Marinomonas arctica sp. nov., a psychrotolerant bacterium isolated from the Arctic

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A novel psychrotolerant, Gram-negative, motile bacterium, designated strain 328^T, was isolated from sea-ice samples collected off the Canadian Basin of the Arctic Ocean (78° 23' 14" N 149° 06' 55" W). Strain 328^T was able to grow at 0–37 °C, with optimum growth at 25–27 °C. It possessed phosphatidylethanolamine and phosphatidylglycerol as major phospholipids and C_{10:0} 3-OH (31.78 %), C_{18:1ω7c} (27.50 %) and iso-C_{15:0} 2-OH and/or C_{16:1ω7c} (19.22 %) as predominant cellular fatty acids. The DNA G + C content of strain 328^T was 45.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 328^T was a member of the genus *Marinomonas* (92.7–96.0 % 16S rRNA gene sequence similarity). On the basis of phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain 328^T was considered to represent a novel species of the genus *Marinomonas*. The name *Marinomonas arctica* sp. nov. is proposed, with strain 328^T (=CGMCC 1.6498^T=JCM 14976^T) as the type strain.

The genus *Marinomonas* was proposed by the reclassification of two species of the genus *Alteromonas* (Baumann *et al.*, 1972), namely *Alteromonas vaga* and *Alteromonas communis*, as *Marinomonas vaga* and *Marinomonas communis*, based on 16S rRNA gene sequences (Van Landschoot & De Ley, 1983). So far, the genus *Marinomonas* comprises ten species: *M. communis*, *M. vaga*, *M. mediterranea*, *M. primoryensis*, *M. pontica*, *M. ushuaiensis*, *M. aquimarina*, *M. dokdonensis*, *M. polaris* and *M. ostreistagnii* (Baumann *et al.*, 1972; Solano & Sanchez-Amat, 1999; Romanenko *et al.*, 2003; Ivanova *et al.*, 2005; Prabakaran *et al.*, 2005; Macián *et al.*, 2005; Yoon *et al.*, 2005; Gupta *et al.*, 2006; Lau *et al.*, 2006). During screening of cold-adapted bacteria from sea-ice samples of the Arctic Ocean, a novel psychrotolerant strain, designated strain 328^T, was isolated. Based on a polyphasic taxonomic approach, strain 328^T was identified as a representative of a novel species of the genus *Marinomonas*.

Abbreviation: ECL, equivalent chain-length.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Marinomonas arctica* 328^T is DQ492749.

A supplementary table showing the fatty acid profiles of strain 328^T and other *Marinomonas* species is available with the online version of this paper.

Strain 328^T was isolated from sea-ice samples collected from the Canadian Basin (78° 23' 14" N 149° 06' 55" W) using a MARK II ice auger during the Second Chinese National Arctic Research Expedition cruise of the USCGC icebreaker *Xue Long* into the Canadian Basin in August 2003. Sea-ice samples were cut carefully into 10–20 cm sections using a sterile saw and placed in sterile plastic bottle to be melted at 4 °C. Strain 328^T was obtained using previously described media and methods (Zhang *et al.*, 2006). The novel isolate was routinely grown on marine agar (MA) for 2 days at 25 °C and maintained as a glycerol suspension (20 %, w/v) at –80 °C. *M. polaris* JCM 12522^T, obtained from the JCM, was routinely grown on MA or in marine 2216 broth (MB) at 22 °C and used as a reference strain.

DNA was extracted from strain 328^T and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR with two universal primers (Zhang *et al.*, 2006). Purified PCR product was ligated to pMD 18-T (TaKaRa Bio) and cloned according to the manufacturer's instructions. Sequencing reactions were carried out using an ABI BigDye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). The almost complete 16S rRNA gene sequence (1465 bp) of strain 328^T was submitted to

