Marinospirillum celere sp. nov., a novel haloalkaliphilic, helical bacterium isolated from Mono Lake

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Two strains of a Gram-negative, helical, haloalkaliphilic bacterium were isolated from Mono Lake (USA). Both strains were mesophilic and grew between 13 and 55 °C, with optimum growth at 35–45 °C. The optimum pH for growth was 9.5. Growth was observed at NaCl concentrations of 0.5–12 % (w/v), with optimum growth at 2 % NaCl. Both isolates were motile by means of bipolar tuft flagella, coccoid body-forming and strictly aerobic. It was concluded that they belong to the same species, based on DNA–DNA hybridization values (95% DNA relatedness). DNA G+C contents of the novel strains were 52.1 and 52.3 mol%. On the basis of 16S rRNA gene sequence similarity, both strains were shown to be related closely to the members of the genus Marinospirillum (family Oceanospirillaceae, class Gammaproteobacteria). Sequence similarity of strain v1C_Sn-red1 to the type strains of Marinospirillum alkaliophilum, Marinospirillum minutulum, Marinospirillum megaterium and Marinospirillum insulare was 95.0, 92.7, 91.8 and 91.8 %, respectively. Chemotaxonomic data [major ubiquinone, Q8; major fatty acids, C18:1(n-7) and C16:0] and physiological and biochemical tests supported the affiliation of the novel strains to the genus Marinospirillum as members of a novel species, for which the name Marinospirillum celere sp. nov. is proposed, with the type strain v1C_Sn-red1 (=LMG 24610T=VKM 2416T).

The genus Marinospirillum was created by Satomi et al. (1998) and at present it comprises four species: Marinospirillum minutulum (Watanabe, 1959; Satomi et al., 1998; type species of the genus), Marinospirillum megaterium (Satomi et al., 1998), Marinospirillum alkaliophilum (Zhang et al., 2002) and Marinospirillum insulare (Satomi et al., 2004). All species that belong to this genus are motile, helical, halophilic and Gram-negative. Organotrophic growth occurs on complex organic substrates such as yeast extract and peptone and on organic acids. Carbohydrates are not utilized. Sorokin et al. (2007) isolated an alkaliophilic strain, Marinospirillum sp. ANL-isoa, utilizing amides and carboxylic acids in an isobutyronitrile-degrading consortium, but this strain was not validly described as representing a novel species.

Strain v1C_Sn-red1 was isolated from a combined water–sediment slurry sample taken from a hot spring (40 °C, pH 9.3, salinity 25 g l−1) on Paoha island on Mono Lake (CA, USA). Strain Spir-10 was isolated from a water sample (10 m depth, pH 9.5, salinity 90 g l−1) of Mono Lake. Samples were collected in August 2000. Subsamples (1 ml) were inoculated into 20 ml aerobic medium containing (1−1): KH2PO4, 0.5 g; NH4Cl, 0.5 g; KCl, 0.5 g; NaCl, 25 g; Na2SO4, 0.5 g; MgSO4·7H2O, 0.2 g; yeast extract, 1 g; peptone, 2 g; trace element solution, 1 ml (Pfenning & Lippert, 1966); vitamin B12, 15 µg. After autoclaving, 0.05 g CaCl2·2H2O l−1, 5 g NaHCO3 l−1 and 5 g Na2CO3 l−1 were added from sterile (10 %, w/v) stock solutions. Enrichment cultures of the strains were incubated at 40 °C (v1C_Sn-red1) or 25 °C (Spir-10). For strain isolation, samples of water were transferred onto plates that contained 2% agar. Growth was followed by monitoring the increase in OD550 in Hungate tubes (anaerobically) or in plain tubes (aerobically). All runs were done in duplicate. The pH was adjusted to 9.5 by varying the amounts of NaHCO3 and Na2CO3 and addition of 1 M HCl and 1 M NaOH. After determination of optimal growth conditions, both strains were cultivated at 35 °C, pH 9.5 and 2 % NaCl (w/v). Substrate-utilization tests were
performed at optimal temperature and in a pH-optimal carbonate-buffered medium of the following composition: mineral stock plus vitamin solution were the same as above; yeast extract, 0.05 g l⁻¹; substrate, 1 g l⁻¹. All substrates were prepared as anaerobic stock solutions in distilled water. Reduction of various inorganic compounds was tested anaerobically on a medium containing pyruvate, peptone and yeast extract (all at 1 g l⁻¹) as described previously (Gorlenko et al., 2004). Poly-β-hydroxybutyrate accumulation, catalase and oxidase tests, casein, gelatin, hippurate and starch hydrolysis and production of DNase, RNase, urease, lipase, phosphatase, indole and H₂S were determined as described by Gerhardt et al. (1994).

