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NOTE

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Psychromonas aquatilis sp. nov., isolated from seawater samples obtained in the Chilean Antarctica

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Abstract

A slightly beige-white pigmented, Gram-staining-negative, rod-shaped bacterium, strain M1A1^T, was isolated from seawater samples obtained in Fildes Bay, Antarctica (62°12′ S 58° 57′ W). Phylogenetic analysis based on nearly full-length 16S rRNA gene sequences showed that the isolate shared 98.4 % 16S rRNA gene sequence identity to the type strain of *Psychromonas arctica*, but less than 97 % 16S rRNA gene sequence similarities to all other species of the genus *Psychromonas*. DNA–DNA hybridization with *Psychromonas arctica* DSM 14288^T showed low values (21 %, reciprocal 27 %). The main cellular fatty acid of strain M1A1^T was summed feature 3 fatty acids ($C_{16:1}\omega7c/C_{16:1}\omega8c$), followed by $C_{16:0}$. Based on phylogenetic, chemotaxonomic, genomic and phenotypic analyses, we propose a novel species of the genus *Psychromonas* with the name *Psychromonas aquatilis* sp. nov. and the strain M1A1^T (=CIP 111183^T=CCM 8710^T=LMG 29766^T) as type strain.

Since the description of *Psychromonas antarctica* [1], additional members of the genus *Psychromonas* have been isolated and described. All members are Gram-negative rods usually exhibiting growth at low temperatures and requiring salt for growth. The G+C content averages 40(±3) mol% and their membranes are enriched in 16-carbon saturated and unsaturated fatty acids [1–12]. Beyond these similarities, the strains of members of the genus *Psychromonas* described to date have a great deal of variety in other physiological characteristics including cell size, temperature range for growth, piezophily, presence of gas vacuoles, and carbon sources utilized. Here we describe a slightly beige-white pigmented bacterial strain, designated M1A1^T, isolated from a water sample from Antarctica and shown to represent a novel species of the genus *Psychromonas*.

Antarctic water samples were collected in March 2014 during the fiftieth Scientific Antarctic Expedition (ECA-50), to King George Island (62° 002′ S 58° 21′ W), Chilean Antarctica. Water samples were collected and transported to the laboratory at Antarctic Research Station *Professor Julio Escudero Base* for bacteriological examination. Samples were directly inoculated onto tryptone soya agar (Oxoid) supplemented with 1 % (w/v) NaCl (TSA-1) and incubated for 1 week at 18 °C under aerobic conditions. Isolation of a pure culture was obtained from plates and a representative colony was selected, which was streaked onto a new TSA-1 plate to obtain pure cultures, and then stored at −80 °C in Criobille tubes (AES Laboratories) as well as in tubes containing tryptone soya broth (TSB) supplemented with 1 % (w/v) NaCl and 20 % (v/v) glycerol. The original colonies of strain M1A1^T had a diameter of approximately 2 mm, with smooth edges and a beige-white pigmentation. The strain was sub-cultivated on TSA-1 at 18 °C and can be maintained for several weeks at 6–8 °C.

The nearly full-length 16S rRNA gene of strain M1A1^T was PCR-amplified with universal 16S rRNA gene targeting primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) [13], and sequenced by the Sanger method with primers 27F (5′-GAGTTTGATCMTGGCTCAG-3′) and E786F (5′-GATTAGATACCCTGGTAG-3′) at LGC Genomics (Berlin, Germany). The manually corrected 16S rRNA gene sequence had a length of 1471 nucleotides spanning gene termini 46 to 1514 (numbered according to the *Escherichia coli rrnB* reference sequence published by Brosius *et al.* [14]). *Psychromonas arctica* was obtained as the closest related species with 98.4 % 16S rRNA gene sequence similarity by a first BLAST search in the EzTaxon 16S rRNA gene sequence type strain database [15]. The phylogenetic placement of strain M1A1^T was investigated in detail by the

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reconstruction of phylogenetic trees using the 'All species living tree project' database (LTPs; [16]) release LTPs123 (September 2015) and the ARB software package release 5.2 [17]. Sequences not included in the LTP database were added after alignment with the SILVA Incremental Aligner (SINA; v1.2.11; [18]). After manual correction of the alignment, considering the secondary structure of the 16S rRNA, phylogenetic trees were calculated with the maximum-likelihood method using RAxML version 7.04 [19] with GTR-GAMMA and rapid bootstrap analysis, the maximum-parsimony method using DNAPARS v. 3.6 [20], and the neighbour-joining method using ARB neighbour-joining and the Jukes–Cantor correction [21]. All phylogenetic trees based on 100 resamplings (bootstrap analysis; [22]) and sequence positions between gene termini 54 and 1454 (according to Brosius *et al.* [14]) were considered. Type strains of members of the next closest related families (*Idiomarinaceae*, *Pseudoalteromonadaceae*, *Moritellaceae*) were included in the phylogenetic analysis to demonstrate the distinct phylogenetic placement of the genus *Psychromonas* as the exclusive member of the family *Psychromonadaceae*. Pairwise sequence similarities were calculated within ARB using the ARB neighbour-joining tool, without considering evolutionary models.

