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1 **_Marinobacterium maritimum_ sp. nov., a marine bacterium isolated from the Arctic**
2 **Sediment**

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14 Running title: *Marinobacterium maritimum* sp. nov.

15

16 The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain AR11^T
17 is EU399548.
18 A maximum likelihood phylogenetic tree based on 16S rRNA gene sequences is available as
19 supplementary material with the online version of this paper.

20

1 **Abstract:**

2 A Gram-negative, aerobic, rod-shaped, motile, marine bacterium was isolated from the Arctic
3 marine sediment. Isolated strain, $AR11^T$, grew with 0.5-7 % NaCl and at 7-37 °C and pH 5.5-
4 9.0. It utilized propionate, 3-hydroxy-benzoate, L-proline, acetate, D- and L-lactate, L-
5 alanine, malate and phenylacetic acid. Alkaline phosphatase, esterase lipase (C8), leucine
6 arylamidase, and acid phosphatase activity tests were positive. Acid was produced from 5-
7 ketogluconate and esculin. Strain $AR11^T$ possessed $C_{16:0}$ (22.0 %), summed feature 4 ($C_{16:1}$
8 $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH, 28.1 %) and summed feature 7 ($C_{18:1}$ $\omega 7c$ and/or $\omega 9t$ and/or $\omega 12t$,
9 34.0 %) as the major cellular fatty acids. The major ubiquinone was Q8. Comparative 16S
10 rRNA gene sequence studies showed that this strain belonged to the *Gammaproteobacteria*.
11 Strain $AR11^T$ was the most closely related to *Marinobacterium stanieri* DSM 7027^T,
12 *Marinobacterium halophilum* $Mano11^T$ and *Marinobacterium georgiense* KW-40^T (97.8 %,
13 97.0 %, and 96.7 % 16S rRNA gene sequence similarities, respectively). The G + C content
14 of the genomic DNA of strain $AR11^T$ was 57.9 mol%. DNA-DNA relatedness data indicated
15 that strain $AR11^T$ represented a distinct species that was separated from *M. stanieri* DSM
16 7027^T, *M. halophilum* $Mano11^T$ and *M. georgiense* KW-40^T. On the basis of polyphasic
17 evidences, it was proposed that strain $AR11^T$ (=KCTC 22254^T=JCM 15134^T) represented the
18 type strain of a novel species, *Marinobacterium maritimum* sp. nov.

19

Main text:

The genus *Marinobacteirum,* within the class *Gammaproteobacteria*, was first 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 this genus was proposed in 1997, *Marinobacterium* spp. have been isolated from several environments, *Marinobacterium halophilum* from tidal flats (Getbol) (Chang *et al*., 2007), *Marinobacterium litorale* from surface sea water of Yellow sea (Kim *et al*., 2007), *Marinobacterium rhizophilum* from roots of plant inhabiting a coastal tidal flat (Kim *et al*., 2008). *Marinobacterium stanieri* (Satomi *et al*., 2002) and *Marinobacterium jannaschii* (Satomi *et al*., 2002) were regrouped from *Pseudomonas stanieri* (Baumann *et al.,* 1983) and *Oceanospirillum jannaschii* (Bowditch *et al.,* 1984) by 16S rRNA and *gyrB* gene sequence analysis. Here, we provide a polyphasic taxonomic characterization of a *Marinobacterium*-like bacterial strain AR11^{T}, which was isolated from the Arctic marine sediments. The isolated strain AR11^{T} is proposed as a novel species of the genus *Marinobacterium*.

During the screening of thiosulfate-oxidizers, one novel bacterial strain, AR11^{T} was isolated from the Arctic marine sediment and selected for further characterization by polyphasic approaches. A sediment sample was placed in a sterile conical tube and serially

