Marinomonas primoryensis sp. nov., a novel psychrophile isolated from coastal sea-ice in the Sea of Japan

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Two bacterial strains (KMM 3633^T and KMM 3634) were isolated from marine coastal sea-ice and characterized by using phenotypic and molecular methods. The isolates were aerobic, Gram-negative, psychrophilic, halophilic and motile by means of a single polar flagellum. The DNA G + C content was 45.3–45.6 mol%. The major cellular fatty acids were C_{16:0}, C_{16:1ω9c} and C_{18:1ω7c}. Comparison of almost-complete 16S rDNA sequences demonstrated that the strains were phylogenetically closely related to each other (99.5 % sequence identity), and related to *Marinomonas* species (94.4–96.4 % identity). DNA–DNA reassociation between KMM 3633^T and KMM 3634 occurred at a level of 92 %. Based on phenotypic, chemotaxonomic and phylogenetic properties, the name *Marinomonas primoryensis* sp. nov. is proposed for strains KMM 3633^T and KMM 3634; the type strain is KMM 3633^T (= JCM 11775^T = NRIC 523^T).

During the study of microbial communities associated with marine environments, two bacterial strains were isolated from a coastal sea-ice sample, obtained from a sea-ice column at a depth of 0.8 m in Amursky Bay of the Sea of Japan, near Vladivostok, Russia, in March 2001. The isolates were aerobic, Gram-negative, heterotrophic micro-organisms with a respiratory metabolism, and had phenotypic characteristics similar to those of the genus *Marinomonas*. Phylogenetic analysis on the basis of 16S rDNA sequence similarity revealed that the new isolates belonged to a novel species of the genus *Marinomonas*, for which the name *Marinomonas primoryensis* sp. nov. is proposed.

Baumann *et al.* (1972) reported aerobic, Gram-negative, non-fermentative, non-pigmented, polarly flagellated, marine bacteria containing DNA with a G + C content of 43–48 mol%, and identified that they belonged to a novel genus, *Alteromonas*. This genus originally comprised four species: *Alteromonas marinopraeensis* that was later renamed *Alteromonas haloplanktis* (Reichelt & Baumann, 1973),

Alteromonas macleodii, *Alteromonas communis* and *Alteromonas vaga* (Baumann *et al.*, 1972). However, *A. communis* and *A. vaga* have characteristics that distinguish them from most of the *Alteromonas* species described: lack of amylase, gelatinase, lipase and chitinase, and utilization of glycerol, lactate, DL-β-hydroxybutyrate, *m*-hydroxybenzoate and other carbon sources. DNA–rRNA hybridization experiments performed by Van Landschoot & De Ley (1983) confirmed the heterogeneity of the genus *Alteromonas*; *A. communis* and *A. vaga* were found to form a separate rRNA branch. A novel genus, *Marinomonas*, was proposed to accommodate *A. communis* and *A. vaga* (Van Landschoot & De Ley, 1983). A novel melanogenic bacterium from the Mediterranean Sea, MMB-1, was described by Solano & Sanchez-Amat (1999) as a novel species of the genus *Marinomonas*, namely *Marinomonas mediterranea*.

The strains tested were isolated from a coastal sea-ice sample, as follows. The sea-ice sample was collected in a sterile flask with a small amount of sterile sea water, kept for 2 days at 15 °C, and melted carefully. An aliquot of melting sea-ice was spread onto a plate of Marine 2216 agar (MA; Difco) and incubated for 7 days at 15 °C. The strains were stored at –80 °C in liquid nutrient medium supplemented with 30 % (v/v) glycerol. The strains,

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designated KMM 3633^T and KMM 3634, were deposited in the Collection of Marine Microorganisms (KMM), Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia. Strains KMM 3633^T and KMM 3634 were routinely grown on MA, Marine Broth (MB; Difco) and Tryptic Soya Agar (TSA; Serva), prepared with natural sea water. 16S rRNA gene sequences of strains KMM 3633^T and KMM 3634 (1419 and 1449 bp, respectively) were determined and compared as described by Shida *et al.* (1997). Previously published 16S rDNA sequences were obtained from the EMBL/GenBank/DBJ databases. The DNA G + C content was determined by the methods of Marmur & Doty (1962) and Owen *et al.* (1969). DNA–DNA relatedness was measured spectrophotometrically by using the renaturation rate method (De Ley *et al.*, 1970).

