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Marinomonas arenicola sp. nov., isolated from marine sediments of the Sea of Japan

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The DDBJ/GenBank accession number for the 16S rRNA gene sequence of the strain KMM 3893^T =NRIC 0752^T =JCM 15737^T is AB467281.

***Marinomonas*-like bacterium KMM 3893^T was isolated from a marine sandy sediment collected from the Sea of Japan offshore and subjected to a phenotypic and phylogenetic study. Comparative 16S rRNA gene sequence analysis confirmed the novel strain assignment to the genus *Marinomonas.* Strain KMM 3893^T constituted a separate phyletic line to the genus *Marinomonas* sharing <97% sequence similarity with respect to other recognized *Marinomonas* species. Chemotaxonomically strain KMM 3893^T contained predominantly fatty acids $C_{18:1\omega7c}$, $C_{16:1\omega7c}$, and $C_{16:0}$, and had a DNA G+C content of 50.0 mol%. On the basis of phylogenetic analysis, physiological and biochemical characterization, strain KMM 3893^T represents a novel species of the genus *Marinomonas*, for which the name *Marinomonas arenicola* sp. nov. is proposed. The type strain of this species is KMM 3893^T (=NRIC 0752^T =JCM 15737^T).**

The genus *Marinomonas* was created by Van Landschoot & De Ley (1983) as the result of the reclassification of two species, *Alteromonas communis* and *Alteromonas vaga* (Baumann *et al*., 1972). Subsequently the genus was expanded with nine additional species, including *Marinomonas mediterranea* (Solano & Sanchez-Amat, 1999), *Marinomonas primoryensis* (Romanenko *et al.*, 2003), *Marinomonas aquamarina* (Macián *et al*., 2005), *Marinomonas pontica* (Ivanova *et al*., 2005), *Marinomonas ushuaiensis* (Prabagaran *et al*., 2005), *Marinomonas dokdonensis* (Yoon *et al*., 2005), *Marinomonas polaris* (Gupta *et al*., 2006), *Marinomonas ostreistagni* (Lau *et al*., 2006), and *Marinomonas arctica* (Zhang *et al*., 2008). Here we report the phenotypic characterization and phylogenetic analysis of a novel marine sediment isolate, designated KMM 3893^T. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain KMM 3893^T belonged to the genus *Marinomonas* and might represent a novel species of this genus.

Strain KMM 3893^T could be distinguished from other recognized *Marinomonas* species on the basis of combined differential phenotypic characteristics and phylogenetic distinctiveness;

therefore, it is proposed that strain KMM 3893^T belongs to a novel species, which is named *Marinomonas arenicola* sp. nov.

Strain KMM 3893^T was isolated from a sandy sediment sample collected from the Sea of Japan offshore at a depth 1 m, as described previously (Romanenko *et al.*, 2004). The bacterium KMM 3893^T was grown aerobically on marine 2216 agar (MA) or marine broth (MB), TSA, and seawater medium agar plates (SWM), containing: 5.0 g l^{-1} peptone, 2.5 g l^{-1} yeast extract, 1.0 g l^{-1} glucose, 0.2 g l^{-1} K_2HPO_4, 0.05 g l^{-1} $MgSO_4$, and 15.0 g l^{-1} agar, 750 ml seawater/250 ml distilled water at 25-28 °C, and stored at –80 °C in the liquid MB supplemented with 30% (v/v) glycerol. The strain KMM 3893^T was deposited in the Collection of Marine Microorganisms (KMM), Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia. Motility was observed by the hanging drop method as described by Gerhardt *et al*. (1994). Phenotypic properties were tested according to the standard methods described by Smibert & Krieg (1994). The oxidation/fermentation medium of Leifson (1963) for marine bacteria was used to test acid production from carbohydrates with 1% (w/v) of each compound. Growth at different temperatures and pH values and in the presence of various NaCl concentrations, and antibiotic resistance were studied as described previously (Romanenko *et al*., 2003, 2004, 2005). In addition, biochemical tests were carried out using API 20NE, API ID32 GN, and API 50 CH test kits (bioMérieux) according to the manufacturer's instructions. For comparative fatty acid analysis, strain KMM 3893^T, *Marinomonas communis* CIP 74.1^T and *Marinomonas primoryensis* KMM 3633^T were cultivated on MA at 28 °C and in MB at 22 °C for 3 d, and lipids were extracted using chloroform-methanol extraction method of Bligh & Dyer (1959). Fatty acid methyl esters (FAMEs) were obtained by alkaline methanolysis (15% NaOH/methanol). The resultant FAMEs were extracted by hexane and analyzed using a GLC-MS Hewlett-Packard model 6890 gas chromatograph equipped with a HP 5 MS 5% Phenyl Methyl Siloxane capillary column (30 m x 250 μm x 0.25 μm) and connected to a Hewlett-Packard model 5973 mass spectrometer. The DNA base composition was determined as