The pairwise 16S rRNA gene sequence similarity of strain M1A1^T was highest to the type strain of *P. arctica* (98.4 %). Sequence similarities to type strains of all other species of the genus *Psychromonas* were below 97.0 % (95.1 to 96.5 %). Phylogenetic trees indicated, independent of the treeing method, the placement of strain M1A1^T into the monophyletic cluster of the genus *Psychromonas*, representing the *Psychromonadaceae* (Fig. 1). Strain M1A1^T and the type strain of *P. arctica* formed a distinct cluster within the genus *Psychromonas*.

DNA–DNA hybridization experiments were performed with strain M1A1^T and the type strain of *P. arctica* DSM 14288^T according to the method of Ziemke *et al.* [23] (except that for nick translation 2 µg DNA were labelled during 3 h of incubation at 15 °C) using DNA extracted by the method of Pitcher *et al.* [24]. Strain M1A1^T showed low DNA–DNA relatedness to *P. arctica* DSM 14288^T (21 %, reciprocal 27 %). The DNA melting temperature method established by Gonzalez and Saiz-Jimenez [25] was used to determine the genomic G+C content of strain M1A1^T. The analysis was performed as described in detail by Glaeser *et al.* [26]. The genomic G+C content of strain M1A1^T was 38.5 mol%, which was in the range of the G+C content reported for species of the genus *Psychromonas* and slightly lower than the G+C content reported for *P. arctica* (40.1 mol%; [6]).

For detailed phenotypic characterization, strain M1A1^T was cultured on TSA-1 at 18 °C for 3 days. Gram-staining was performed by the modified Hucker method according to Gerhardt *et al.* [27]. Cell morphology was analysed by light microscopy at ×1000 magnification with a Leica DFC 3000G microscope. Cytochrome oxidase activity was tested with

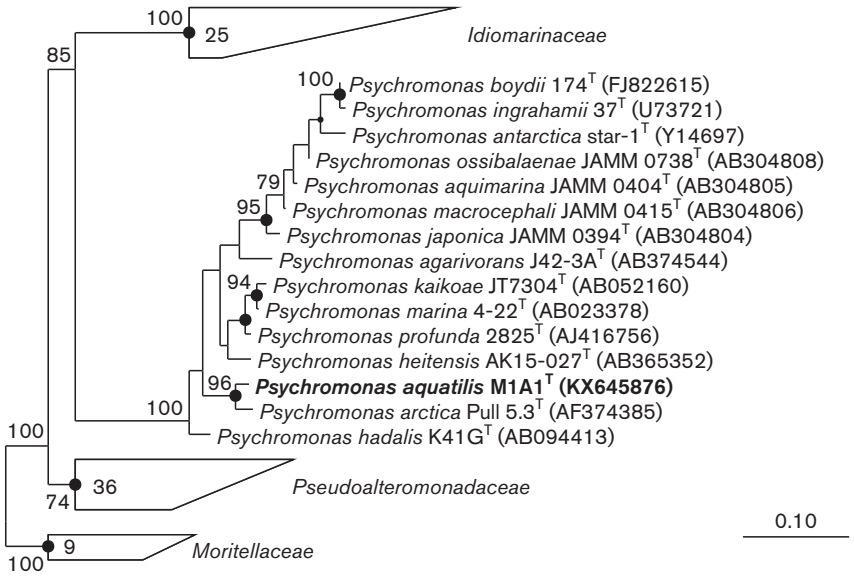


Fig. 1. Phylogenetic tree based on nearly full-length 16S rRNA gene sequences showing the placement of strain M1A1^T within the genus *Psychromonas* (family *Psychromonadaceae*). The phylogenetic tree was generated in ARB with the maximum-parsimony method and based on sequence termini 54 and 1454 (numbering according to Brosius *et al.* [14]) and 100 replications (bootstrap support). Bootstrap values >70 % are depicted at nodes. Filled circles mark nodes which were also present in the maximum-likelihood tree; larger circles represent nodes which had a bootstrap value >70 % in the maximum-likelihood tree. Numbers in clusters represent number of species included in the cluster. The family *Moritellaceae* was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

Microbiology Bactident oxidase test strips (Merck), and catalase activity was tested by gas bubble formation after dropping 3 % H_2O_2 (v/v) onto a fresh culture grown on TSA-1.

