Marinomonas ushuaiensis sp. nov., isolated from coastal sea water in Ushuaia, Argentina, sub-Antarctica

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A Gram-negative, rod-shaped, psychrophilic, motile, non-spore-forming bacterium, strain U1^T, was isolated from Ushuaia located at the southernmost tip of Argentina. On the basis of 16S rRNA gene sequence similarity, strain U1^T was found to be closely related to *Marinomonas communis* (DSM 5604^T) and *Marinomonas primoryensis* (IAM 15010^T). At the DNA–DNA level, however, the values for similarity were 41 and 25 %, respectively. The major fatty acids present were iso-C_{16:0}, C_{16:1}ω7c, iso-C_{17:1} and C_{18:1}ω7c and the G + C content of the DNA was 43.6 mol%. All of the above characteristics support the affiliation of strain U1^T to the genus *Marinomonas*. Furthermore, on the basis of phenotypic features, chemotaxonomic characteristics and phylogenetic analysis of the 16S rRNA gene sequence, it appears that strain U1^T is distinct from the four *Marinomonas* species with validly published names. Strain U1^T, therefore, represents a novel species, for which the name *Marinomonas ushuaiensis* sp. nov. is proposed. The type strain of *M. ushuaiensis* is U1^T (=MTCC 6143^T=DSM 15871^T=JCM 12170^T).

The genus *Marinomonas* was extracted from the genus *Alteromonas* (Baumann *et al.*, 1972) to accommodate *Alteromonas communis* and *Alteromonas vaga*, which formed a separate and a distinct rRNA branch when compared with the other species of *Alteromonas*, namely *Alteromonas macleodii*, *Alteromonas haloplanktis* and *Alteromonas putrefaciens* (van Landschoot & De Ley, 1983). Two of these species, namely *A. haloplanktis* and *A. putrefaciens*, were reclassified as *Pseudoalteromonas haloplanktis* (Gauthier *et al.*, 1995) and *Shewanella putrefaciens* (MacDonell & Colwell, 1985), respectively. Species assigned to the genus *Marinomonas* are rod-shaped, motile, metabolize *p*-hydroxybenzoate and *m*-hydroxybenzoate, utilize acetate but not butyrate or valerate and have DNA G + C contents in the range 46–48 mol% (Baumann *et al.*, 1972). To date, four species of *Marinomonas* have been described: *Marinomonas communis*, *Marinomonas vaga*, *Marinomonas mediterranea* and *Marinomonas primoryensis* (Baumann *et al.*, 1972; Solano & Sanchez-Amat, 1999; Romanenko *et al.*, 2003). In

this report, a bacterial strain, U1^T, isolated from sea water collected from a sub-Antarctic region, Ushuaia, has been identified, on the basis of polyphasic taxonomy, as a novel species of the genus *Marinomonas*; the name *Marinomonas ushuaiensis* sp. nov. is proposed.

Ushuaia is a sub-Antarctic region located at the southernmost tip of Argentina (54° 47' S, 68° 20' W). Strain U1^T was isolated from the surrounding sea water using marine agar (2216; Difco). About 50 ml of sea water collected in a sterile container was filtered using a 0.45 µm filter; the filter was then placed directly on marine agar plates and incubated at 2 °C until the appearance of colonies. A total of 17 representative colonies were isolated and purified from sea water of Ushuaia; strain U1^T was one of the representative colonies. On marine agar, colonies of U1^T are circular (2–3 mm in diameter), convex, cream in colour and are able to grow between 4 and 25 °C, exhibiting optimum growth at 20 °C. No growth is observed above 30 °C. Strain U1^T does not grow at pH 5.0 but grows between pH 7.0 and 12.5, showing maximum growth at pH 8.0. Furthermore, it can tolerate up to 6 % (w/v) NaCl but can not grow in the absence of NaCl, indicating that it is halophilic. Cell morphology was observed under a Leitz Diaplan phase-contrast microscope: cells of U1^T grown at

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain U1^T is AJ627909.

Table 1. Phenotypic characteristics that differentiate *M. ushuaiensis* sp. nov. U1^T from related species

Strains: 1, *M. ushuaiensis* U1^T; 2, *M. primoryensis* IAM 15010^T; 3, *M. communis* DSM 5604^T; 4, *M. vaga* ATCC 27119^T (data from Baumann *et al.*, 1972); 5, *M. mediterranea* ATCC 700492^T (Solano & Sanchez-Amat, 1999). Unless indicated otherwise, data are from the present study. +, Positive; –, negative; ND, no data available; W, weakly positive; V, variable.