described by Marmur & Doty (1962) and Owen *et al*. (1969). The 16S rRNA gene sequence of 1523 nucleotides was determined for the strain KMM 3893^T as described by Shida *et al*. (1997). The sequence obtained was compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DDBJ databases by using the FASTA program (Pearson & Lipman, 1988). Phylogenetic analysis of 16S rRNA gene sequences was performed using the software package MEGA 4 (Tamura *et al*., 2007) after multiple alignment of data by CLUSTALX (version 1.83; Thompson *et al*., 1997). Phylogenetic trees were constructed by the neighbor-joining and maximum-parsimony methods and the distances were calculated according to the Kimura two-parameter model. The robustness of phylogenetic trees was estimated by the bootstrap analysis of 1000 replicates.

Comparative 16S rRNA gene sequence analysis showed that strain KMM 3893^T belonged to the genus *Marinomonas* and formed a distinct lineage (Fig. 1). The same relationship was also evident in 16S rRNA gene sequence dendrogram generated using the maximum-parsimony algorithm (Supplementary Fig. S1 on IJSEM Online). Strain KMM 3893^T shared 16S rRNA gene sequence similarities of 96.9% to *Marinomonas pontica* 46-16^T, 96.7% to *Marinomonas dokdonensis* DSW10-10^T, and a somewhat less values of 96.1% to *Marinomonas ushuaiensis* U1^T, 95.6% to each of *Marinomonas vaga* ATCC 27119^T and *Marinomonas communis* LMG 2864^T, and less than 95% similarity to other members of the genus *Marinomonas*. The 16S rRNA gene sequence similarity value of 97.0% was proposed by Stackebrandt & Goebel (1994) and subsequently re-evaluated to 98.7% by Stackebrandt & Ebers (2006) as a criterion for species discrimination. Taking into consideration cut-off values above we concluded that 16S rRNA gene sequence similarities obtained for the strain KMM 3893^T are low enough to exclude its assignment to any of recognized *Marinomonas* species. The detailed fatty acids composition of strain KMM 3893^T is given in Table 2. For comparison purposes fatty acid profiles of three strains, KMM 3893^T, *M. communis* CIP 74.1^T and *M. primoryensis* KMM 3633^T grown in/on

MA and MB at different temperatures were examined. Noticeable differences in FA compositions of strains tested were not found regardless of culture conditions applied. *M. communis* CIP 74.1^T, and *M. primoryensis* KMM 3633^T, and strain KMM 3893^T contained $C_{18:1\omega7c}$, $C_{16:1\omega7c}$, and $C_{16:0}$ as major fatty acids (Table 2). These results obtained in this study are in accordance with the data previously reported for *M. communis, M. vaga, M. mediterranea* (Mikhailov *et al.,* 2002), *M. primoryensis* (Romanenko *et al.*, 2003), *M. dokdonensis* (Yoon *et al., 2005), M. pontica* (Ivanova *et al.*, 2005), *M. ostreistagni* (L*au et al.,* 2006), and *M. arctica* (Zhang *et al.,* 2008). However, the data obtained disagreed with the results found by Prabagaran *et al*. (2005) and by Gupta *et al*. (2006) who reported the presence of significant percentage of iso-$C_{16:0}$ (13.5%; 11.3%; 16.1%, and 18.5%) and minor amounts of $C_{16:0}$ (1.1%; 1.1%, 2.1%, and 6.2%) in FA profiles of *M. communis*, *M. primoryensis,* and *M. ushuaiensis*, and *M. polaris*, respectively. In the present study FA patterns of strain KMM 3893^T, *M. communis*, and *M. primoryensis* appeared to be similar in terms of the presence of $C_{18:1\omega7c}$, $C_{16:1\omega7c}$, and $C_{16:0}$ as predominant fatty acids and the absence of iso-$C_{16:0}$ independent of growth conditions. In addition, analysis here of *M. communis* CIP 74.1^T revealed a slight difference in the absence of $C_{12:1}$; strain KMM 3893^T differed in possessing a small amount of $C_{17:1.}$ The DNA G+C content of 50 mol% determined for the strain KMM 3893^T is in line with those reported for recognized *Marinomonas*, but significantly higher a G+C value reported for *M. usuaiensis* U1^T (43.6 mol%) (Table 1). The differential phenotypic features of strain KMM 3893^T and related species of the genus *Marinomonas* are listed in Table 1 and in the species description. It is interesting to mention that strain KMM 3893^T was susceptible to 16 of 20 antibiotics tested as listed in the species description.

