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 U1T, was isolated from Ushuaia located at the southernmost tip of Argentina. On the basis of 16S rRNA gene sequence similarity, strain U1T was found to be closely related to Marinomonas communis (DSM 5604T) and Marinomonas primoryensis (IAM 15010T). At the DNA–DNA level, however, the values for similarity were 41 and 25 %, respectively. The major fatty acids present were iso-C16 : 0, C16 : 1ω7c, iso-C17 : 0 and C18 : 1ω7c and the G + C content of the DNA was 43.6 mol%. All of the above characteristics support the affiliation of strain U1T 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 U1T is distinct from the four Marinomonas species with validly published names. Strain U1T, therefore, represents a novel species, for which the name Marinomonas ushuaiensis sp. nov. is proposed. The type strain of M. ushuaiensis is U1T (= MTCC 6143T = DSM 15871T = JCM 12170T).

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, U1T, 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 U1T 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 U1T was one of the representative colonies. On marine agar, colonies of U1T 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 U1T 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 U1T grown at...
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<sup>T</sup> and related species

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>1</th>
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<th>3</th>
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<tr>
<td>C&lt;sub&gt;12:0&lt;/sub&gt;</td>
<td>2-7</td>
<td>1-0</td>
<td>1-2</td>
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<td>iso-C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>1-2</td>
<td>1-9</td>
<td>0-9</td>
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<tr>
<td>anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>1-2</td>
<td>1-4</td>
<td>1-0</td>
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<tr>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>16-1</td>
<td>13-5</td>
<td>11-3</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω7c&lt;/sub&gt;</td>
<td>2-1</td>
<td>1-1</td>
<td>1-1</td>
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<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>28-4</td>
<td>23-4</td>
<td>21-9</td>
</tr>
<tr>
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<td>1-8</td>
<td>1-3</td>
<td>0-9</td>
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<tr>
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<td>5-0</td>
<td>7-2</td>
<td>8-0</td>
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<td>1-0</td>
<td>1-6</td>
<td>1-4</td>
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<tr>
<td>C&lt;sub&gt;18:1ω7c&lt;/sub&gt;</td>
<td>36-6</td>
<td>46-8</td>
<td>51-0</td>
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</tbody>
</table>

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<sup>T</sup> and *M. communis* DSM 5604<sup>T</sup> were used as controls in studies relating to biochemical tests, identification of fatty acids and DNA–DNA hybridization.

Strain U1<sup>T</sup> conforms to the characteristics of the genus *Marinomonas*, based on the above morphological features and the fact that U1<sup>T</sup> is negative for oxidase, positive for catalase, and utilizes benzoate, *m*-hydroxybenzoate and acetate but does not utilize *p*-hydroxybenzoate, *ω*-hydroxybenzoate or butyrate (Table 1). In addition, strain U1<sup>T</sup> possesses iso-C<sub>16:0</sub> (16-1 %), C<sub>16:1ω7c</sub> (28-4 %), iso-C<sub>17:1</sub> (5-0 %) and C<sub>18:1ω7c</sub> (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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> and *M. communis* and *M. primoryensis* is also evident from the BLAST analysis of the 16S rRNA gene sequence of U1<sup>T</sup>, which demonstrated 97 and 96 % similarity to *M. communis* (DSM 5604<sup>T</sup>) and *M. primoryensis* (IAM 15010<sup>T</sup>), respectively. Despite the high level of similarity at the 16S rRNA gene sequence level, U1<sup>T</sup>, at the DNA–DNA level as determined by DNA–DNA hybridization, shares only 41 and 25 % similarity with *M. communis* (DSM 5604<sup>T</sup>) and *M. primoryensis* (IAM 15010<sup>T</sup>), respectively. Thus, it appears that U1<sup>T</sup>, 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 *ω*-hydroxybenzoate, *p*-hydroxybenzoate, butyrate, cellobiose, erythritol, sorbose, sorbitol, *L*-rhamnose, arabinose, xylitol, DL-malate, *D*-mannitol, raffinose, glycerol,
Acids are iso-C₁₆ : ₀ (16 mol%), C₁₈ : ₀ (7 mol%), C₁₈ : ₁ (28 mol%), 3-OH C₁₇ : ₁ (5 mol%) and C₁₈ : ₁ (36–5 mol%). The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence is AJ627909.

The type strain is U₁ᵀ (= MTCC 6143ᵀ = DSM 15871ᵀ = JCM 12170ᵀ). Isolated from coastal sea water collected from Ushuaia (sub-Antarctica).

References


