Marinobacter bryozorum sp. nov. and Marinobacter sediminum sp. nov., novel bacteria from the marine environment

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Two marine, Gram-negative, aerobic, halophilic strains, designated KMM 3657T and KMM 3840T, were isolated and found to be phylogenetically closely related to each other, showing 96-6 % 16S rRNA gene sequence similarity. Both strains are members of the genus Marinobacter in the γ-Proteobacteria (94-7–98-0 % 16S rRNA gene sequence similarity). Strain KMM 3657T and Marinobacter lipolyticus SM19T were closely related, with 98-0 % sequence similarity. The novel strains shared generic physiological and chemotaxonomic properties with Marinobacter species, but differed in their temperature range for growth, inability to grow in 20 % NaCl and at > 43 °C, metabolic properties and fatty acid composition. On the basis of phenotypic and phylogenetic analysis data, it is proposed that the strains represent two novel species, Marinobacter bryozorum sp. nov., with the type strain KMM 3840T (= DSM 15401T), and Marinobacter sediminum sp. nov., with the type strain KMM 3657T (= R65T = DSM 15400T).

The genus Marinobacter was described by Gauthier et al. (1992) and currently comprises eight species, four of which were described in 2003 [Marinobacter excellens (Gorskhova et al., 2003), Marinobacter litoralis (Yoon et al., 2003), Marinobacter lipolyticus (Martín et al., 2003) and Marinobacter lutaoensis (Shieh et al., 2003)] and two in 2004 [Marinobacter daepoensis and Marinobacter flavimaris (Yoon et al., 2004)]. The type species is Marinobacter hydrocarbonoclasticus (Gauthier et al., 1992). In the course of studying the diversity of halophilic bacteria associated with the marine environment, strains KMM 3657T (= R65T) and KMM 3840T (= 50-11T) were isolated from a sediment sample obtained from Peter the Great Bay, Sea of Japan, Russia, and from a Bryozoa specimen collected in the Bering Sea, August 1991, respectively. Aliquots of the diluted sediment sample and homogenized internal tissues of the Bryozoa specimen were spread on agar plates of sea water medium (SWM), containing (1-1): 5-0 g peptone, 2-5 g yeast extract, 1-0 g glucose, 0-2 g K2HPO4, 0-05 g MgSO4, 500 ml sea water, 500 ml distilled water and 15-0 g agar. Samples were incubated for 7 days at 28 °C. Each colony was streaked and cultivated on nutrient medium containing sodium ions or natural sea water. Strains KMM 3657T and KMM 3840T, deposited in the Collection of Marine Micro-organisms (KMM), Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia, were stored at −80 °C in liquid nutrient medium supplemented with 20 % (v/v) glycerol. Strains were routinely grown on marine 2216 agar (MA; Difco), marine broth (MB; Difco) and SWM. Standard tests, Gram-reaction evaluation and determination of oxidase, catalase, amylase, caseinase, chitinase and gelatinase activities were carried out as described by Smibert & Krieg (1994). Growth at different temperatures (4–50 °C) and pH values (pH 5-0–10-0) was examined on MA and MB media, respectively. Requirement and tolerance of various NaCl concentrations were determined using SWM prepared on the artificial sea water base supplemented with the appropriate NaCl concentrations (0, 0-5, 1, 3, 5, 8, 10, 15, 18, 18-5, 20, 25 and 30 %, w/v). Leifson’s medium was used to test acid production from carbohydrates with 1 % (w/v) of each compound (Leifson, 1963). In addition, biochemical tests were performed using the API

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of KMM 3657T and KMM 3840T are AJ609270 and AJ609271, respectively.
20NE and API ZYM test kits (bioMérieux) according to the manufacturer’s instructions, except that the cultures were suspended in 3 % (w/v) NaCl solution. The isolates were also characterized using the Biolog GN MicroPlate panel. Strains were grown on MA medium at 28 °C for 24 h and the microtitre plates were inoculated with cells suspended in 3 % (w/v) NaCl solution. Results were read automatically with a spectrophotometer after 24, 48 and 96 h and for up to 7 days incubation at 28 °C. The DNA G + C content was determined as described by Marmur & Doty (1962) with the modification of Owen et al. (1969). Fatty acid methyl esters were analysed using the standard procedure of the Microbial Identification system (Microbial ID) and compared to the fatty acid database. For fatty acid methyl ester analysis, bacteria were grown on MA for 3 days at 28 °C. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out as described by Rainey et al. (1996). Purified PCR products were sequenced directly using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer’s instructions.