The isolate KMM 3893^T could be distinguished from recognized *Marinomonas* species in not being able to assimilate most compounds which are included in the API 32 ID, API 20 NE and 50 CH panels. Strain KMM 3893^T was similar to the *M. ushuaiensis* (Prabagaran *et al*., 2005) in oxidase reaction, nitrate reduction and carbon assimilation pattern, but differed in not being able

to degrade starch and utilize D-glucose and *m*-hydroxybenzoate; in minimal and maximal growth temperatures which supported its growth; and in the tolerance to 8-10 % NaCl.

Based on the results obtained it is proposed to assign strain KMM 3893^T to the genus *Marinomonas* as representing novel species, *Marinomonas arenicola* sp. nov.

Description of *Marinomonas arenicola* sp. nov.

Marinomonas arenicola (a.re.ni'co.la. L. n. *arena*, sand; L. suff. -*cola*, inhabitant, dweller; N.L. n. *arenicola*, a sand-dweller).

An aerobic, Gram-negative, oxidase-negative, catalase-positive, motile rod-shaped bacterium (approximately 2 μm in length). Colonies are non-pigmented, hemi-transparent, shiny, and smooth with the regular edges of 2-3 mm in diameter on MA. Strain KMM 3893^T could grow at 4-37 °C with an optimum of 25-28 °C, and did not grow at temperatures above 38 °C. Sodium ions are essential for growth. Growth is observed in 0.5-10% (w/v) NaCl. No growth observed in 12% NaCl. The pH range is 5.5-9.5 with pH optimum 6.5-8.0. Negative for casein, gelatin, Tween-80, starch, chitin, and DNA hydrolysis, and for H_2S production. On the L-tyrosine containing medium strain did not produce melanin-like pigments and/or clearance zone. Acid formed from D-xylose; no acid produced from D-glucose, D-mannitol, D-sucrose, D-lactose, D-maltose, D-galactose, D-mannose, D-cellobiose, D-sorbitol, L-arabinose, and L-rhamnose. According to API 20 NE tests strain KMM 3893^T is positive for PNPG test; and negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease production, gelatin hydrolysis, aesculin hydrolysis, and assimilation of D-glucose, D-mannitol, maltose, L-arabinose, D-mannose, N-acetylglucosamine, D-gluconate, caprate, adipate, L-malate, citrate, and phenylacetate. In the API 50 CH tests, strain KMM 3893^T was weakly positive for potassium 5-ketogluconate and potassium 2-ketogluconate utilization; and negative for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-

glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, amidon, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, D-arabitol, and gluconate utilization. According to the API ID 32GN tests, assimilation of itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, propionic acid, capric acid, trisodium citrate, L-alanine, L-proline, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, suberic acid, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, D-mannitol, L-fucose, D-sorbitol, L-rhamnose, inositol, L-arabinose, L-serine, valeric acid, L-histidine, and potassium 2-ketogluconate is negative.