Cellular fatty acid analysis was performed as described by Svetashev *et al.* (1995). Standard phenotypic characterization of the strains was carried out as described by Baumann *et al.* (1984), Gauthier & Breittmayer (1992) and Smibert & Krieg (1994). Cell morphology was examined by transmission electron microscopy of exponential-phase cells grown in MB. The cells were negatively stained with potassium phosphotungstate (1 %, w/v; pH 7.0). Motility was observed by the hanging drop method. Growth at 4–41 °C was examined on MA. Sea water requirement and tolerance of 0–20 % NaCl were determined using nutrient

medium prepared with an artificial sea water base, supplemented with appropriate amounts of NaCl. Glucose utilization and acid production from sugars (1 %, w/v) were tested in Leifson's medium (Leifson, 1963). Utilization of various compounds as sole carbon and energy sources was tested in a mineral liquid medium containing (g l⁻¹): NH₄Cl, 1.0; K₂HPO₄, 0.075; CaCl₂, 1.45; NaCl, 30.0; MgCl₂, 6.15; KCl, 0.75; FeSO₄, 0.028; supplemented with 0.2 % (w/v) test substrate. Growth was determined spectrophotometrically after 2 days cultivation.

Strains KMM 3633^T and KMM 3634 were aerobic, Gram-negative, halophilic, motile by a single polar flagellum, 0.4–0.6 µm wide and 1.6–1.8 µm long. They have positive or weakly positive oxidase activity and weak catalase activity. Strains KMM 3633^T and KMM 3634 were psychrophilic and grew at 4–30 °C, but not at 35 °C. Sodium ions were essential for growth. Growth was supported at 1–6 % NaCl. Smooth, transparent, yellowish colonies were formed on MA. Slight diffusion of yellowish pigment into the medium was observed after 2 days incubation. Both strains grew very poorly on the medium containing L-tyrosine as the sole carbon source, and did not produce melanin-like pigments. Phenotypic and biochemical characteristics of the new strains and other *Marinomonas* species are listed in Table 1. The major cellular fatty acids of KMM 3633^T and KMM 3634 were (respectively): C_{16:0}

Table 1. Phenotypic characteristics of KMM 3633^T, KMM 3634 and *Marinomonas* species

Data from Baumann *et al.* (1972), Solano *et al.* (1997), Solano & Sanchez-Amat (1999) and this study. All strains are positive for motility by a single polar flagellum, Na⁺ requirement for growth and utilization of glycerol, mannitol, citrate, acetate and succinate. All strains are negative for arginine dihydrolase, amylase production, lactose and L-tyrosine utilization. +, Positive; –, negative; W, weak reaction; V, variable reaction between strains; ND, no available data.

Characteristic	KMM 3633 ^T	KMM 3634	<i>M. communis</i>	<i>M. vaga</i>	<i>M. mediterranea</i>
Pigment	Yellowish	Yellowish	None	None	Melanin-like
Oxidase	+	W	+	–	–
Nitrate reduction	–	–	–	–	+
Growth at:					
4 °C	+	+	–	–	–
35 °C	–	–	+	+	–
40 °C	–	–	+	–	–
Hydrolysis of:					
Gelatin	–	–	–	–	+
Casein	–	–	ND	ND	ND
Tween 80	–	–	–	–	+
Utilization of:					
D-Glucose	W	+	+	+	+
Maltose	+	+	V	V	–
N-Acetylglucosamine	+	+	–	+	ND
m-Hydroxybenzoate	+	+	+	+	–
Mannitol	+	+	+	+	ND
Malate	W	+	+	+	+
L-Arginine	–	+	+	V	ND
L-Lysine	+	+	+	V	ND
DNA G + C content (mol%)	45.3	45.6	46–48	47–49	46.3 ± 0.9

Fig. 1. Phylogenetic relationships of KMM 3633^T, KMM 3634 and some related taxa based on 16S rRNA gene sequences. The branching pattern was generated by the neighbour-joining method. The significances for particular nodes were obtained by boot-strap analysis (1000 replications). Bar, 0.01 nucleotide substitutions per site.

