Marinobacterium maritimum sp. nov., a marine bacterium isolated from Arctic sediment

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A Gram-negative, aerobic, rod-shaped, motile, marine bacterium, strain AR11T, was isolated from Arctic marine sediment. Strain AR11T grew with 0.5–7% NaCl and at 7–37 °C and pH 5.5–9.0. It utilized propionate, 3-hydroxybenzoate, L-proline, acetate, D- and L-lactate, L-alanine, malate and phenylacetic acid. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase and acid phosphatase activity tests were positive. Acid was produced from 5-ketogluconate and aesculin. Strain AR11T possessed C16:0 (22.0%), summed feature 4 (C16:1ω7c and/or iso-C15:0 2-OH; 28.1%) and summed feature 7 (one or more of C18:ω7c, ω9t and ω12t; 34.0%) as the major cellular fatty acids. The major ubiquinone was Q-8. Comparative 16S rRNA gene sequence studies showed that strain AR11T belonged to the Gammaproteobacteria and was most closely related to Marinobacterium stanieri DSM 7027T, Marinobacterium halophilum man011T and Marinobacterium georgiense KW-40T (97.8, 97.0 and 96.7% similarity, respectively). The G+C content of the genomic DNA of strain AR11T was 57.9 mol%. DNA–DNA relatedness data indicated that strain AR11T represented a distinct species that was separated from M. stanieri DSM 7027T, M. halophilum KCTC 12240T and M. georgiense JCM 21687T. On the basis of evidence from this polyphasic study, it is proposed that strain AR11T (=KCTC 22254T=JCM 15134T) represents the type strain of a novel species, Marinobacterium maritimum sp. nov.

The genus Marinobacterium, with the type species Marinobacterium georgiense, within the class Gammaproteobacteria, was described by González et al. (1997) to accommodate a Gram-negative, strictly aerobic, rod-shaped bacterium that was isolated from marine pulp mill effluent enrichment cultures. Since then, further species have been isolated from several environments: Marinobacterium halophilum from tidal flats (getbol) (Chang et al., 2007), Marinobacterium litorale from surface water of the Yellow Sea (Kim et al., 2007) and Marinobacterium rhizophilum from roots of a plant inhabiting a coastal tidal flat (Kim et al., 2008). Pseudomonas stanieri (Baumann et al., 1983) and Oceanospirillum jannaschii (Bowditch et al., 1984) were reclassified as Marinobacterium stanieri and Marinobacterium jannaschii (Satomi et al., 2002) as a result of 16S rRNA and gyrB gene sequence analysis. Here, we provide a polyphasic taxonomic characterization of a Marinobacterium-like bacterial strain, AR11T, which was isolated from Arctic marine sediment.

During the screening of thiosulfate-oxidizers, a novel bacterial strain, AR11T, was isolated from an Arctic marine sediment and selected for further characterization by a polyphasic approach. A sediment sample was placed in a sterile conical tube and serially diluted with filter-sterilized (0.22 μm pore size; Millipore) natural sea water containing 1 mM thiosulfate. After incubation at 25 °C under aerobic conditions for 2 weeks, an aliquot of the last dilution showing turbidity was spread onto artificial seawater medium (ASW; 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl2·6H2O and 15 g agar per litre distilled water) (Levring, 1946) and incubated at 25 °C for 2 weeks. Single colonies were purified by transferring them onto marine agar 2216 (MA; Difco) and subjecting them to an additional incubation at 25 °C for 3 days. Isolated strains were stocked as glycerol suspensions (20%, w/v) at −70 °C.

