-1^T (Mountfort et al. 1998) was isolated from the sediment of Salt Pond near Bratina Island on the McMurdo Ice Shelf. A complex medium was prepared as the basal medium, except that trypticase and yeast extract were each

present at 0.2 % (w/v). The sporulation medium was according to that described by Duncan and Strong (1968). The solid basal or complex medium for slants contained 2 % agar; the sterile reduced medium was dispensed in 3-ml amounts into sterile 13-mm × 100-mm culture tubes fitted with no. 00 black rubber stoppers. The composition of liquid aerobic complex medium was the same as for the anaerobic medium except that the medium was not boiled during its preparation, a reducing agent was not added prior to use, and air replaced N₂–CO₂ as the gas phase. The solid complex medium for plates contained 2 % agar.

P. aquimarina JAMM 0404^T (Miyazaki et al. 2008) was isolated from the sediment on the seabed adjacent to a sperm whale carcass off Kagoshima, Japan, after a 1-week incubation at 15 °C on marine agar 2216.

P. arctica Pull 5.3^T (Groudieva et al. 2003) was isolated from a seawater sample taken near Svalbard, Spitzbergen. The complex medium contained (L⁻¹): NaCl, 28.13 g; KCl, 0.77 g; CaCl₂·2H₂O, 0.02 g; MgSO₄·7H₂O, 0.5 g; NH₄Cl, 1.0 g; iron ammonium citrate, 0.02 g; yeast extract, 0.5 g; 10-fold concentrated trace element solution (DSM 141), 1 ml; 10-fold concentrated vitamin solution (DSM 141), 1 ml; KH₂PO₄, 2.3 g; Na₂HPO₄·2H₂O, 2.9 g; starch, 5 g. The pH was adjusted with NaOH to 7.2.

P. boydii 174^T (Auman et al. 2010) was isolated from an open water site at Point Barrow, Alaska, USA, about 40–60 cm from the ice/water interface from a 1.8-m ice core grown on Ordal's seawater cytophaga medium (SWCm) using full-strength artificial seawater (Irgens et al. 1989).

P. hadalis K41G^T (Nogi et al. 2007) was isolated from sediment collected from the bottom of the Japan Trench on marine agar 2216 (Difco) and was grown at 6 °C and 60 MPa. High-pressure cultivation was achieved using a liquid hydraulic

system. Piezophilic bacteria were cultivated in a plastic bag containing liquid medium in a pressure vessel made of stainless steel (SUS304). If necessary, oxygen-saturated fluorinert (FC-72; Sumitomo-3 M) was added to supply oxygen to the cultures (20 % of total volume). This was performed according to the procedure reported previously (Kato et al. 1994; Yanagibayashi et al. 1999).

P. heitensis AK15-027^T (Hosoya et al. 2008) was isolated from seawater taken from Heita Bay off Kamaishi in Japan. Strain AK15-027^T was isolated from seawater taken from Heita Bay in July 2003, using full-strength seawater agar that contained 100 % (v/v) filtered seawater and 1.5 % (w/v) agar, supplemented with 5 % (v/v) vitamin solution of the DSM141 medium (DSMZ 1993). The isolates were cultured and maintained on 1/5-strength marine agar [800 ml filtered seawater, 200 ml distilled water, 7.48 g marine broth 2216 (Difco) and 15 g agar].

P. ingrahamii 37^T (Auman et al. 2006) was isolated from a sea ice core collected from Point Barrow, Alaska, USA. Ordal's seawater cytophaga medium (SWCm), prepared in full-strength artificial seawater, was used for the isolation and routine growth of strain 37^T (Irgens et al. 1989).

P. japonica JAMM 0394^T (Miyazaki et al. 2008) was isolated from the sediment on the seabed adjacent to a sperm whale carcass off Kagoshima, Japan, after a 1-week incubation at 15 °C on marine agar 2216.

P. kaikoae AK15-027^T (Nogi et al. 2002) was isolated from sediment collected from the deepest cold-seep environment with chemosynthesis-based animal communities within the Japan Trench, at a depth of 7,434 m. The isolated piezophilic strains were grown in marine broth 2216 (Difco) at 10 °C and 50 MPa. High-pressure cultivation was achieved using a liquid hydraulic system. Piezophilic bacteria were cultivated in a plastic bag containing liquid medium in a pressure vessel made of stainless steel (SUS304). If necessary, oxygen-saturated Fluorinert (FC-72; Sumitomo-3 M) was added to supply oxygen to the cultures (20 % total volume). This method was performed according to the procedure reported previously (Kato et al. 1994; Yanagibayashi et al. 1999).

P. macrocephali JAMM 0415^T (Miyazaki et al. 2008) was isolated from the sediment on the seabed adjacent to a sperm whale carcass off Kagoshima, Japan, after a 1-week incubation at 15 °C on marine agar 2216.