Strain KMM 3893^T contained predominant fatty acids $C_{18:1\omega7c}$, $C_{16:0}$, and $C_{16:1\omega7c}$. The detailed fatty acid composition of strain KMM 3893^T is given in Table 2. Strain KMM 3893^T was susceptible to antibiotics (content per disc): ampicillin (10 μg), benzylpenicillin (10 U), vancomycin (30 μg), gentamicin (10 μg), kanamycin (30 μg), carbenicillin (100 μg), chloramphenicol (30 μg), neomycin (30 μg), ofloxacin (5 μg), polymyxin (300 U), rifampicin (5 μg), streptomycin (30 μg), erythromycin (15 μg), tetracycline (30 μg), cephazolin (30 μg), and cephalexin (30 μg); and resistant to nalidixic acid (30 μg), lincomycin (15 μg), oxacillin (10 μg), and oleandomycin (15 μg). The DNA G+C content of the type strain KMM 3893^T is 50 mol% (determined by the thermal denaturation method). The type strain, KMM 3893^T (=NRIC 0752^T =JCM 15737^T), was isolated from a marine sandy sample, collected from the Sea of Japan off shore, Russia.

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Table 1. Phenotypic characteristics of *Marinomonas arenicola* sp. nov. KMM 3893^T and related *Marinomonas* species.

1, KMM 3893^T (data from this study); **2**, *M. ushuaiensis* (data from Prabagaran *et al*., 2005); **3**, *M. pontica* (Ivanova *et al.*, 2005); **4***, M. dokdonensis* (Yoon *et al.*, 2005); **5**, *M. primoryensis* (Romanenko *et al.*, 2003); **6**, *M. communis*; **7**, *M. vaga* (Baumann *et al*., 1972) **8**, *M. mediterranea* (Solano & Sanchez-Amat, 1999). All strains are positive for motility and sodium ions requirement for growth; and negative for arginine dihydrolase, indole and H_2S production. +, Positive; -, negative; W, weak reaction; V, variable reaction between strains; ND, no available data.

Feature	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Pigment	None	None	None	None	Yellowish	None	None	Melanin-like
Oxidase	-	-	+	+	+	+	-	-
Nitrate reduction	-	-	-	-	-	-	-	+
Tolerance to NaCl (%):								
8	+	-	+	+	W*	+	+	ND
10	+	-	+	+	-	+*	+*	ND
Growth at (°C):								
4	+	+	+	+	+	-	-	-
35	+	-	-	+	-	+	+	-
37	+	-	-	+	-	+	-	-
40	-	-	-	-	-	+	-	-

Hydrolysis of:								
Gelatin	-	-	-	-	-	-	-	+
Tween-80	-	-	-	+	-	-	-	+
Starch	-	+	-	-	-	-	-	-
Utilization of:								
D-Glucose	-	+	+	ND	+[†]	+	+	+
Maltose	-	W	+	+	+	V	V	-
N-Acetyl-D-glucosamine	-	ND	ND	ND	+	-	+	ND
m-Hydroxybenzoate	-	+	+	-	+	+	+	-
Mannitol	-	-	ND	ND	+	+	+	ND
Malate	-	-	+	+	+[†]	+	+	+
L-Arginine	-	-	+	ND	V	+	V	ND
L-Lysine	-	-	+	ND	+	+	V	ND
DNA G+C content (mol%)	50.0	43.6	46.5	45.3-45.7	45.3- 45.6	46-48	47-49	46.3±0.9

[*] Data for the type strains of *M. primoryensis* KMM 3633^T, *M. communis* CIP 74.1^T, and *M. vaga* CIP 103202^T were obtained in this study.

[†] Weakly positive for the type strain of *M. primoryensis* KMM 3633^T.

Table 2. Cellular fatty acid composition (%) of strains KMM 3893^T and *M. communis* CIP 74.1^T and *M. primoryensis* KMM 3633^T.

1, KMM 3893^T; **2**, *M. communis* CIP 74.1^T; **3**, *M. primoryensis* KMM 3633^T.

Designation: a, strains were grown on MA at 28 °C; b, strains were grown in MB at 22 °C.