1 diluted with filter-sterilized (0.22 μm pore size, Millipore) natural sea water containing 1 mM
2 thiosulfate. After incubation at 25 °C in aerobic condition for 2 weeks, an aliquot of the last
3 dilution showing turbidity was spread onto the artificial sea water medium (AM; per liter
4 distilled water 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2{\cdot}6H_2O$, and 15 g agar) (Levring, 1946)
5 and incubated 25 °C for 2 weeks. Single colonies were purified by transferring them onto
6 marine agar 2216 (MA; Difco), and subjecting them to an additional incubation at 25 °C for 3
7 days. Isolated strains were stocked as a glycerol suspension (20 %, w/v) at -70 °C.
8
9 Bacterial genomic DNA was extracted using a commercial genomic DNA extraction
10 kit (Solgent, Daejeon, Korea). The 16S rRNA gene was amplified from the chromosomal
11 DNA using the universal bacterial primer 27F (5’-AGAGTTTGATCMTGGCTCAG-3’; *E.*
12 *coli* position 8-27) and 1492R (5’-TACGGYTACCTTGTTACGACTT-3’; *E. coli* position
13 1492-1510) (Park *et al*., 2006; Weisburg *et al*., 1991) and the purified PCR products were
14 sequenced by Solgent Co. Ltd. (Daejeon, Korea). The 16S rRNA gene sequence of strain
15 AR11^T determined in this study was about 1425 bp in length. The 16S rRNA gene full
16 sequences were compiled using SeqMan software (DNAStar). The 16S rRNA gene sequences
17 of related taxa were obtained from the GenBank database. Sequence alignments were
18 performed using the CLUSTAL_X program (Thompson *et al*., 1997). Gaps were edited in the

1 BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura
2 two-parameter model (Kimura, 1983). Phylogenetic trees were constructed based on the
3 neighbour-joining (Saitou & Nei, 1987) and the maximum likelihood method (Felsenstein,
4 1981) algorithms by using the MEGA 3 Program (Kumar *et al*, 2004) and PHYLIP package
5 (Felsenstein, 1993), respectively. In the analysis of phylogeny, strain AR11^T was determined
6 to belong to the *Gammaproteobacteria*, and the highest degrees of sequence similarity was
7 found to be with *Marinobacterium stanieri* DSM 7027^T (97.8 % sequence similarity),
8 *Marinobacterium halopilum* Mano11^T (97.0 % sequence similarity) and *Marinobacterium*
9 *georgiense* KW-40^T (96.7 % sequence similarity) (Fig.1). Strain AR11^T clearly belonged to
10 the lineage *Marinobacterium,* as shown by the bootstrap value of neighbour-joining (Fig. 1)
11 and maximum likelihood phylogenetic trees (sFig. 1) of 16S rRNA gene sequences (100 %
12 and 95 %, respectively).
13
14 The Gram reaction was determined by using a Gram-stain kit (Difco) according to
15 the manufacturer's instructions. Cell morphology was examined by light microscopy (Eclipse
16 80i; Nikon) and transmission electron microscope (EM-109; Carl Zeiss) after negative
17 staining with 1 % (wt/v) phosphotungstic acid. Catalase activity was determined by bubble
18 production in 3% (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v)

1 tetramethyl-*p*-phenylenediamine. Strain AR11^T was Gram-negative, aerobic and rod-shaped.
2 Cells possessed a polar flagellum (Fig. 2). Colonies were ivory, circular, convex, with regular
3 edges and a diameter of 2-3 mm when grown on MA 2216 (Difco) at 25 °C for 3 days.

4

5 Cellular fatty acids were analyzed in the isolated strain and reference organisms
6 (*Marinobacterium stanieri* DSM 7027^T, *Marinobacterium halophilum* Mano11^T and
7 *Marinobacterium georgiense* KW-40^T) grown on MA 2216 (Difco) at 25 °C at pH 7.8 for 3
8 days. The cellular fatty acids were saponified, methylated, and extracted according to the
9 protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acids were
10 analyzed by gas chromatography (Hewlett Packard 6890) and identified using the Microbial
11 Identification software package. Respiratory quinones were isolated, purified and analysed as
12 described by Lee *et al*. (2001). Chromosomal DNA extracted for 16S rRNA gene
13 amplification was used for determination of G + C content. RNA in the DNA solution was
14 removed by incubation with a mixture of ribonuclease A and T1 (each, 20 units ml^{-1}) at 30
15 °C for 1 hr. The G + C content of the chromosomal DNA was analyzed as described by
16 Mesbah *et al*. (1989) using a reverse-phase HPLC. The major cellular fatty acid profiles of
17 strain AR11^T were composed of $C_{16:0}$ (22.0 %), summed feature 4 ($C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$
18 2-OH, 28.1 %) and summed feature 7 ($C_{18:1}$ $\omega 7c$ and/or $\omega 9t$ and/or $\omega 12t$, 34.0 %). These fatty

acid profiles were similar to those of closely related type strains in Table 1. There were differences in the proportion of fatty acids between this study and the study of *M. halophilum* Mano11^T (Chang *et al.,* 2007) and *M. georgiense* KW-40^T (González *et al.,* 1997), which may be caused by different cultivation, extraction or analytical conditions. The major ubiquinone of AR11^T was Q8. The G + C content of genomic DNA of strain AR11^T was 57.9 mol%.