GenBank and EMBL to search for similar sequences using the BLAST algorithm. A phylogenetic tree was constructed using the Kimura two-parameter and pairwise-deletion model analysis implemented in the MEGA version 3.0 program (Kumar *et al.*, 2004). Stability of clustering of the tree was evaluated by bootstrap analysis of 1000 datasets. Phylogenetic analysis (Fig. 1) showed that strain 328^T was grouped with members of the genus *Marinomonas*. Sequence similarity calculations based on neighbour-joining analysis indicated that the closest relatives of strain 328^T were the type strains of *M. pontica* (96.0%), *M. polaris* (95.9%) and *M. primoryensis* (95.7%). Lower sequence similarities (<97.0%) were found with all recognized species of the genus *Marinomonas*. It has been suggested that in bacterial strains with less than 97% 16S rRNA gene sequence similarity, levels of DNA–DNA hybridization are less than 70% (Stackebrandt & Goebel, 1994), which defines a genomic species (Wayne *et al.*, 1987). Thus, based on 16S rRNA gene sequence analysis, strain 328^T represents a novel species.

Cell morphology was examined with an Axioplan 2 microscope. Colony morphology was observed on MA after incubation at 25 °C for 24–36 h. The growth temperature was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined for the culture in MB at various pH values adjusted with HCl or NaOH (1 mol l⁻¹). General physiological tests were performed using conventional methods (Dong & Cai, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). In addition, biochemical properties were assessed using the API kits (API 20 E, API 20NE and API ZYM; bioMérieux) according to the manufacturer's instructions, except that the inoculum was prepared by suspending cells in a 3% (w/v) NaCl solution. All of the tests were performed in duplicate. Strain 328^T demonstrated an ability to utilize a comparatively wide range of carbon compounds as the sole source of carbon and energy compared with those utilized by its phylogenetic relatives. The morphological, cultural, physiological and biochemical characteristics of strain 328^T are given in the species description (see below) or are shown in Table 1.

Cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by TLC (Kates, 1986).

For fatty acid methyl ester analysis, cell mass of strain 328^T was harvested from MA plates after incubation at 25 °C for 3 days. Fatty acid methyl esters were prepared and analysed according to the standard protocol described in the MIDI (Microbial Identification) system (Sasser, 1990). The main phospholipids present included phosphatidylethanolamine and phosphatidylglycerol. The predominant cellular fatty acids of strain 328^T were C_{10:0} 3-OH (31.78%), C_{18:1} ω7c (27.50%), iso-C_{15:0} 2-OH and/or C_{16:1} ω7c (19.22%), an unknown fatty acid with an equivalent chain-length (ECL) of 11.799 (7.44%), C_{16:0} (5.89%), C_{10:0} (2.99%), C_{12:0} 3-OH (1.43%) and C_{18:0} (1.11%). The fatty acid profile of the novel strain resembled those determined for the other species of the genus *Marinomonas*, but differed in terms of relative amounts of unknown fatty acid (ECL 11.799) and C_{10:0} 3-OH (Ivanova *et al.*, 2005; Gupta *et al.*, 2006; Lau *et al.*, 2006). The fatty acid profiles of strain 328^T and other *Marinomonas* species are given in Supplementary Table S1, available in IJSEM Online.

The DNA G+C content was determined using a DU800 spectrophotometer (Beckman) with a thermal controller by the thermal denaturation (*T*_m) method (Marmur & Doty, 1962) using *Escherichia coli* K-12 as the reference. The DNA G+C content of strain 328^T was 45.2 mol%. This value is consistent with the G+C content of members of the genus *Marinomonas*, which ranges from about 41 to 50 mol%.

Strain 328^T could be distinguished from other species of the genus *Marinomonas* by a combination of physiological and biochemical properties (Table 1). Based on these results, it is concluded that strain 328^T represents a novel species of the genus *Marinomonas*, for which the name *Marinomonas arctica* sp. nov. is proposed.

Description of *Marinomonas arctica* sp. nov.

Marinomonas arctica (arc'ti.ca. L. fem. adj. *arctica* from the Arctic, referring to the site where the type strain was isolated).

Cells are Gram-negative, psychrotolerant, curved rods, 0.3–0.4 × 1.2–2.6 µm in size. Colonies are non-pigmented, convex, circular and smooth with entire edges. Growth occurs at 0–37 °C and pH 5.0–10.0, with optimum growth at 25–27 °C and at approximately pH 6.0–9.0. Growth

Fig. 1. Phylogenetic dendrogram of *Marinomonas arctica* sp. nov. and related species based on 16S rRNA gene sequences. The tree was constructed using the neighbour-joining method. Numbers at nodes represent levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 1% sequence divergence.