Growth was tested on R2A (Oxoid), nutrient agar (NA; Oxoid), K7 [0.1 % (w/v) yeast extract, peptone and glucose, 15 g l^{-1} agar, pH 6.8], medium 65 according to DSMZ (4.0 g yeast extract, 10.0 g malt extract, 4.0 g D-glucose, 2.0 g $CaCO_3$, 12 g l^{-1} agar, pH 6.8) and PYE [0.3 % (w/v) yeast extract and 0.3 % (w/v) casein peptone, respectively, 15 g agar l^{-1}, pH 7.2], Luria–Bertani (LB; Sigma-Aldrich), tryptic soy agar with and without the addition of 1 % (w/v) NaCl (TSA-1 and TSA , respectively; Becton Dickinson), malt agar (Merck), DEV agar (DEV; 'Deutsche Einheitsverfahren' Merck), marine agar 2216 (MA; Becton Dickinson), Columbia agar with sheep blood (BA; Liofilchem), glycine arginine agar, MacConkey agar (McC; Oxoid), thiosulfate-citrate-bile salts agar (TCBS; Oxoid) and Müller-Hinton agar with and without the addition of 1 % (w/v) NaCl (MH and MH-1, respectively; Oxoid). Temperature-dependent growth was tested on TSA-1 at 0.5, 4, 10, 15, 20, 25, 28, 30, 33, 37, 45, 50 and 55 °C, salinity dependent growth was tested at 18 °C in TSB bouillon supplemented with 0 to 10.0 % (w/v) NaCl, and pH-dependent growth was tested using R2A bouillon supplemented with 2 % (w/v) NaCl and adjusted to pH values of pH 4 to 9 (in 0.5 pH units intervals). The pH values were adjusted using 1 M HCl and 1 M KOH and stabilized by the addition of 5 mM phosphate buffer adjusted to the same pH values. Further physiological tests were performed with API 20NE, API ZYM and API 50CH test strips (bioMérieux) as described by the manufacturer's instructions, except for the incubation temperature that was set at 18 °C. API 50CH was used to test acid production; therefore a loop of fresh biomass was suspended in pure water to McFarland 4 and added to a tube of API 50CHB/E medium for inoculation of the test panels. All panels were analysed after incubation for 3 days at 18 °C, except nitrate reduction and indole production (API 20NE) were analysed after 48 h. Further comparative phenotypic characterization of the strain was carried out using the 96-well physiological test panel according to Kämpfer *et al.* [28]. For inoculation of the panel, strains were suspended in 0.9% NaCl to a McFarland value of 0.5. Analysis of acid production, carbon substrate assimilation, and enzyme activity was performed after 3 days of growth at 25 °C.

Strain M1A1^T showed a rod-shaped morphology, single or in pairs, with a cell size of 1.2×3.0 µm. The strain grew well at 0.5 to 28 °C on BA, MA, MH, MH-1, TSA and TSA-1, but there was no growth on Malt, CASO, glycine arginine, NA, McC, R2A, TCBS, K7, PYE and DEV agars, weak growth was observed on LB agar. Good growth was obtained after incubation for 2 days between 4 and 28 °C (no growth at 30 °C or above), in the presence of 0–7.0 % (w/v) NaCl. The best growth occurred with 1–3 % NaCl in which the formation of cell clumps was observed. Growth was observed between pH 6.5 and 8.0.

Results of the test panel according to Kämpfer *et al.* [28] showed growth with the different carbon sources but acid production from several sugars and sugar-related compounds was not observed (Table 1). Physiological tests performed with the API test systems resulted in differences in comparison to the type strains of species of the genus *Psychromonas*. The differentiating physiological characteristics of strain M1A1^T compared to type strains of other species of the genus *Psychromonas* are listed in Table 1. All physiological properties of the novel strain are provided in the species description.