The marine isolates KMM 3840T and KMM 3657T were Gram-negative, aerobic, oxidase- and catalase-positive, non-pigmented, halophilic bacteria. The strains studied were motile, rod-shaped organisms that differed slightly in their cell morphology. Cells of KMM 3840T were ovoid, 1·0–1·3 μm long and 0·4–0·5 μm in diameter (Fig. 1a), whereas those of KMM 3657T were long rods, 1·8–2·5 μm long and 0·3–0·4 μm in diameter (Fig. 1b). Isolates KMM 3840T and KMM 3657T required sodium ions for growth and grew in 1·0–18·0 % (w/v) NaCl at 7–42 °C and 0·5–18·0 % (w/v) NaCl at 4–42 °C, respectively.

According to 16S rRNA gene sequence analysis, strains KMM 3840T and KMM 3657T were closely related to each other phyletically (96·6 % sequence similarity) and showed moderate to close gene sequence relatedness to some Marinobacter type strains, i.e. M. lipolyticus SM19T, M. hydrocarbonoclasticus DSM 8798T and Marinobacter aquacoeI VTB7 (sequence similarity values of 96·6, 96·1 and 96·1 % to KMM 3840T and 98·0, 96·4 and 96·6 % to KMM 3657T, respectively). The sequence divergence values of >2 % obtained for the novel isolates and previously described Marinobacter type strains exceeded the value accepted as criteria for the delineation of different species (Stackebrandt & Goebel, 1994). The phylogenetic position of the two isolates was identical using two different treeing algorithms. Fig. 2 displays the dendrogram based on neighbour-joining analysis (Felsenstein, 1993). Ribotyping analysis (Fig. 3), which served as a basis for molecular identification, revealed individual patterns for the two novel isolates, thus confirming the differences between strain KMM 3657T and M. lipolyticus DSM 15157T.

The physiological and genomic characteristics of strains KMM 3840T and KMM 3657T were compared with those of other species of Marinobacter (Table 1). Some biochemical characteristics and carbon sources for growth were similar between the novel isolates and other Marinobacter species, but many differences were found that allowed them to be distinguished from each other (Table 1). Both strains examined could be distinguished from related Marinobacter species in their maximal growth temperature, salinity

![Fig. 1. Scanning electron micrographs of strains KMM 3840T (a) and KMM 3657T (b). Bars, 2 μm.](image-url)
range for growth, narrow spectrum of organic substrates that could be utilized as sole carbon sources, weak or negative enzymic reactions and DNA G+C content (Table 1). Other reactions obtained by API 20NE, API ZYM and Biolog are indicated in the species description.

The fatty acid compositions of the isolates are shown in Table 2. The fatty acids C12:0 3-OH, C16:0, C16:1ω9c and C18:1ω9c have been reported to be predominant in the cellular fatty acid compositions of known Marinobacter species (Spröer et al., 1998; Nguyen et al., 1999; Martín et al., 2003; Yoon et al., 2003). Strain KMM 3657T possessed significant amounts of C16:1ω7c (15·87 %), whereas strain KMM 3840T differed from other Marinobacter strains by having a high proportion of C18:1ω9c and a low level of C16:1ω9c (Table 2). The DNA G+C content of KMM 3657T was 56·5 mol%, whereas that of strain KMM 3840T was found to be slightly higher (59·6 mol%) than those of the other Marinobacter strains, which range between 55 and 57 mol%.

On the basis of the physiological and molecular properties, it is proposed that isolates KMM 3840T and KMM 3657T represent two novel species in the genus Marinobacter, Marinobacter bryozoorum sp. nov. and Marinobacter sediminum sp. nov., respectively.

Description of Marinobacter bryozoorum sp. nov.