Characteristic	1	2	3	4	5
Cell shape	Straight rod	Straight rod	Curved rod	Straight rod	Straight rod
Pigment	Absent	Yellowish	Absent	Absent	Melanin-like
Growth at:					
4 °C	+	+	–	–	–
30 °C	+	+	+	+	ND
35 °C	–	–	+	+	–
pH range for growth	7.0–12.5	7.0–12.0	7.0–11.0	ND	ND
NaCl requirement	+	+	+	ND	+
NaCl tolerance (%)	6	6	6	ND	6
Oxidase	–	+	+	–	–
Nitrate reduction	–	–	–	–	+
Denitrification	–	–	–	–	ND
Lipase	–	–	–	–	+
Amylase	+	–	–	–	–
Urease	–	+	–	ND	ND
Gelatinase	–	–	–	–	+
Phosphatase	+	–	–	ND	ND
Citrate	–	+	–	ND	ND
Carbon-source utilization:					
Butyrate	–	–	+	+	ND
Cellobiose	–	–	–	ND	ND
Erythritol	–	–	–	ND	ND
Glucose	+	W	+	+	+
Glycerol	–	+	–	ND	ND
<i>o</i> -Hydroxybenzoate	–	–	–	ND	ND
<i>m</i> -Hydroxybenzoate	+	+	+	+	–
<i>p</i> -Hydroxybenzoate	–	–	+	ND	ND
DL-Malate	–	+	+	+	+
D-Maltose	W	+	V	V	–
Mannitol	–	+	+	+	ND
D-Mannose	+	+	+	ND	+
D-melibiose	+	–	–	ND	ND
Succinate	–	–	+	+	+
Valerate	–	–	–	ND	ND
Acetate	+	+	+	ND	ND
Benzoic acid	+	+	–	ND	ND
L-Arginine	–	–	+	V	ND
L-Lysine	–	+	+	V	ND

22 °C for 48 h are Gram-negative, motile, non-sporulating rods (0.5–0.7 µm wide and 2–3 µm long).

Marine agar was used for growth and maintenance of the strain and for the determination of the phenotypic and chemotaxonomic characteristics listed in Table 1, Table 2 and in the species description. For biochemical tests, the culture was grown at 22 °C in marine agar and the tests were performed as described by Baumann *et al.* (1984), Gauthier

& Breittmayer (1992) and Smibert & Krieg (1994). The utilization of various carbon compounds as sole carbon sources was tested in mineral liquid medium containing ammonium chloride (1 g l^{–1}), dipotassium hydrogen phosphate (0.075 g l^{–1}), calcium chloride (1.45 g l^{–1}), sodium chloride (30.0 g l^{–1}), magnesium chloride (6.15 g l^{–1}), potassium chloride (0.75 g l^{–1}) and ferrous sulfate (0.028 g l^{–1}), supplemented with 0.2 % carbon source (Romanenko *et al.*, 2003). Fatty acid methyl esters

Table 2. Fatty acid composition (%) of *M. ushuaiensis* sp. nov. U1^T and related species

Strains: 1, *M. ushuaiensis* U1^T; 2, *M. communis* DSM 5604^T; 3, *M. primoryensis* IAM 15010^T. All cultures were grown in marine broth at 22 °C and the cell pellet was used for fatty acid analysis.

Fatty acid methyl ester	1	2	3
C _{12:0}	2.7	1.0	1.2
iso-C _{14:0}	1.2	1.9	0.9
anteiso-C _{15:0}	1.2	1.4	1.0
iso-C _{16:0}	16.1	13.5	11.3
C _{16:0}	2.1	1.1	1.1
C _{16:1} ω7c	28.4	23.4	21.9
iso-C _{17:0}	1.8	1.3	0.9
iso-C _{17:1}	5.0	7.2	8.0
C _{18:0}	1.0	1.6	1.4
C _{18:1} ω7c	36.6	46.8	51.0

were prepared from cells grown at 22 °C for 48 h, according to the method of Sato & Murata (1988), and analysed as described previously by Reddy *et al.* (2002). The isolation of DNA and estimation of the G+C content (mol%) of the DNA was determined as described previously (Reddy *et al.*, 2000). DNA–DNA hybridization was performed by using the membrane filter method of Tourova & Antonov (1987), as described by Shivaji *et al.* (1992). *M. primoryensis* IAM 15010^T and *M. communis* DSM 5604^T were used as controls in studies relating to biochemical tests, identification of fatty acids and DNA–DNA hybridization.

Strain U1^T conforms to the characteristics of the genus *Marinomonas*, based on the above morphological features and the fact that U1^T is negative for oxidase, positive for catalase, and utilizes benzoate, *m*-hydroxybenzoate and acetate but does not utilize *p*-hydroxybenzoate, *o*-hydroxybenzoate or butyrate (Table 1). In addition, strain U1^T possesses iso-C_{16:0} (16.1%), C_{16:1}ω7c (28.4%), iso-C_{17:1} (5.0%) and C_{18:1}ω7c (36.6%) as the major fatty acids, like the members of the genus *Marinomonas* (Table 2). Furthermore, the G+C content of the DNA of U1^T is 43.6 mol%, which is in agreement with the characteristically low G+C content of the genus *Marinomonas* (Baumann *et al.*, 1972). The other morphological, biochemical and chemotaxonomic characteristics are given in Table 1, Table 2 and the detailed species description.