Gram staining was performed as described by Gerhardt et al. (1994). Cell morphology was observed under an Olympus BX-41 phase-contrast microscope at × 1000 with cells grown overnight. Micrographs were taken by C-7070 (Olympus) photo attachment on slides coated with 1 % (w/v) ultrapure agar. The fine structure of cells was studied after fixation of cells with a 1 % (w/v) aqueous solution of uranyl acetate with subsequent treatment with lead citrate (Reynolds, 1963). A JEM-100CXII electron microscope was used at magnification of 20 000–27 000. Negative staining of cells was achieved with 0.1 % uranyl acetate and cells were examined under a transmission electron microscope. Membrane fatty acids were extracted from freeze-dried cells with methanol/chloroform and analysed by GC-MS as described by Zhilina et al. (1997). Analysis of isoprenoid quinones was performed as described by Collins (1985).

DNA was isolated according to Marmur (1961). The G+C content was determined by the thermal-denaturation method of Marmur & Doty (1962). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by De Ley et al. (1970). Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene were done as described by Rainey et al. (1996). PCR products were sequenced by using a CEQ DTCs kit and run on a CEQ 2000XL DNA sequencing system (Beckman-Coulter). The sequences were aligned by using the CLUSTAL_X software (Thompson et al., 1997). An evolutionary-distance matrix was calculated by using the algorithm of Jukes & Cantor (1969). The phylogenetic tree was constructed by using the TREECON package (Van de Peer & De Wachter, 1994) and the neighbour-joining algorithm (Saitou & Nei, 1987). Bootstrap analyses were based on 500 resamplings.

Physiological and biochemical characteristics of the strains studied are listed in the species description and in Table 1. Both strains require NaCl, with growth being observed at NaCl concentrations of 0.5–12 % (w/v) with an optimum at 2 % for both strains. Growth of both strains was seen at 13–55 °C, with a wide temperature optimum of 35–45 °C. The pH range for growth of both strains is 8.0–10.5, with an optimum at 9.5. Best growth occurred on caprilate (up to 3 × 10⁸ cells ml⁻¹). The shortest doubling time on caprilate-containing medium under optimal conditions was 20 min. At the early stages of development, cells were motile with a swimming speed of up to 60 μm s⁻¹. After about 4 days cultivation, thin-walled coccoid bodies are observed, but no spores are produced. In old cultures, cells become shorter and thinner and a protuberance arises from

### Table 1. Characteristics of *Marinospirillum* species and strain v1c_Sn-red<sup>T</sup>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Cell length (μm)</td>
<td>1.4–15</td>
<td>2.7–4.0</td>
<td>2–2.8</td>
<td>5–15</td>
<td>2.5–7.5</td>
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<td>Cell diameter (μm)</td>
<td>0.2–0.4</td>
<td>0.2–0.3</td>
<td>0.3–0.4</td>
<td>0.8–1.2</td>
<td>0.1–0.2</td>
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<tr>
<td>Colony formation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Oxygen requirement</td>
<td>Ae</td>
<td>Ae</td>
<td>Ae</td>
<td>Mi</td>
<td>Ae</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td>13–55</td>
<td>8–49</td>
<td>11–37</td>
<td>4–25</td>
<td>4–37</td>
</tr>
<tr>
<td>Optimum</td>
<td>35–45</td>
<td>37</td>
<td>30</td>
<td>20–25</td>
<td>25–30</td>
</tr>
<tr>
<td>pH for growth</td>
<td>8.0–10.5</td>
<td>7.0–11.0</td>
<td>7.0–10.5</td>
<td>7.5–9.0</td>
<td>6.5–10.0</td>
</tr>
<tr>
<td>Optimum</td>
<td>9.5</td>
<td>9.5</td>
<td>9.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>0.5–12</td>
<td>0.2–5.0</td>
<td>0.2–8.0</td>
<td>0.5–9.0</td>
<td>0.5–10.0</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>− or (+)</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>Isoprenoid quinone</td>
<td>Q8</td>
<td>Q8, MK6</td>
<td>Q8</td>
<td>Q8</td>
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<td>Major fatty acids</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;(&lt;i&gt;ω-7&lt;/i&gt;), C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>ND</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;, C&lt;sub&gt;18:1&lt;/sub&gt;(&lt;i&gt;ω-7&lt;/i&gt;)</td>
<td>ND</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;, C&lt;sub&gt;18:1&lt;/sub&gt;(&lt;i&gt;ω-7&lt;/i&gt;)</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>52.3</td>
<td>46.8</td>
<td>42.5</td>
<td>44–45</td>
<td>42–43</td>
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</table>
the outer membrane at the end of the cell. The protuberance enlarges and eventually forms a single coccoid body as the helical cell is absorbed (Fig. 1). Similarities in phenotypic characteristics support the inclusion of the studied strains in the genus Marinospirillum. However, the new strains clearly differed from other species by a higher growth-temperature range and optimum and an inability to grow at pH values <8.0.