Biomass for fatty acid analysis was harvested after growth on TSA at 28 °C for 48 h. The analysis was performed as described by Kämpfer and Kroppenstedt [29]. Fatty acids were separated with a 5898A gas chromatograph (Hewlett Packard), respective peaks were automatically integrated and fatty acid names and percentages were determined with the Sherlock MIDI software v. 2.1 (TSBA v. 4.1). The fatty acid profile of strain M1A1^T was consistent with the profiles described for species of the genus *Psychromonas* as shown in Table 2, with the predominant unsaturated fatty acid $C_{16:1}\omega7c$/$C_{16:1}\omega8c$ shown as summed feature 3 and lower amounts of the straight-chain saturated fatty acid $C_{16:0}$ (Table 2).

Based on genotypic, chemotaxonomic and physiological data, strain M1A1^T represents a novel species of the genus *Psychromonas*, for which the name *Psychromonas aquatilis* sp. nov. is proposed.

DESCRIPTION OF *PSYCHROMONAS AQUATILIS* SP. NOV.

Psychromonas aquatilis (a.qua'ti.lis. L. fem. adj. *aquatilis* living in water).

Gram-staining-negative, rod-shaped with a cell size of approximately 1.2×3.0 µm. Colonies grown after 3 days on TSA-1 at 18 °C are circular, shiny, and beige-white-pigmented with a smooth border. Motile, aerobic and catalase- and cytochrome oxidase-positive. Good growth at 18 °C after incubation for 3 days on BA, MA, MH, MH-1, TSA and TSA-1, no growth on Malt, CASO, glycine arginine, NA, McC, R2A TCBS, K7, PYE and DEV agars and weak growth on LB agar. Optimal growth occurs between 5 and 22 °C, no growth at 30 °C and above. Good growth in the presence of 0–3.0 % (w/v) NaCl, and at pH 6.5 to 7.0, slight growth up to 7.0 % NaCl and at pH 8.0, but not at 8.0 % NaCl and pH 5.0 or below or at pH 8.5 and above. Negative for indole production, arginine dihydrolase, β-galactosidase, acid production from L-arabinose, potassium gluconate and malic acid, and assimilation of *N*-acetylglucosamine (API 20NE), capric acid, adipic acid, trisodium citrate and phenylacetic acid, but positive for aesculin hydrolysis. Positive for alkaline phosphatase, leucine arylamidase, acid phosphatase and naphtol-AS-BI-phosphydrolase activity (API ZYM). According to API 50 CH tests, the type strainis only positive for aesculin and ferric citrate. The fatty acids

Table 1. Comparison of characteristics of strain M1A1^T and other members of the genus *Psychromonas*

Strains: 1, M1A1^T; 2, *P. arctica* Pull 5.3^T; 3, *P. ingrahamii* 37^T; 4, *P. antarctica* DSM 10704^T; 5, *P. kaikoae* JT7304^T; 6, *P. marina* 4-22^T; 7, *P. profunda* 2825^T. Data for strain M1A1^T and *P. arctica* from this study, data for other species of the genus *Psychromonas* were taken from Breezee *et al.* [4], Mountfort *et al.* [1], Nogi *et al.* [10], Groudieva *et al.* [6], Xu *et al.* [12], and Brenner *et al.* [5]. All strains are oxidase-positive, Gram reaction-negative and negative for utilization of D-sorbitol as sole carbon source. Characteristics are scored as: +, positive; −, negative; W, weakly positive after 3 weeks; (W), weakly positive after 6 weeks; NR, not reported; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Cell morphology and arrangement	Rods singles, pairs	Rods singles, pairs	Large rods singles, pairs	Ovoid rods singles, pairs	Ovoid rods	Rods	Rods
Cell length (μm)	1.2–3.0	1.3–2.6	6–14	2.5–6	2–4	1.5–2.0	2.0–5.5
Production of gas vesicles	−	−	+	−	−	−	−
Colony colour	Beige-white	White	White	White	NR	Colourless	Colourless
Motility	+	+	−	+	+	+	+
Carbon sources utilized							
D-Fructose	−	+	+	+	+	+	+
D-Galactose	−	+	+	+	+	+	+
D-Glucose	−	+	+	+	+	+	+
D-Mannose	−	+	−	−	+	−	−
D-Mannitol	−	+	+	+	+	+	W
N-Acetylglucosamine	−	−	+	+	ND	+	ND
Arabinose	−	−	−	ND	−	−	−
D-Xylose	−	−	−	−	−	+	+
Cellobiose	−	+	+	−	+	+	+
Lactose	−	+	−	−	−	+	+
Maltose	−	+	ND	+	+	+	+
Sucrose	−	+	+	+	+	+	W
Fumarate	−	+	+	−	ND	ND	+
DL-Lactate	−	−*	+	−	ND	ND	(W)
DL-Malate	−	−*	−	−	ND	ND	ND
Glycerol	ND	+	+	−	−	+	(W)
Carbon sources fermented							
D-Glucose (Gas)	−	+ (G)	+	+ (G)	+	+	+
myo-Inositol	−	−	−	−	−	−	+
Lactose	−	−	+	−	−	+	+
Trehalose	−	−	+	+	+	−	+
D-Xylose	−	−	−	−	−	+	+
Polymers hydrolysed							
Starch	+	+	−	+	−	+	W
Gelatin	−	−	−	+	+	−	−
[NaCl] allowing growth (%)	0–7	1–7	1–12 †	0–4	>0	>0–7	>0
Growth temperature range (°C)	0.5 to 28	0 to 25	−12 to 10‡	2 to 17	4 to 15	0 to 25	2 to 14
pH range (optimum)	6.5 to 8.0 (7.0)	6.5 to 9.8 (8.8)	6.5 to 7.4	(6.5)	ND	ND	ND
O_2 requirement for growth	Aerobe	Aerobe	Facultative anaerobe	Aerotolerant anaerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe
Catalase	+	+	+/−	+	+	+	+
Indole	−	ND	−	−	−	−	+
Nitrate reduction	−	−	+	−	+	+	+