Bacterial genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified from chromosomal DNA using the universal
bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'; Escherichia coli positions 8–27) and 1492R (5'-TACGTYACCTTGTTACGACTT-3'; positions 1492–1510) (Park et al., 2006; Weissburg et al., 1991) and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Korea). The 16S rRNA gene sequence of strain AR11T determined in this study was about 1425 bp long. Full 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Sequence alignments were performed using the CLUSTAL_X program (Thompson et al., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura’s two-parameter model (Kimura, 1983). Phylogenetic trees were constructed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods by using the MEGA 3 program (Kumar et al., 2004) and PHYLIP package (Felsenstein, 1993), respectively. In the analysis of phylogeny, strain AR11T was determined to belong to the Gammaproteobacteria, and the highest degrees of 16S rRNA gene sequence similarity were found with M. stanieri DSM 7027T (97.8 % sequence similarity), M. halophilum man011T (97.0 %) and M. georgiense KW-40T (96.7 %) (Fig. 1). Strain AR11T clearly belonged to the lineage of Marinobacterium, as shown by the bootstrap values of neighbour-joining (Fig. 1) and maximum-likelihood (Supplementary Fig. S1, available in IJSEM Online) phylogenetic trees (100 and 95 %, respectively).

The Gram reaction was determined by using a Gram-stain kit (Difco) according to the manufacturer’s instructions. Cell morphology was examined by light microscopy (Eclipse 80i; Nikon) and transmission electron microscopy (EM-109; Carl Zeiss) after negative staining with 1 % (w/v) phosphotungstic acid. Catalase activity was determined by bubble production in 3 % (v/v) H2O2 and oxidase activity was determined using 1 % (w/v) tetramethyl p-phenylenediamine. Cells of strain AR11T stained Gram-negative and were aerobic rods. Cells possessed a polar flagellum (Fig. 2). Colonies were ivory, circular and convex with regular edges and a diameter of 2–3 mm when grown on MA 2216 (Difco) at 25 °C for 3 days.

Cellular fatty acids were analysed in the isolated strain and reference organisms (M. stanieri DSM 7027T, M. halophilum KCTC 12240T and M. georgiense JCM 21667T) grown on MA 2216 (Difco) at 25 °C at pH 7.8 for 3 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MSI, 1999). The fatty acids were analysed by gas chromatography (Hewlett Packard 6890) and identified using the Microbial Identification software package. Respiratory quinones were isolated, purified and analysed as described by Lee et al. (2001). Chromosomal DNA extracted for 16S rRNA gene amplification was used for determination of G+C content. RNA in the DNA solution was removed by incubation with a mixture of RNases A and T1 (each at 20 U ml⁻¹) at 30 °C for 1 h. The G+C content of the chromosomal DNA was analysed as described by Mesbah et al. (1989) using reversed-phase HPLC. The major cellular fatty acids of strain AR11T were C₁₆ : 0 (22.0 %), summed feature 4 (C₁₆ : 1ω₇c and/or iso-C₁₅ : 0 2-OH; 28.1 %) and summed feature 7 (one or more of C₁₇ : 1ω₇c, C₁₇ : 0ω₉t and C₁₂ : 0 3-OH; 34.0 %). This fatty acid profile is similar to those of closely related type strains (Table 1). There were differences in the proportion of fatty acids between this study and the original descriptions of M. halophilum man011T (Chang et al., 2007) and M. georgiense KW-40T (González et al., 1997), which may have been caused by different cultivation, extraction or analytical conditions. The major ubiquinone of AR11T was Q-8. The G+C content of genomic DNA of strain AR11T was 57.9 mol%.