Fatty acid	**1**		**2**		**3**	
	a	**b**	**a**	**b**	**a**	**b**
$C_{10:0}$ 3-OH	**9.7**	**8.9**	**10.4**	**3.7**	**4.8**	**5.0**
$C_{12:1}$	2.1	3.0	-	-	4.4	6.0
$C_{12:0}$	11.0	10.7	5.6	4.4	0.8	-
$C_{13:0}$	1.3	-	-	-	-	-
$C_{14:0}$	1.3	-	2.2	1.9	-	-
$C_{16:1\omega7c}$	**10.2**	**13.0**	**15.9**	**22.2**	**22.1**	**23.5**
$C_{16:0}$	**17.4**	**16.0**	**11.4**	**10.8**	**11.9**	**14.4**
$C_{17:1}$	3.2	2.8	-	0.2	-	-
$C_{18:1\omega7c}$	**31.0**	**38.8**	**44.2**	**53.2**	**37.0**	**38.2**
$C_{18:1\omega 9c}$	-	-	0.1	0.4	-	-
$C_{18:0}$	4.2	4.1	2.5	2.6	5.6	5.8

References

Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **3,** 402-429.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911-917.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). ***Methods for General and Molecular Bacteriology***. Washington, DC: American Society for Microbiology.

Gupta, P., Chaturvedi, P., Pradhan, S., Delille, D. & Shivaji, S. (2006). *Marinomonas polaris* sp. nov., a psychrohalotolerant strain isolated from coastal sea water off the subantarctic Kerguelen islands. *Int J Syst Evol Microbiol* **56**, 361-364.

Ivanova, E. P., Onyshchenko, O. M., Christen, R., Lysenko, A. M., Zhukova, N. V., Shevchenko, L. S. & Kiprianova, E. A. (2005). *Marinomonas pontica* sp. nov., isolated from the Black Sea. *Int J Syst Evol Microbiol* **55**, 275–279.

Lau, K. W. K., Ren, J., Wai, N. L. M., Lau, S. C. L., Qian, P.-Y., Wong, P.-K. & Wu, M. (2006). *Marinomonas ostreistagni* sp. nov., isolated from a pearl-oyster culture pond in Sanya, Hainan Province, China. *Int J Syst Evol Microbiol* **56**, 2271–2275.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183-1184.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109-118.

Macián, M. C., Arahal, D. R., Garay, E. & Pujalte, M. J. (2005). *Marinomonas aquamarina* sp. nov., isolated from oysters and seawater. *Syst Appl Microbiol* **28**, 145–150.

Mikhailov, V. V., Romanenko, L. A. & Ivanova, E. P. (2002). The genus *Alteromonas* and related *Proteobacteria*. In *The Prokaryotes*, 3rd edn, release 3.10. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.

Owen, J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base composition from melting profiles in dilute buffers. *Biopolymers* 7, 503-516.

Pearson, W. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA* **85,** 2444-2448.

Prabagaran, S. R., Suresh, K., Manorama, R., Delille, D. & Shivaji, S. (2005). *Marinomonas ushuaiensis* sp. nov., isolated from coastal sea water in Ushuaia, Argentina, sub-Antarctica. *Int J Syst Evol Microbiol* **55**, 309–313.

Romanenko, L. A., Uchino, M., Mikhailov, V. V., Zhukova, N. V. & Uchimura, T. (2003). *Marinomonas primoryensis* sp. nov., a new psychrophilic bacterium isolated from coastal sea-ice of the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 829-832.

Romanenko, L. A., Schumann, P., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2004). *Reinekea marinisedimentorum* gen. nov., sp. nov., a novel gammaproteobacterium from marine coastal sediments. *Int J Syst Evol Microbiol* **54**, 669-673.

Romanenko, L. A., Uchino, M., Falsen, E., Frolova, G. M., Zhukova, N. V. & Mikhailov, V. V. (2005). *Pseudomonas pachastrellae* sp. nov. isolated from a marine sponge. *Int J Syst Evol Microbiol* **55**, 919-924.

Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289-298.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607-655. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Solano, F. & Sanchez-Amat, A. (1999). Studies on the phylogenetic relationships of melanogenic marine bacteria: proposal of *Marinomonas mediterranea* sp. nov. *Int J Syst Bacteriol* **49**, 1241-1246.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846-849.

Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microb Today* **45**, 153-155.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596-1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**, 4876-4882.

Van Landschoot, A. & De Ley, J. (1983). Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas, Marinomonas* (Gen. nov.) and some other Gram-negative bacteria. *J Gen Microbiol* **129**, 3057-3974.

Yoon, J.-H., Kand, S.-J. & Oh, T.-K. (2005). *Marinomonas dokdonensis* sp. nov., isolated from sea water. *Int J Syst Evol Microbiol* **55**, 2303-2307.