*Results not in congruence with those reported by Auman *et al.* [3].

†Weak growth was seen at concentrations up to 20 %.

‡Attempts to grow *P. ingrahamii* at temperatures lower than −12 °C were inconclusive because the culture medium froze.

Table 2. Fatty acid contents of strain M1A1^T and other members of the genus *Psychromonas*

Strains: 1, M1A1^T; 2, *P. arctica* Pull 5.3^T; 3, *P. ingrahamii* 37^T; 4, *P. antarctica* DSM 10704^T; 5, *P. kaikoae* JT7304^T; 6, *P. marina* 4-22^T; 7, *P. profunda* 2825^T. Data for strain M1A1^T and *P. arctica* are from this study. Values are percentages of total fatty acids. Isomers are shown in parentheses if known. Results <1 % not shown.

Fatty Acid	1	2	3	4	5	6	7
$C_{12:0}$	4.2	3.6 (2.7–5.2)	2.5	1	1	–	–
$C_{14:0}$	–	–	–	–	6	–	–
$C_{15:0}$	–	–	–	–	1	–	–
$C_{16:0}$	25.2	21.4 (7.0–16.2)	18.7	24	15	43.6	31
$C_{14:1}$	–	2.7–5.2 ($\omega5t$)	–	8 ($\omega7t$)	10 ($\omega7t$)	3.2	15
$C_{16:1}$	55.9 ($\omega7c$)	61.9 (app. 50) ($\omega7c$), 7.0–16.2 ($\omega7t$)	67 ($\omega7c$)	58 ($\omega7c$)	52 ($\omega7c$), 2 ($\omega9c$)	39.4	44
$C_{18:1}$	4.6 ($\omega7$)	3.6 (7.0–16.2) ($\omega7$)	3.6	3 ($\omega7c$)	2 ($\omega7c$)	3.1	–
$C_{20:5}\omega3$	–	–	–	–	2	–	–
$C_{22:6}$	–	–	–	–	2	1.6	–
$C_{12:0}$ 3-OH	–	–	–	–	2	–	–
$C_{12:0}$ alde, iso-$C_{16:1}$, or $C_{14:0}$ 3-OH	10.1	9.5	4.5	6 ($C_{14:0}$ 3-OH)	4 ($C_{14:0}$ 3-OH)	2.7 (iso-$C_{16:1}$)	–

Data for other *Psychromonas* species were taken from Breezee *et al.* [4], Nogi *et al.* [10], Groudieva *et al.* [6] (data of *P. arctica* given in brakets) and Xu *et al.* [12].

profile contains summed feature 3 fatty acids ($C_{16:1}\omega7c$/$C_{16:1}\omega8c$) and $C_{16:0}$ and $C_{12:0}$ 3-OH as predominant fatty acids.

The type strain M1A1^T (=CIP 111183^T=CCM 8710^T=LMG 29766^T) was isolated from seawater samples collected in an area surrounding King George Island, Fildes Bay, Chilean Antarctica in the summer of 2014. The genomic G+C content of the type strain is 38.5 mol%.

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Conflicts of interest
The author declare that there are no conflicts of interest.

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