Marinomonas polaris sp. nov., a psychrohalotolerant strain isolated from coastal sea water off the subantarctic Kerguelen islands

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Two aerobic, psychrohalotolerant, motile bacterial isolates, CK13^T and CK16, isolated from sea-water samples collected off the subantarctic Kerguelen island, were characterized by using a polyphasic taxonomic approach. On the basis of 16S rRNA gene sequence data, the strains were 99.6% similar and exhibited 93–97% similarity with the seven recognized species of *Marinomonas*. The most closely related species were *Marinomonas pontica* and *Marinomonas primoryensis*, with 97 and 96% similarity at the 16S rRNA gene sequence level, respectively. DNA–DNA hybridization values between strain CK13^T and *M. pontica* and *M. primoryensis* were only 58 and 40%, respectively. The major fatty acids present in strain CK13^T were iso-C_{16:0}, C_{16:0}, C_{16:1ω7c} and C_{18:1ω7c}. The DNA G+C content of strain CK13^T was 41.2 mol%. Phosphatidylethanolamine and phosphatidylglycerol were identified as the predominant phospholipids. All the above characteristics support the affiliation of strain CK13^T to the genus *Marinomonas*. Phylogenetic analysis and phenotypic and genotypic distinctiveness confirmed that strains CK13^T and CK16 are members of a novel species of the genus *Marinomonas*, for which the name *Marinomonas polaris* sp. nov. is proposed. The type strain is CK13^T (=MTCC 6645^T=DSM 16579^T=JCM 12522^T).

The genus *Marinomonas* was created to accommodate two reclassified *Alteromonas* species, namely *Alteromonas communis* and *Alteromonas vaga* (Baumann *et al.*, 1972), as these species formed a separate and distinct rRNA branch when compared with the other true alteromonads (Van Landschoot & De Ley, 1983). Species assigned to the genus *Marinomonas* are rod-shaped, motile, lack amylase, gelatinase and lipase activities and have the ability to utilize glycerol, lactate and *m*-hydroxybenzoate, but not butyrate or valerate as carbon sources. To date, seven species of *Marinomonas* have been described, *Marinomonas communis*, *M. vaga*, *M. mediterranea*, *M. primoryensis*, *M. ushuaiensis*, *M. pontica* and *M. aquimarina* (Baumann *et al.*, 1972; Solano & Sanchez-Amat, 1999; Romanenko *et al.*, 2003; Ivanova *et al.*, 2005; Macian *et al.*, 2005; Prabakaran *et al.*, 2005). In the present study, a bacterial strain CK13^T, isolated from sea water off the subantarctic Kerguelen island, has been identified as representing a novel species of the genus *Marinomonas*.

Strains CK13^T and CK16 were isolated from sea water collected at a site located 110 km south-west of the subantarctic Kerguelen islands (50° 40' S 68° 25' E). The strains were isolated along with 45 other pure strains by plating 200 µl sea water on marine agar 2216 (Difco) and incubating the plates at 12 °C for 10 days. Marine agar was used for growth and maintenance of the strains and for the determination of the phenotypic and chemotaxonomic characteristics as listed in Table 1 and Supplementary Table S1 (see IJSEM Online). Luria–Bertani medium [1.0% (w/v) tryptone, 0.5% (w/v) yeast extract and 1.0% (w/v) NaCl] was used to test the salt tolerance of the organism. The shape, size and motility of the cells was ascertained using a Leitz Diaplan phase-contrast microscope with an oil-immersion objective (× 100). The sensitivity of the cultures to antibiotics was checked by using antibiotic discs (Himedia). For biochemical tests, cultures were grown at 22 °C on marine agar 2216 and tests were performed as described by Baumann *et al.* (1984) and Smibert & Krieg (1994). Utilization of various carbon compounds as sole carbon sources was tested in liquid minimal medium containing (l⁻¹) 1 g ammonium chloride, 0.075 g dipotassium hydrogen phosphate, 1.45 g calcium chloride, 30.0 g sodium chloride, 6.15 g magnesium chloride, 0.75 g

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CK13^T is AJ833000.

A supplementary table detailing the cellular fatty acid content of strain CK13^T and related *Marinomonas* species is available in IJSEM Online.