(24 and 22.3 %), C_{16:1}ω_{9c} (27.1 and 30.2 %) and C_{18:1}ω_{7c} (35.8 and 35.5 %). C_{18:0} (8.7 and 7.6 %) and C_{12:1} (1.9 and 1.7 %) were detected as minor fatty acids. The following fatty acids were at level < 1.0 %: C_{12:0}, C_{14:0}, C_{15:0}-a, C_{17:0} and C_{17:1}-i.

The DNA G + C content was 45.3–45.6 mol%. DNA–DNA reassociation was 92 % between KMM 3633^T and KMM 3634, indicating that the new strains represent the same species (Wayne *et al.*, 1987).

Strains KMM 3633^T and KMM 3634 were phylogenetically closely related (99.5 % 16S rDNA sequence similarity), related to *Marinomonas communis* with 96.4 % similarity and to other members of this genus with 94.4–94.5 % similarity (Fig. 1). The sequence similarity values between KMM 3633^T and previously described *Marinomonas* type strains were all < 97 %, the value that is accepted as indicative of the distinction of different species (Stackebrandt & Goebel, 1994). Based on both sequence dissimilarity values (> 3 %) and placement in the phylogenetic tree, the isolates do not belong to any other described species and represent a novel *Marinomonas* species. In addition, there are several phenotypic features that allow the clear differentiation of KMM 3633^T and KMM 3634 from other *Marinomonas* species (Table 1).

Strains KMM 3633^T and KMM 3634 differed from *M. mediterranea* by pigments, oxidase reaction, growth at 4–5 °C, inability to produce lipase or gelatinase or reduce nitrate and ability to utilize maltose and *m*-hydroxybenzoate. Differential characteristics between the new strains and the other previously described *Marinomonas* species, *Marinomonas vaga* and *M. communis*, were the maximum and minimum growth temperatures, oxidase reaction, pigmentation, utilization of some carbon compounds and lower DNA G + C contents.

Thus, on the basis of the phenotypic, genotypic and phylogenetic characteristics, we propose a novel species, *Marinomonas primoryensis* sp. nov.

Description of *Marinomonas primoryensis* sp. nov.

Marinomonas primoryensis (pri.mo.ry.en'sis. N.L. fem. adj. *primoryensis* pertaining to Primorye, Far-Eastern region of Russia).

Cells are Gram-negative, strictly aerobic, 0.4–0.6 µm wide, 1.6–1.8 µm long and motile by a single polar unsheathed flagellum. Endospores are not formed. Oxidase reaction is positive or weakly positive. Catalase activity is weak. Sodium ions are required for growth; grows in 1–6 % NaCl. Psychrophilic, neutrophilic and chemoorganotrophic. Growth occurs at 4–30 °C but not at 35 °C. Transparent colonies are formed, which become yellowish after 36–48 h incubation. Pigment diffuses slightly into the agar. Lipase, caseinase, DNase, gelatinase and amylase are not produced. Negative for arginine dihydrolase, nitrate reduction and acid production from carbohydrates. Utilizes D-glucose, maltose, mannitol, glycerol, *N*-acetylglucosamine, L-asparagine, L-lysine, L-methionine, citrate, malate, *m*-hydroxybenzoate, acetate and succinate; does not utilize arabinose, valine or L-glutamic acid. The predominant fatty acids are C_{16:0}, C_{16:1}ω_{9c} and C_{18:1}ω_{7c}. The DNA G + C content is 45.3–45.6 mol%.

The type strain is KMM 3633^T (=JCM 11775^T=NRIC 523^T). Isolated from coastal sea-ice in Amursky Bay of the Sea of Japan.

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