Utilization of various substrates as sole carbon sources and enzyme activities were determined with API 20NE, API 32GN and API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Bacterial suspensions were made in sterile, chilled ASW (Levring, 1946) by addition of 0.005 % yeast extract (González et al., 1997). Acid production from different carbohydrates was determined using 1 % (w/v) tetramethyl phosphotungstic acid. Acid utilization was done in the isolated strain and reference organisms (M. stanieri DSM 7027T, M. halophilum KCTC 12240T and M. georgiense JCM 21667T) grown on MA 2216 (Difco) at 25 °C at pH 7.8 for 3 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MSI, 1999). The fatty acids were analysed by gas chromatography (Hewlett Packard 6890) and identified using the Microbial Identification software package. Respiratory quinones were isolated, purified and analysed as described by Lee et al. (2001). Chromosomal DNA extracted for 16S rRNA gene amplification was used for determination of G+C content. RNA in the DNA solution was removed by incubation with a mixture of RNases A and T1 (each at 20 U ml⁻¹) at 30 °C for 1 h. The G+C content of the chromosomal DNA was analysed as described by Mesbah et al. (1989) using reversed-phase HPLC. The major cellular fatty acids of strain AR11T were C₁₆ : 0 (22.0 %), summed feature 4 (C₁₆ : 1ω₇c and/or iso-C₁₅ : 0 2-OH; 28.1 %) and summed feature 7 (one or more of C₁₇ : 1ω₇c, C₁₇ : 0ω₉t and C₁₂ : 0 3-OH; 34.0 %). This fatty acid profile is similar to those of closely related type strains (Table 1). There were differences in the proportion of fatty acids between this study and the original descriptions of M. halophilum man011T (Chang et al., 2007) and M. georgiense KW-40T (González et al., 1997), which may have been caused by different cultivation, extraction or analytical conditions. The major ubiquinone of AR11T was Q-8. The G+C content of genomic DNA of strain AR11T was 57.9 mol%.

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**Fig. 1.** Neighbour-joining tree showing the phylogenetic positions of AR11T and its nearest neighbours based on 16S rRNA gene sequences. Bar, 2 substitutions per 100 nucleotide positions. Bootstrap values are expressed as percentages of 500 replications; values >50 % are shown.
mined by employing the API 50 CH system (bioMérieux) according to the manufacturer’s instructions. The suspension medium (using the CHB medium) was supplemented with 2% NaCl (final concentration). After inoculation, the galleries were incubated at 25°C for 3 days and reactions were read. Hydrolysis of starch and Tween 80 was determined as described by Cowan & Steel (1965) with modified ASW (Levring, 1946). DNA hydrolysis was observed by using DNase test agar with methyl green (Difco). The production of H2S was tested on peptone iron agar (Difco). These media were supplemented with 2% NaCl (final concentration). Discs containing the following antibiotics were tested: ampicillin (10μg), chloramphenicol (25μg), erythromycin (15μg), gentamicin (10μg), kanamycin (30μg), penicillin G (10μg), streptomycin (10μg) and tetracycline (30μg).

Growth at different temperatures was assessed after 3 days of incubation on MA 2216 (Difco). Strain AR11T was able to grow at 7–37°C, but not at 4 or above 40°C (optimum, 25–28°C). Growth at different NaCl concentrations (0–12%, w/v) was measured using ASW (Levring, 1946) without NaCl, adding 0.005% yeast extract. Strain AR11T required sodium ions for growth and grew in 0.5–7% NaCl (optimum 1–2%). The response to pH (pH 5.0–9.5 at intervals of 0.5 pH units) was determined in marine broth 2216 (Difco) at 25°C for 3 days. The pH was adjusted with 1 M HCl or NaOH. It grew at pH 5.5–9.0 but not at or below pH 5.0 or at or above pH 9.5 (optimum, pH 7.5–8.0). The physiological characteristics of strain AR11T are summarized in the species description, and selective characteristics are compared with those of closely related type strains in Table 2.

DNA–DNA hybridization experiments were carried out with AR11T, M. stanieri DSM 7027T, M. halophilum KCTC 12240T, M. georgiense JCM 21667T, using the method described by Ezaki et al. (1989). Genomic DNA of strain AR11T and the reference strains was extracted using a genomic DNA extraction kit (Solgent) and used as a probe. Probe DNAs were biotinylated with photobiotin and hybridized with single-stranded, unlabelled chromosomal DNA fragments of reference or test micro-organisms. Means were determined from three independent determinations of DNA–DNA hybridization. The level of hybridization of strain AR11T with M. stanieri DSM 7027T, M. halophilum KCTC 12240T and M. georgiense JCM 21667T was respectively 28.8, 25.2 and 29.3%.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, strain AR11T should be placed in a novel species, for which we propose the name Marinobacterium maritimum sp. nov.