The 16S rRNA gene was amplified from genomic DNA, purified and sequenced as described previously (Shivaji *et al.*, 2000). The partial sequence of the 16S rRNA gene was then aligned with closely related sequences from the EMBL database, using CLUSTAL W (Thompson *et al.*, 1994). The pair-wise evolutionary distances were computed using the DNADIST program with the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were constructed using four tree-making algorithms (Neighbour-Joining, KITSCH, FITCH and DNAPARS) of the PHYLIP software package

(Felsenstein, 1993). The stability among the clades of the phylogenetic tree was assessed by taking 1000 replicates and analysing the dataset using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package (Felsenstein, 1993).

Assignment of U1^T to the genus *Marinomonas* is further confirmed by the phylogenetic analysis based on the 16S rRNA gene sequence (1496 nt). Using the neighbour-joining algorithm, it is observed that strain U1^T is within the radiation of the cluster comprising the *Marinomonas* species, and formed a clade with *M. communis* and *M. primoryensis* with a bootstrap value of >98% (Fig. 1). The close relationship between U1^T and *M. communis* and *M. primoryensis* is also evident from the BLAST analysis of the 16S rRNA gene sequence of U1^T, which demonstrated 97 and 96% similarity to *M. communis* (DSM 5604^T) and *M. primoryensis* (IAM 15010^T), respectively. Despite the high level of similarity at the 16S rRNA gene sequence level, U1^T, at the DNA–DNA level as determined by DNA–DNA hybridization, shares only 41 and 25% similarity with *M. communis* (DSM 5604^T) and *M. primoryensis* (IAM 15010^T), respectively. Thus, it appears that U1^T, which exhibits <97% similarity at the 16S rRNA gene level, <70% similarity at the DNA–DNA level (the threshold value used to discriminate between strains at the species level; Stackebrandt & Goebel, 1994) and a number of phenotypic differences with respect to previously described *Marinomonas* species, should be classified as a novel species of the genus *Marinomonas*. The name *Marinomonas ushuaiensis* sp. nov. is proposed for this species.

Description of *Marinomonas ushuaiensis* sp. nov.

Marinomonas ushuaiensis (ush.u.ai.en'sis. N.L. fem. adj. *ushuaiensis* pertaining to the place Ushuaia, sub-Antarctica).

Aerobic, Gram-negative, motile, non-spore-forming and rod-shaped. Colonies on marine agar medium are non-pigmented, cream, circular, raised, smooth and 2–3 mm in diameter. Able to grow between 4 and 25 °C but does not grow above 30 °C, indicating that the species is psychrophilic. Does not grow at pH 5.0 but grows between pH 7.0 and 12.5, with an optimum pH of 8.0, indicating the alkaliphilic nature of the species. The maximum salt tolerance observed for the species is 6%; it does not grow below 1% NaCl, indicating that it is also halophilic. Positive for phosphatase, catalase, amylase and starch hydrolysis but negative for gelatin hydrolysis, lipase, urease, oxidase, indole production, nitrate reduction to nitrite, in the Voges–Proskauer test and for citrate utilization. Utilizes benzoate, *m*-hydroxybenzoate, acetate, D-glucose, galactose, D-melibiose, D-mannose, lactose, maltose and L-leucine as sole carbon sources but does not utilize *o*-hydroxybenzoate, *p*-hydroxybenzoate, butyrate, cellobiose, erythritol, sorbose, sorbitol, L-rhamnose, arabinose, xylose, DL-malate, D-mannitol, raffinose, glycerol,

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (1491 bases) showing the phylogenetic relationship between *M. ushuaiensis* (U1^T) and other related species of the genus *Marinomonas* and related reference micro-organisms. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are given at the nodes.

D-ribose, succinate, valerate, lactic acid, L-arginine or L-lysine as sole carbon source. Sensitive to the antibiotics amikacin (30 µg), ciprofloxacin (30 µg), kanamycin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), streptomycin (30 µg), erythromycin (30 µg), lincomycin (30 µg), penicillin (30 µg) and nalidixic acid (30 µg), but resistant to vancomycin (30 µg) and ampicillin (10 µg). The G+C content of the DNA is 43.6 mol%. The major fatty acids are iso-C_{16:0} (16.1%), C_{16:1ω7c} (28.4%), C_{17:1} (5.0%) and C_{18:1ω7c} (36.6%). The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence is AJ627909.

The type strain is U1^T (=MTCC 6143^T=DSM 15871^T=JCM 12170^T). Isolated from coastal sea water collected from Ushuaia (sub-Antarctica).

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