Utilization of various substrates as sole carbon source 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 AM (Levring, 1946) by addition of 0.005 % yeast extract (González *et al*., 1997). Acid production from different carbohydrates was determined by employing the API 50 CH system (bioMe´rieux) according to the manufacturer's instructions. The suspension media (using the CHB medium) were 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 AM (Levring, 1946). DNA hydrolysis was observed by using DNase test agar with methyl green (Difco). The production of H_2S was tested on peptone iron agar (Difco). These media were

1 supplemented with 2 % NaCl (final concentration). The following antibiotics were tested:

2 ampicillin (10 μg), chloramphenicol (25 μg), erythromycin (15 μg), gentamicin (10 μg),

3 kanamycin (30 μg), penicillin G (10 μg), streptomycin (10 μg) and tetracycline (30 μg).

4

5 Growth at different temperatures was assessed after 3 days incubation on MA 2216

6 (Difco). Strain AR11^T was able to grow at 7-37 °C, but not at 4 or above 40 °C (optimum,

7 25-28 °C). Growth at different NaCl concentrations (0-12 %, w/v) was measured using AM

8 (Levring, 1946) without NaCl adding 0.005 % yeast extract. Strain AR11^T required sodium

9 ions for growth and grew in 0.5-7 % NaCl (optimum 1-2 %). The response to pH (pH 5.0-9.5

10 at intervals of 0.5 pH units) was determined in marine broth 2216 (Difco) at 25 °C for 3 days.

11 The pH was adjusted with 1N HCl or 1N NaOH. It grew at pH 5.5-9.0 but not at pH values

12 below 5.0 or above 9.5 (optimum, pH 7.5-8.0). The physiological characteristics of strain

13 AR11^T were summarized in the species description and selective characteristics were

14 compared with those of closely related type strains in Table 2.

15

16 DNA-DNA hybridization experiments were carried out with AR11^T, *M. stanieri*

17 DSM 7027^T, *M. halophilum* Mano11^T and *M. georgiense* KW-40^T, using the method

18 described by Ezaki *et al*. (1989). The genomic DNA of strain AR11^T or reference

microorganisms (*M. stanieri* DSM 7027^{T}, *M. halophilum* Mano11^{T} and *M. georgiense* KW-40^{T}) was extracted using a genomic DNA extraction kit (Solgent, Korea) and used as a probe. Probe DNAs were biotinylated with photobiotin and hybridized with single-stranded unlabelled chromosomal DNA fragments of reference or test microorganisms. Means from three independent determinations of DNA–DNA hybridization levels were determined. The hybridization levels of strain AR11^{T} with *M. stanieri* DSM 7027^{T}, *M. halophilum* Mano11^{T} and *M. georgiense* KW-40^{T} were 28.8 %, 25.2 % and 29.3 %, respectively.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparison described above, strain AR11^{T} should be placed into a novel species, for which we proposed 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).

Cells are Gram-negative, aerobic, slightly halophilic, oxidase- and catalase-positive, straight-rod-shaped (5.0-6.0 × 8.0-9.0 μm), and motile by means of a single polar flagellum. Favorable growth occurs aerobically with forming circular colonies with regular edges within 3 days, with diameters of approximately 2.0-3.0 mm. Growth occurs at 7-37 °C (optimum, 25-28 °C) and at pH 5.5-9.0 (optimum, pH 7.5-8.0) and in 0.5-7 % (w/v) NaCl (optimum, 1-2 %). Strain AR11^T has Na^+ requirement for growth. Reduction of nitrates to nitrites and nitrogen, indole production, Methyl red and Voges–Proskauer tests, arginine dihydrolase, urease, hydrolysis for gelatin, esculin, starch and tween 80 are negative but not DNA is hydrolysed. Utilize propionate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, acetate, L-alanine, malate and phenylacetic acid. D- and L-lactate are weakly utilized. But this strain does not utilize D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, caprate, valerate, citrate, L-histidine, 2-ketogluconate, L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, L-serine, D-mannose, gluconate or adipate (API 32GN, API 20NE). Alkaline phosphatase, esterase lipase (C8), leucine arylamidase and acid phosphatase enzyme activity tests are positive, but esterase (C4), lipase (C14), valine arylamidase, crystine arylamidase, trypsin, *α*-chymotrypsin, naphtol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase or *α*-fucosidase activity tests are