Strain v1c_Sn-redT contains ubiquinones that consist mainly of Q8. Menaquinone and methylmenaquinone were not detected. Other Marinospirillum species also have Q8 as the major isoprenoid quinone (Satomi et al., 1998, 2004; Zhang et al., 2002). The fatty acid profile of strain v1c_Sn-redT is composed of C18:1(n-7) (48.52 %), C16:0 (36.22 %), C16:1(n-7) (8.05 %), C14:1(n-3) (3.16 %) and C14:0 (1.07 %). This novel strain shows a combination of fatty acids found in other species of the genus Marinospirillum.

A DNA–DNA hybridization experiment revealed that the new isolates are related closely to each other (95 % DNA relatedness), suggesting that they belong to the same species. The G + C contents of the new isolates v1c_Sn-redT and Spir-10 were respectively 52.3 and 52.1 mol%, i.e. 5–10 mol% higher than those of other Marinospirillum species. Strains v1c_Sn-redT and Spir-10 have a high level of 16S rRNA gene sequence similarity to each other (99.6 %) and form a stable phylogenetic cluster, with a bootstrap value of 100 %, within the genus Marinospirillum (Fig. 2). Sequence-similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain v1c_Sn-redT were M. alkaliphilum Z4T (95.0 %), M. minututum ATCC 19193T (92.7 %), M. megaterium JCM 10129T (91.8 %) and M. insulare K3 (91.8 %). Lower sequence similarities (<89.5 %) were found with other Gram-negative spirilla.

On the basis of the low 16S rRNA gene sequence-similarity values (<95 %) to other species of the genus Marinospirillum, a higher G+C content and their distinct physiological characteristics compared with other species of this genus, strains v1c_Sn-redT and Spir-10 represent a novel species. We propose the name Marinospirillum celere sp. nov. for this taxon, with the type strain v1c_Sn-redT.

Sequence-similarity calculations, a higher G+C content and higher pH and temperature optima show that both novel strains are related more closely to M. alkaliphilum than to other Marinospirillum species. Although M. minututum, M. megaterium and M. insulare were isolated previously from an ocean environment and artificial brines (Satomi et al., 1998, 2004), the novel strains and M. alkaliphilum were isolated from soda lakes. The last two species could form a novel genus in the family Oceanospirillaceae, but we believe it is better to wait until more haloalkaliphilic Marinospirillum species have been isolated before naming this taxon.

Description of Marinospirillum celere sp. nov.

Marinospirillum celere (ce’le.re. L. neut. adj. celere rapid, indicating the rapid growth on nutrient media and high motility).

Gram-negative, helical, aerobic and chemoheterotrophic. Motile by bipolar flagella. Cells are 0.2–0.4 μm in diameter and 1.4–15 μm in length. Coccoid bodies are observed after

![Fig. 1. (a, b) Electron micrographs of ultrathin-sectioned cells of strain v1c_Sn-redT from (a) the exponential-growth phase, showing the invagination of the cell membrane during cell division (arrow), and (b) the stationary-growth phase, showing the formation of a coccoid body. (c) Phase-contrast photograph of cells of strain v1c_Sn-redT from the exponential-growth phase. Bars, 0.5 μm (a, b); 2 μm (c).](http://ijs.sgmjournals.org)

![Fig. 2. Phylogenetic tree constructed by the neighbour-joining method based on 16S rRNA gene sequences of the new isolates v1c_Sn-redT and Spir-10 and type strains of related species. Bar, 0.02 genetic distance. Numbers at nodes are bootstrap-support values (percentages of 500 resamplings). The type strain of Alkalispirillum mobile was used as the outgroup.](http://ijs.sgmjournals.org)
4 days culture, but no spores are produced. The helix type is counterclockwise. Halophilic, NaCl is required for growth; growth occurs at NaCl concentrations of 0.5–12 % (w/v) and is optimal at 2 % NaCl. Temperature range for growth is 13–55 °C; optimal temperature is 35–45 °C. Alkaliphilic, pH range for growth is 8.0–10.5; optimal pH is 9.5. Colonies are circular, smooth, opalescent and 1 mm in diameter after overnight culture. Catalase- and oxidase-positive. Able to utilize caprilate, acetate, pyruvate, lactate, Casamino acids, yeast extract and peptone. D-Ribose, D-glucose, sucrose, rhamnose, cellulose, glycerol, citrate, glycolate and cysteine are not utilized. Casein, gelatin, hirpurate and starch are not hydrolysed. Indole, H2S, DNase, RNase, phosphatase, urease and lipase are not produced. Reduction of thiolsulfate, sulfate, sulfite, polysulfide, sodium selenite, ferric citrate, ferric hydroxide and elemental sulfur is not observed. Nitrate reduction and denitrifying activity are negative. Does not grow autotrophically on H2 + CO2. Intracellular poly-β-hydroxybutyrate is formed. Isoprenoid quinone type is Q8. Whole-cell fatty acids consist mainly of C18:1(n-7) and C16:0. DNA G+C content of the type strain is 52.3 mol%.

The type strain is v1c_Sn-redT (=LMG 24610T=VKM 2416T), isolated from Mono Lake (CA, USA).

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References


Z. Namsaraev and others