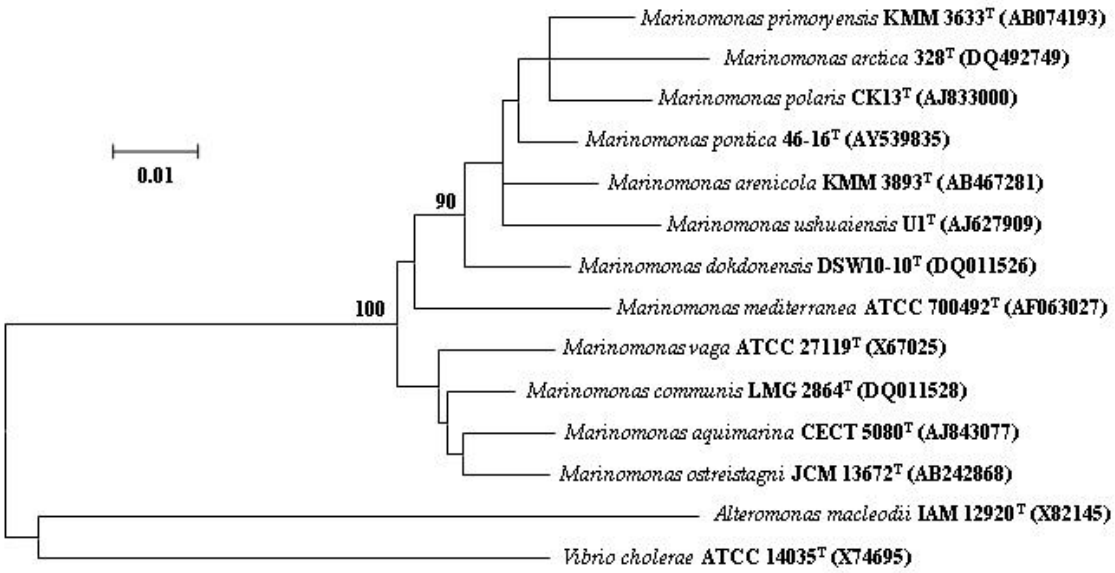
Zhang, D.C., Li, H. R. , Xin, Y. H., Liu, H. C., Chen, B., Chi, Z. M., Zhou, P. J. & Yu, Y. (2008). *Marinomonas arctica* sp. nov., a psychrotolerant bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **58**, 1715-1718.

Legend of Figures

Fig 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing relationship of isolate KMM 3893^T and *Marinomonas* species. Phylogenetic analysis was performed using the software package MEGA 4 (Tamura *et al*., 2007) after multiple alignment of data by CLUSTALX (version 1.83; Thompson *et al*., 1997). Bootstrap values based on 1000 replications are given as percentages at the branching points. Numbers indicate percentages greater than 90%. Bar, 0.01 substitutions per nucleotide position.

Supplementary Figure S1. Maximum parsimony phylogenetic tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing relationship of isolate KMM 3893^T and *Marinomonas* species. Phylogenetic analysis was performed using the software package MEGA 4 (Tamura *et al*., 2007) after multiple alignment of data by CLUSTALX (version 1.83; Thompson *et al*., 1997). Bootstrap values based on 1000 replications are given as percentages at the branching points and numbers indicate percentages greater than 90%. Bar, 20 % nucleotide sequence divergence.

Fig. 1.


0.01
90
100
Marinomonas primoryensis **KMM 3633^T (AB074193)**
Marinomonas arctica **328^T (DQ492749)**
Marinomonas polaris **CK13^T (AJ833000)**
Marinomonas pontica **46-16^T (AY539835)**
Marinomonas arenicola **KMM 3893^T (AB467281)**
Marinomonas ushuaiensis **U1^T (AJ627909)**
Marinomonas dokdonensis **DSW10-10^T (DQ011526)**
Marinomonas mediterranea **ATCC 700492^T (AF063027)**
Marinomonas vaga **ATCC 27119^T (X67025)**
Marinomonas communis **LMG 2864^T (DQ011528)**
Marinomonas aquimarina **CECT 5080^T (AJ843077)**
Marinomonas ostreistagni **JCM 13672^T (AB242868)**
Alteromonas macleodii **IAM 12920^T (X82145)**
Vibrio cholerae **ATCC 14035^T (X74695)**

Supplementary figure S1.