**Description of Marinobacterium maritimum sp. nov.**

*Marinobacterium maritimum* (ma.ri’ti.mum. L. neut. adj. maritimum living near the sea).

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**Table 1. Cellular fatty acid compositions of strain AR11T and related type strains**

Strains: 1, AR11T; 2, *M. stanieri* DSM 7027T; 3, *M. halophilum* KCTC 12240T; 4, *M. georgiense* JCM 21667T. Data are from this study. Values are percentage of total fatty acids; values less than 0.4% are not shown. --, Not detected (<0.4%); ECL, equivalent chain length.

<table>
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<th>3</th>
<th>4</th>
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<td>C10 : 0</td>
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<td>C16 : 0</td>
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<td>21.0</td>
<td>24.3</td>
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<td>C18 : 0</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>C17 : 0</td>
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<td>1.0</td>
<td>0.4</td>
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*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained C16 : 07c and/or iso-C15 : 0 2-OH. Summed feature 7 contained one or more of C18 : 1ω7c, ω9t and ω12t.
Cells are Gram-negative, aerobic, slightly halophilic, oxidase- and catalase-positive, straight rods (0.5–0.6 x 0.8–0.9 μm), motile by means of a single polar flagellum. Favourable growth occurs aerobically, with circular colonies with regular edges forming within 3 days, approx. 2.0–3.0 mm in diameter. Growth occurs at 7–37 °C (optimum, 25–28 °C), at pH 5.5–9.0 (optimum, pH 7.5–8.0) and in 0.5–7% (w/v) NaCl (optimum, 1–2%). Requires sodium ions for growth. Negative for reduction of nitrates to nitrites and nitrogen, indole production, methyl red and Voges–Proskauer tests, arginine dihydrolase, urease and hydrolysis of gelatin, aesculin, starch and Tween 80. DNA is hydrolysed. Utilizes propionate, 3-hydroxybutyrate, 4-hydroxybenzoate, L-proline, acetate, L-alanine, malate and phenylacetic acid. Utilizes D- and L-lactate weakly. Does not utilize D-mannitol, D-glucose, salicin, melibiose, L-fucose, D-sorbitol, L-arabinose, caprate, valerate, citrate, L-histidine, 2- or 5-ketogluconate, L-rhamnose, N-acetylglucosamine, D-ribose, inositol, sucrose, maltose, itaconate, suberate, malonate, glycerol, 3-hydroxybenzoate, L-serine, D-mannose, gluconate or adipate (API 32GN, API 20NE). Enzyme activity tests for alkaline phosphatase, esterase lipase (C8), leucine arylamidase and acid phosphatase are positive; activity tests for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, x-chymotrypsin, naphthol-AS-BI-phosphohydrolase, 3-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, β-galactosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative (API ZYM).

Acid is produced from 5-ketogluconate and asesculin and is produced weakly from D- and L-xylene, methyl β-d-xlyopyranoside, methyl α-d-mannopyranoside, amygdalin, arbutin, cellobiose, maltose, lactose, turanose, D-tagatose, D- and L-fucose and D- and L-arabitol. Acid is not produced from glycerol, erythritol, D- or L-arabinose, D-ribose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-d-glucopyranoside, N-acetylglucosamine, salicin, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-l-lyxose, gluconate or 2-ketogluconate (API 50 CH). Susceptible to ampicillin, chloramphenicol, erythromycin, gentamicin, lincomycin, streptomycin and tetracycline, but resistant to kanamycin. Ubiquinone Q-8 is detected as the major respiratory quinone. The major cellular fatty acids are C16:0, summed feature 4 (C16:1ω7t and/or C18:1ω9t C8-OH and/or C18:2ω6t η9t and η12t). The DNA G+C content of the type strain is 57.9 mol% (as determined by HPLC).

The type strain, AR11T (=KCTC 22254T=JCM 15134T), was isolated from Arctic marine sediment.

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**References**