1 negative (API ZYM). Acid is produced from 5-ketogluconate and esculin, and produced
2 weakly from D- and L-xylose, methyl β-D-xylopyranoside, methyl α-D-mannopyranoside,
3 amygdalin, arbutin, D-cellobiose, D-maltose, D-lactose, D-turanose, D-tagatose, D- fucose,
4 L-fucose, D- arabitol and L-arabitol. Acid is not produced from glycerol, erythritol, D-
5 arabinose, L-arabinose, D-ribose, D-adonitol, D-galactose, D-glucose, D-fructose, D-
6 mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-
7 glucopyranoside, *N*-acetylglucosamine, salicin, D-melibiose, sucrose, D-trehalose, inulin, D-
8 melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, gluconate or 2-
9 ketogluconate (API 50 CH). Susceptible to ampicillin, chloramphenicol, erythromycin,
10 gentamicin, penicillin G, streptomycin and tetracycline, but resistant to kanamycin.
11 Ubiquinone-8 was detected as the major respiratory quinone. The major cellular fatty acids of
12 AR11^T are $C_{16:0}$, summed feature 4 ($C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and summed feature 7
13 ($C_{18:1}$ $\omega7c$ and/or $\omega9t$ and/or $\omega12t$). DNA G + C content is 57.9 mol% (as determined by
14 HPLC).

15

16 The type strain, AR11^T (=KCTC 22254^T=JCM 15134^T), isolated from the Arctic marine
17 sediment.

18

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References

Baumann, P., Bowditch, R. D., Baumann, L. & Beaman, B. (1983). Taxonomy of marine *Pseudomonas* species: *P. stanieri* sp. nov.; *P. perfectomarina* sp. nov., nom. rev.; *P. nautica*; and *P. doudoroffii*. *Int J Syst Bacteriol* **33**, 857-865.

Bowditch, R. D., Baumann, L. & Baumann, P. (1984). Description of *Oceanospirillum kriegii* sp. nov. and *O. jannaschii* sp. nov. and assignment of two species of *Alteromonas* to this genus as *O. commune* comb. nov. and *O. vagum* comb. nov. *Curr Microbiol* **10**, 221-230.

Chang, H.-W., Nam, Y.-D., Kwon, H.-Y., Park, J. R., Lee, J.-S., Yoon, J.-H., An, K.-G. & Bea, J.-W. (2007). *Marinobacterium halophilum* sp. nov., a marine bacterium isolated from the Yellow Sea. *Int J Syst Evol Microbiol* **57**, 77-80.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224-229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368-376.

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5. Seattle:

1 University of Washington.

2

3 **González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997).**
4 *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp.
5 nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst*
6 *Bacteriol* **47**, 369-376.

7

8 **Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis
9 program for Windows 95/98/NT. *Nucl Acids Symp Ser* **41**, 95-98.

10

11 **Kim, H., Choo, Y.-J., Song, J., Lee, J.-S., Lee, K. C. & Cho J.-C. (2007).**
12 *Marinobacterium litorale* sp. nov. in the order *Oceanospirillales. Int J Syst Evol Microbiol* **57**,
13 1659-1662.

14

15 **Kim, Y.-G., Jin, Y.-A., Hwang C. Y. & Cho B. C. (2008).** *Marinobacterium rhizophilum* sp.
16 nov., isolated from the rhizosphere of the coastal tidal-flat plant *Suaeda japonica*. *Int J Syst*
17 *Evol Microbiol* **58**, 164-167.

18

19 **Kimura, M. (1983).** The Neutral Theory of Molecular Evolution. Cambridge: Cambridge
20 University Press.

21

22 **Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for molecular
23 evolutionary genetics analysis and sequence alignment. *Brief bioinform* **5**, 150-163.

24

25 **Lee, J.-S., Shin, Y. K., Yoon, J.-H., Takeuchi, M., Pyun, Y.-R. & Park, Y.-H. (2001).**