Table 1. Phenotypic characteristics that differentiate *Marinomonas polaris* sp. nov. CK13^T from other *Marinomonas* species

Strains: 1, *M. polaris* CK13^T; 2, *M. pontica* LMG 22531^T; 3, *M. primoryensis* IAM 15010^T; 4, *M. ushuaiensis* MTCC 6143^T (data from Prabakaran *et al.*, 2005); 5, *M. mediterranea* ATCC 700492^T (Solano & Sanchez-Amat, 1999); 6, *M. communis* DSM 5604^T (Baumann *et al.*, 1972); 7, *M. vaga* ATCC 27119^T (Baumann *et al.*, 1972); 8, *M. aquimarina* CECT 5080^T (Macian *et al.*, 2005). Data for taxa 1–3 are from this study. All of these *Marinomonas* species are Gram-negative, rod-shaped, motile bacteria which test negative for gelatin hydrolysis and for denitrification and test positive for glucose and mannose utilization. Strains CK13^T and CK16 share similar phenotypic characteristics. +, Positive; –, negative; ND, no data available; w, weakly positive; v, variable.

Characteristic	1	2	3	4	5	6	7	8
Pigment	Absent	Absent	Absent	Absent	Melanin-like	Absent	Absent	Absent
Growth at:								
4 °C	+	+	+	+	–	–	–	–
37 °C	+	–	–	–	–	+	ND	+
pH range for growth	6.0–10.0	6.0–10.0	6.0–9.5	7.0–12.5	ND	7.0–11.0	ND	ND
NaCl requirement	–	+	+	+	+	+	ND	+
NaCl tolerance (%)	11	10	8	6	6	6	ND	10
Lipase	–	–	–	–	+	–	–	–
Amylase	–	–	–	+	–	–	–	–
Phosphatase	+	+	+	+	ND	–	ND	ND
Simmon's citrate test	+	+	–	–	ND	–	ND	ND
Nitrate reduction	–	–	–	–	+	–	–	–
Carbon source utilization:								
L-Alanine	–	+	–	ND	ND	+	+	+
L-Arginine	+	+	+	–	ND	+	v	ND
Benzoate	–	–	–	+	ND	–	–	ND
Butyrate	–	–	–	–	ND	–	–	ND
Cellobiose	+	+	+	–	ND	–	ND	–
Citrate	–	–	–	ND	+	+	+	+
D-Galactose	+	–	–	ND	ND	ND	ND	–
Erythritol	+	–	–	–	ND	–	+	ND
Glycerol	+	+	+	–	+	+	+	–
L-Glycine	+	–	–	ND	ND	–	–	+
m-Hydroxybenzoate	–	–	–	+	–	+	+	ND
Lactose	+	+	–	+	ND	–	–	–
L-Lysine	–	–	–	–	ND	+	v	–
Malate	–	–	–	–	+	+	+	+
Maltose	+	+	+	w	–	ND	ND	ND
L-Rhamnose	–	+	–	–	ND	ND	+	–
D-Ribose	+	–	+	–	ND	–	ND	w
Sorbitol	+	–	–	–	+	+	+	–
Succinate	–	–	–	–	+	+	+	+
L-Threonine	+	–	–	ND	ND	+	ND	–

potassium chloride and 0.028 g ferrous sulphate, supplemented with 0.2 % of the filter-sterilized carbon source (Romanenko *et al.*, 2003). Fatty acid methyl esters were prepared from cells grown at 22 °C for 48 h according to the method of Sato & Murata (1988) and analysed as described by Kiran *et al.* (2004). The modified method of Bligh & Dyer (1959) was employed to extract polar lipids and molybdenum blue reagent was used to detect lipids containing phosphate esters. DNA was isolated and the DNA G+C content was determined as described by Gupta *et al.* (2004). DNA–DNA hybridization was performed by the membrane filter method of Tourova & Antonov (1987) as described by Shivaji *et al.* (1992). *M. pontica* LMG 22531^T and *M.*

primoryensis IAM 15010^T were used as controls in biochemical tests, in the identification of fatty acids and polar lipids and in DNA–DNA hybridization experiments.

The 16S rRNA gene was amplified from genomic DNA, purified and sequenced as described by Shivaji *et al.* (2000). To determine the phylogenetic affiliation of the novel strains, the almost-complete 16S rRNA gene sequence (1475 bp) of strain CK13^T was aligned with related species of the genus *Marinomonas* using CLUSTAL W (Thompson *et al.*, 1994). Pairwise evolutionary distances were computed using the DNADIST program with the Kimura two-parameter model as developed by Kimura (1980). Phylogenetic trees were

constructed using the neighbour-joining and UPGMA tree-making algorithms from the PHYLIP package (Felsenstein, 1993). The stability among clades of the phylogenetic tree was assessed by performing 1000 replicates and analysing the dataset using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE from the PHYLIP package.