Marinobacter bryozoorum [bry.o.zo’orum. N.L. gen. pl. n. bryozoorum of Bryozoan (bryozoans), marine invertebrate specimen, source of isolation].

Gram-negative, aerobic, heterotrophic, oxidase- and catalase-positive, motile and rod-shaped (1·0–1·3 μm long and 0·4–0·5 μm in diameter). Requires sodium ions for growth. Grows in 1·0–18·0 % (w/v) NaCl at 7–42 °C. No growth observed in >18·5 % NaCl or at >43 °C. Colonies on MA are non-pigmented, whitish, translucent and smooth,
2–3 mm in diameter. In addition to the phenotypic characteristics indicated in Table 1, the type strain does not produce caseinase, amylase or chitinase. Does not produce acid from glucose, sucrose, lactose, maltose, galactose, arabinose, rhamnose, N-acetylgalosamine, glycerol or mannitol (determined by Leifson’s method). According to API 20NE, positive for nitrate reduction and adipate utilization, and negative for indole production, arginine dihydrolase, urease production, aesculin, gelatin, β-galactosidase and utilization of D-glucose, D-mannitol, maltose, L-arabinose, D-mannose, N-acetylgalosamine, D-gluconate, caprate, L-malate, citrate and phenylacetate. Biolog GN MicroPlate tests were positive for utilization of Tween 40 and methyl pyruvate and negative for the other organic compounds included in the Biolog panel. The whole-cell fatty acid composition is given in Table 2.

**Table 1. Phenotypic characteristics of KMM 3840T, KMM 3657T and phylogenetically related *Marinobacter* species**

Species/strains: 1, KMM 3840T; 2, KMM 3657T; 3, *M. hydrocarbonoclasticus*; 4, *M. lipolyticus*; 5, *M. aquaeolei*; 6, *M. litoralis*. Data were obtained from this study and the following references: Gauthier et al. (1992), Nguyen et al. (1999), Yoon et al. (2003) and Martín et al. (2003). All strains are positive for motility, catalase, oxidase, alkaline phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase (strain SM19T was not tested). All strains are negative for arginine dihydrolase, lipase C14, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase (strain SM19T was not tested), and utilization of D-fructose, D-mannitol, D-gluconate and L-arginine (strain SM19T was not tested). +, Positive; −, negative; W, weak reaction; V, variable; ND, not determined.

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<td><strong>Cell size (µm)</strong></td>
<td>0.4–0.5 × 1.0–1.3</td>
<td>0.3–0.4 × 1.8–2.5</td>
<td>0.3–0.6 × 2.0–3.0</td>
<td>0.3–0.5 × 2.5–3.5</td>
<td>0.4–0.5 × 1.4–1.6</td>
<td>0.5–0.8 × 1.5–3.0</td>
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<td>−</td>
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<td>Esterase C4</td>
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<td>59.6</td>
<td>56.5</td>
<td>57.5</td>
<td>57.0</td>
<td>55.7</td>
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Table 2. Fatty acid composition (%) of KMM 3840T, KMM 3657T and some Marinobacter species

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<tr>
<td>C12:0</td>
<td>5.58</td>
<td>4.15</td>
<td>4.66</td>
<td>5.5</td>
<td>7.89</td>
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<td>C12:0 3-OH</td>
<td>10.68</td>
<td>8.04</td>
<td>7.74</td>
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<tr>
<td>C14:0</td>
<td>0.81</td>
<td>0.92</td>
<td>2.24</td>
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<td>C15:0</td>
<td>0</td>
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<td>1.36</td>
<td>0.7</td>
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<td>1.0</td>
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<tr>
<td>C16:0</td>
<td>17.45</td>
<td>21.78</td>
<td>23.59</td>
<td>25.0</td>
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<td>28.5</td>
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<td>C16:1ω9c</td>
<td>3.67</td>
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<td>10.6</td>
<td>11.62</td>
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<tr>
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<td>C19:0cyclo 10c19o6</td>
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<td>0</td>
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Type strain is KMM 3840T (=50-11T=DSM 15401T). The DNA G+C content of the type strain is 56-5 mol%. Isolated from marine coastal sediments obtained from Peter the Great Bay, Sea of Japan, Russia.

Acknowledgements

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References