Table 1. Phenotypic characteristics that differentiate strain 328^T from other *Marinomonas* species

Strain/species: 1, strain 328^T; 2, *M. pontica* (Ivanova *et al.*, 2005; Gupta *et al.*, 2006); 3, *M. polaris* (Gupta *et al.*, 2006); 4, *M. dokdonensis* (Yoon *et al.*, 2005); 5, *M. ushuaiensis* (Prabakaran *et al.*, 2005); 6, *M. primoryensis* (Romanenko *et al.*, 2003; Gupta *et al.*, 2006); 7, *M. communis* (Baumann *et al.*, 1972). +, Positive; –, negative; w, weak reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Growth at:							
4 °C	+	+	+	+	+	+	–
37 °C	+	–	+	+	–	–	+
NaCl requirement	–	+	–	+	+	+	+
NaCl tolerance (%)	12	10	11	10	6	6	6
Amylase	–	–	–	–	+	–	–
Lipase	–	–	–	+	–	–	–
Phosphatase	+	+	+	ND	+	+	–
Utilization of:							
Cellobiose	+	+	+	+	–	+	–
Erythritol	+	–	+	ND	–	–	–
Galactose	+	–	+	–	+	–	ND
Glycerol	+	+	+	ND	–	+	+
L-Alanine	+	+	–	ND	ND	–	+
Lactose	+	+	+	ND	+	–	–
Mannitol	+	+	ND	+	–	+	+
Malate	+	–	–	+	–	w	+
Rhamnose	+	+	–	ND	–	–	ND
Ribose	+	–	+	+	–	+	–
Sorbitol	+	–	+	+	–	–	+
DNA G+C content (mol%)	45.2	46.5	41.2	45.3–45.7	43.6	45.3–45.6	45.9–47.9

occurs in the presence of 0–12% (w/v) NaCl. Grows aerobically and produces catalase and cytochrome oxidase. Indole production and Voges–Proskauer are negative. Does not reduce nitrate or produce hydrogen sulfide. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, gelatinase, agarase, caseinase, amylase, chitinase, lecithinase, esterase (C4), esterase lipase (C8), lipase (C14), α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, cystine arylamidase, valine arylamidase, leucine arylamidase, α -galactosidase, β -glucuronidase and *N*-acetyl- β -glucosaminidase are negative. Tests for citrate utilization, α -glucosidase, trypsin, acid phosphatase and alkaline phosphatase are positive. The following substrates are utilized as sole carbon sources: glucose, mannitol, D-mannose, sucrose, D-sorbitol, D-arabinose, L-rhamnose, maltose, fructose, D-galactose, erythritol, melibiose, glycerol, cellobiose, raffinose, xylose, melezitose, trehalose, L-proline, L-alanine, L-histidine, gluconate, malate and phenylacetic acid. The following substrates are not utilized as sole carbon sources: salicin, sorbinose, arabinol, amygdalin, dulcitol, inulin, L-leucine, capric acid and adipic acid. Acid is produced from glucose, mannitol, D-mannose, sucrose, D-sorbitol, D-arabinose, L-rhamnose, maltose, fructose, D-galactose, erythritol, melibiose, glycerol, cellobiose, raffinose, xylose, melezitose and trehalose. The main phospholipids present include phosphatidylethanolamine and phosphatidylglycerol. The predominant cellular fatty acids of strain 328^T are C_{10:0} 3-OH (31.78%), C_{18:1} ω 7c (27.50%), iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c

(19.22%), unknown fatty acid (ECL 11.799; 7.44%) and C_{16:0} (5.89%). The DNA G+C content of the type strain is 45.2 mol%.

The type strain is 328^T (=CGMCC 1.6498^T=JCM 14976^T), isolated from the Canadian Basin (78° 23' 14" N 149° 06' 55" W) in the Arctic.

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