Marine isolates CK13^T and CK16 are aerobic, Gram-negative, halotolerant, rod-shaped and motile bacteria which are able to grow between 4 and 37 °C. Colony appearance on marine agar is smooth, non-pigmented and convex. Morphological features and some biochemical properties characteristic of members of the genus *Marinomonas*, such as lack of amylase, lipase and gelatinase activities, the presence of catalase activity, but weak oxidase activity and the utilization of glycerol, but not butyrate, are also exhibited by both novel strains (Table 1). The fatty acid content, with iso- and branched-chain fatty acids C_{16:0} (6.2 %), iso C_{16:0} (18.5 %), C_{16:1ω7c} (26.5 %) and C_{18:1ω7c} (40.0 %) as the major fatty acids, and the presence of phosphatidylethanolamine and phosphatidylglycerol as the major phospholipid constituents also indicate that CK13^T and CK16 are similar to the previously described *Marinomonas* species (Romanenko *et al.*, 2003; Ivanova *et al.*, 2000, 2005; Prabakaran *et al.*, 2005) (see Supplementary Table S1 in IJSEM Online). Other morphological and biochemical characteristics that differentiate CK13^T from recognized *Marinomonas* species are given in Table 1 and in the description of the novel species. Fatty acid content was similar in CK13^T, *M. pontica* LMG 22531^T and *M. primoryensis* IAM 15010^T (see Supplementary Table S1 in IJSEM Online).

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence (1475 nucleotides) of strain CK13^T using the neighbour-joining algorithm confirmed the affiliation of this marine strain to the genus *Marinomonas*. The phylogenetic tree based on the above analysis showed that strain CK13^T is within the species cluster comprising the genus *Marinomonas* and forms a clade with *M. primoryensis* IAM 15010^T and *M. pontica* LMG 22531^T, with a bootstrap value of >90 % (Fig. 1). The close relationship between CK13^T, *M. primoryensis* and *M. pontica* was further evident from a BLAST analysis of the 16S rRNA gene sequence of CK13^T, which exhibits 97 and 96 % similarity with *M. primoryensis* and *M. pontica*, respectively. Despite the high similarity at the 16S rRNA gene sequence level, in terms of DNA–DNA hybridization strain CK13^T shares only 58 and 40 % relatedness with *M. pontica* LMG 22531^T and *M. primoryensis* IAM 15010^T, respectively. Further, CK13^T differs from these closely related species in regard to a number of phenotypic characteristics (Table 1). According to the criteria recommended for discriminating species (Stackebrandt & Goebel, 1994), strain CK13^T, which exhibits >3 % difference at the 16S rRNA gene sequence level with all previously described *Marinomonas* species, <70 % relatedness in DNA–DNA hybridization with the closely related species *M. pontica* and *M. primoryensis* and which also differs in phenotypic traits

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (1475 bp) showing the phylogenetic relationship between *Marinomonas polaris* CK13^T and related species of the genus *Marinomonas*. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. *Vibrio cincinnatiensis* was used as an outgroup. Bar, 0.1 nucleotide substitutions per nucleotide position.

from these two *Marinomonas* species, should be classified as a member of the genus *Marinomonas*. It is concluded that strain CK13^T represents a novel species, for which the name *Marinomonas polaris* sp. nov. is proposed.

Description of *Marinomonas polaris* sp. nov.

Marinomonas polaris (po.la'ris. N.L. fem. adj. *polaris* polar, referring to the origin of the type strain).

Cells are Gram-negative, motile, rod-shaped and are 0.6–0.8 µm wide and 2–3.2 µm in length. On marine agar, colonies are non-pigmented, cream, circular, raised, smooth and 2–3 mm in diameter. Grows between 4 and 37 °C and with 0–11 % salt in the medium. The optimum pH and salt concentration for growth are pH 7.0 and 5 % salt, respectively. CK13^T tests positive for phosphatase, catalase, β-galactosidase, aesculinase and arginine dihydrolase activities and for citrate utilization, but negative for gelatin hydrolysis, indole production and lipase and lysine decarboxylase activities. Negative reaction in the Voges–Proskauer test, does not reduce nitrate to nitrite and no denitrification. Produces acid from sucrose, D-maltose, D-mannose, D-xylose, D-ribose and D-fructose, but not from glucose, melibiose or rhamnose. Utilizes D-glucose, D-fructose, D-xylose, D-galactose, D-ribose, D-mannose, sucrose, lactose, D-maltose, D-sorbitol, inositol, erythritol, adonitol, glycerol, D-cellobiose, dextrin, acetate, D-glucuronic acid, sodium