P. marina 4-22^T (Kawasaki et al. 2002) was isolated from a cold current off the Monbetsu coast of the Okhotsk Sea in Hokkaido, Japan, after a 4-week incubation at 0 °C on marine agar 2216.

P. ossibalaenae JAMM 0738^T (Miyazaki et al. 2008) was isolated from the sediment on the seabed adjacent to a sperm whale carcass off Kagoshima, Japan, after a 1-week incubation at 15 °C on marine agar 2216.

P. profunda 2825^T (Xu et al. 2003) was isolated from deep Atlantic sediments at a depth of 2,770 m and a temperature of 2 °C. Sediment from the upper 2-cm layer was suspended in cold 75 % sterile seawater and spread onto chilled seawater agar plates prepared with a medium containing 1.5 g peptone, 0.5 g

yeast extract, 0.01 g FePO₄·4H₂O, 750 ml seawater, and 250 ml distilled water. Sampling and isolation methods were described in detail by Ruger and Tan (1992).

Maintenance

Serial transfers of each medium on agar or in liquid medium at 4-week intervals followed by maintenance at 4 °C are recommended for medium-term storage, as is maintenance of cells as 15–20 % (w/v) glycerol suspensions in an appropriate medium at –80 °C. Long-term preservation methods include freeze-drying in skim milk or maintenance in liquid nitrogen at –196 °C.

Ecology

The main habitat of family *Psychromonadaceae* appears to be marine, sea ice, or the saline aquatic environments from which the original cultures were isolated. This applies also to *Psychromonas* species described or reclassified recently: *P. antarcticus* was isolated from Antarctic saline pond (Mountfort et al. 1998); *P. agarivorans* (Hosoya et al. 2009), *P. heitensis* (Hosoya et al. 2008) and *P. marina* (Kawasaki et al. 2002) were isolated from the coast of Japan; and *P. ingrahamii* (Auman et al. 2006) and *P. boydii* (Auman et al. 2010) were isolated from the ice core at Point Barrow, Alaska, USA. *P. ingrahamii* and *P. boydii* grow above 10 % (w/v) NaCl, and *P. ingrahamii* grows at –12 °C (Breezee et al. 2004). *P. aquimarina*, *P. japonica*, *P. macrocephali*, and *P. ossibalaenae* (Miyazaki et al. 2008) were isolated from the sediment on the seabed adjacent to a sperm whale carcass off Kagoshima, Japan. This environment has very abundant organic matter (Fujiwara et al. 2007). *P. hadalis* (Nogi et al. 2007), *P. kaikoae* (Nogi et al. 2002), and *P. profunda* (Xu et al. 2003) were isolated from deep sea sediment. Increased pressure enhances the growth of *P. profunda* (Xu et al. 2003) and is required for the growth of *P. hadalis* (Nogi et al. 2007) and *P. kaikoae* (Nogi et al. 2002). The barophilic strain CNPT3 (Delong et al. 1997) proved to be closely related to *P. kaikoae* (Margesin and Nogi 2004). *P. arctica* (Groudieva et al. 2003) was from a seawater sample taken near Svalbard, Spitzbergen. In this way, all species were isolates from a natural environment that was rich in aqueous and mineral salts. Most of the species were grown at minimum temperatures of 0–4 °C and exhibit both fermentative and respiratory metabolism.

Pathogenicity, Clinical Relevance

The information on antibiotic sensitivity and resistance is limited to the family *Psychromonadaceae*. *Psychromonas profunda* cells are susceptible to penicillin G, tetracycline, chloramphenicol, furazolidone, and polymyxin B. No information on antibiotic sensitivity and resistance is available for the other species.

Application

Serine hydroxymethyltransferase from *Psychromonas ingrahamii* was characterized with respect to its spectroscopic, catalytic, and thermodynamic properties (Angelaccio et al. 2012). The properties of the psychrophilic enzyme have been contrasted with the characteristics of its homologous counterpart from *Escherichia coli*, which has been structurally and functionally characterized in depth and with which it shares 75 % of its sequence identity. Spectroscopic measures confirmed that the psychrophilic enzyme displays structural properties that are almost identical to those of its mesophilic counterpart. At variance, the *P. ingrahamii* enzyme showed decreased thermostability and high specific activity at low temperatures, both of which are typical features of cold-adapted enzymes. Furthermore, the enzyme was a more efficient biocatalyst compared to *E. coli* serine hydroxymethyltransferase (SHMT), particularly for side reactions. Many β -hydroxy- α -amino acids are SHMT substrates and represent important compounds in the synthesis of pharmaceuticals, agrochemicals, and food additives.

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