gluconate, sodium malonate, L-glycine, L-histidine, L-threonine, L-arginine, L-asparagine and glutamine, but not L-sorbose, L-rhamnose, L-raffinose, D-arabinose, xylitol, glycogen, citrate, lactate, succinate, butyrate, sodium formate, benzoate, *m*-hydroxybenzoate, 2-oxoglutarate, propionate, L-lysine, L-alanine, L-serine, cysteine, L-aspartic acid or L-glutamic acid as sole carbon sources. The type strain is sensitive to amikacin (30 µg), ciprofloxacin (30 µg), kanamycin (30 µg), chloramphenicol (30 µg), tobramycin (10 µg), nitrofurantoin (300 µg), vancomycin (30 µg), ampicillin (10 µg), tetracycline (30 µg), streptomycin (30 µg), erythromycin (30 µg), lincomycin (30 µg), penicillin (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), chloramphenicol (30 µg), gentamicin (30 µg), cephatoxime (30 µg), cefoperazone (75 µg), cefazolin (30 µg), oleanomycin (15 µg), polymyxin B (50 U), spectinomycin (100 µg), tobramycin (30 µg) and rifampicin (30 µg), but resistant to bacitracin (10 U). The predominant phospholipids are phosphatidylethanolamine and phosphatidylglycerol. Major cellular fatty acids are C_{14:0} (1.2%), anteiso C_{15:0} (1.2%), iso C_{16:0} (18.5%), C_{16:0} (6.2%), C_{16:1ω7c} (26.5%), anteiso C_{17:0} (2.5%), C_{18:0} (2.4%) and C_{18:1ω7c} (40.0%). The DNA G + C content is 41.2 mol%.

The type strain, CK13^T (= MTCC 6645^T = DSM 16579^T = JCM 12522^T), was isolated from coastal sea water collected from the subantarctic Kerguelen island.

References

- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **3**, 402–429.
- Baumann, P., Gauthier, M. J. & Baumann, L. (1984). Genus *Alteromonas* Baumann, Baumann, Mandel and Allen. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 343–352. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gupta, P., Reddy, G. S. N., Delille, D. & Shivaji, S. (2004). *Arthrobacter gangotriensis* sp. nov. and *Arthrobacter kerguelensis* sp. nov. from Antarctica. *Int J Syst Evol Microbiol* **54**, 2375–2378.
- Ivanova, E. P., Zhukova, N. V., Svetashev, V. I., Gorshkova, N. M., Kurilenko, V. V., Frolova, G. M. & Mikhailov, V. V. (2000). Evaluation of phospholipid and fatty acid compositions as chemotaxonomic markers of *Alteromonas*-like proteobacteria. *Curr Microbiol* **41**, 341–345.
- 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.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kiran, M. D., Prakash, J. S. S., Annapoorni, S., Dube, S., Kusano, T., Okuyama, H., Murata, N. & Shivaji, S. (2004). Psychrophilic *Pseudomonas syringae* required trans-monounsaturated fatty acid for growth at higher temperature. *Extremophiles* **8**, 401–410.
- Macian, M. C., Arahal, D. R., Garay, E. & Pujalte, M. J. (2005). *Marinomonas aquamarina* sp. nov., isolated from oysters and sea water. *Syst Appl Microbiol* **28**, 145–150.
- Prabakaran, 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, A. L., Uchino, M., Mikhailov, V. V., Zhukova, N. V. & Uchimura, T. (2003). *Marinomonas primoryensis* sp. nov., a novel psychrophile isolated from coastal sea-ice in the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 829–832.
- Sato, N. S. & Murata, N. (1988). Membrane lipids. *Methods Enzymol* **167**, 251–259.
- Shivaji, S., Ray, M. K., Saisree, L., Jagannadham, M. V., Seshu Kumar, G., Reddy, G. S. N. & Bhargava, P. M. (1992). *Sphingobacterium antarcticus* sp. nov. a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica. *Int J Syst Bacteriol* **42**, 102–116.
- Shivaji, S., Bhanu, N. V. & Aggarwal, R. K. (2000). Identification of *Yersinia pestis* as the causative organism of plague in India as determined by 16S rDNA sequencing and RAPD-based genomic fingerprinting. *FEMS Microbiol Lett* **189**, 247–252.
- Smibert, R. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. 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.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tourova, T. P. & Antonov, A. S. (1987). Identification of microorganisms by rapid DNA-DNA hybridization. *Methods Microbiol* **19**, 333–355.
- 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**, 3057–3074.