1 *Sphingomonas aquatilis* sp. nov., *Sphingomonas koreensis* sp. nov. and *Sphingomonas*
2 *taejonensis* sp. nov., yellow-pigmented bacteria isolated from natural mineral water. *Int J Syst*
3 *Evol Microbiol* **51**, 1491–1498.
4
5 **Levring, T. (1946).** Some culture experiments with *Ulva* and artificial seawater. *Kungl*
6 *Fysiografiska Sällsk Lund Förhandlingar* **16**, 45-56.
7
8 **Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G
9 + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst*
10 *Bacteriol* **39**, 159-167.
11
12 **MIDI (1999).** *Sherlock, Microbial Identification System*, Operating Manual, version 3.0.
13 Newark, DE: MIDI.
14
15 **Park, S.-J., Kang, C.-H., & Rhee S.-K. (2006).** Characterization of the Microbial Diversity
16 in a Korean Solar Saltern by 16S rRNA Gene Analysis. *J Microbiol Biotechnol* **16**, 1640-
17 1645.
18
19 **Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing
20 phylogenetic trees. *Mol Bio Evol* **4**, 406-425.
21
22 **Satomi, M., Kimura, B., Hamada, T., Harayama, S. & Fujii, T. (2002).** Phylogenetic
23 study of the genus *Oceanospirillum* based on 16S rRNA and *gyrB* genes: emended
24 description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov.,
25 *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum*

1 *jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii*
2 comb. nov. and *Marinobacterium stanieri* comb. nov. *Int J Syst Evol Microbiol* **52**, 739-747.
3
4 **Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).**
5 The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment
6 aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876-4882.
7
8 **Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA
9 amplification for phylogenic study. *J Bacteriol* **173**, 697-703.
10
11

1 **Table 1.** Cellular fatty acid compositions of strains AR11^T, *M. stanieri* DSM 7027^T, *M.*
2 *halophilum* Mano11 ^{T} and *M. georgiense* KW-40^T.

3 Strains/species: 1, AR11^T; 2, *M. stanieri* DSM 7027^T; 3, *M. halophilum* Mano11^T; 4, *M.*
4 *georgiense* KW-40^T. Values are percentage of total fatty acid; values less than 0.4% are not
5 shown.

Fatty acid	**1**	**2**	**3**	**4**
Straight-chain fatty acid:				
$C_{10:0}$	3.1	0.6	2.6	2.6
$C_{12:0}$	3.9	5.6	2.9	2.1
$C_{14:0}$	1.2	-	1.0	-
$C_{16:0}$	21.9	21.0	24.3	30.0
$C_{18:0}$	-	-	0.9	0.9
Unsaturated fatty acid:				
$C_{20:1}$ $\omega 9t$	-	-	0.7	-
Hydroxy fatty acids				
$C_{18:1}$ 2-OH	-	-	0.5	-
$C_{10:0}$ 3-OH	6.2	6.7	5.6	4.8
CYCLO				
$C_{17:0\ CYCLO}$	1.2	-	-	-
Summed features*:				
4	28.1	21.8	38.8	22.5
7	34.0	43.3	21.7	36.2
Unknown fatty acid:				
11.798	0.5	1.0	0.4	0.9

6 *Summed features represent groups of two or three fatty acids that could not be separated by
7 GLC with the MIDI system. Summed feature 4 contained one or more of $C_{16:1}$ $\omega 7c$ and/or
8 iso-$C_{15:0}$ 2-OH. Summed feature 7 contained one or more of $C_{18:1}$ $\omega 7c$ and/or $\omega 9t$ and/or $\omega 12t$.

9

10

1 **Table 2.** Selective phenotypic characteristics of strain AR11^T and phylogenetically related

2 *Marinobacterium* species.

3 Strains/species: 1, AR11^T; 2, *M. stanieri* DSM 7027^T; 3, *M. halophilum* Mano11^T; 4, *M.*

4 *georgiense* KW-40^T. Characteristics are scored as: W, weak reaction; +, positive reaction; -,

5 negative reaction; ND, not determined. All strains have motility by single polar flagellum.

6 These are aerobic, rod-shaped, polar flagellum, oxidase- and catalase-positive, utilize 3-

7 hydroxy-butyrate and possess quinone type Q-8.

Characteristic	**1**	**2**	**3**	**4**
Growth at 4 ℃	-	-	+	+
Growth at 40 ℃	-	+	-	+
Production of acid from :				
Glucose	-	-	+	-
Lactose	-	+	+	-
Hydrolysis activity of :				
Acid phospatase*	+	+	-	-
Esterase lipase (C8)*	+	-	-	-
Hydrolysis of :				
DNA	+	-	+	+
Starch	-	-	+	-
Tween 80	-	+	+	+
Utilization of :				
4-hydroxy-benzoate	+	-	-	+
Acetate	+	-	-	+
Citrate	-	+	+	+
D-, L-Lactate	W	-	-	+
L-Alanine	+	-	-	+
L-Proline	+	-	-	+
N-acetyl-glucosamine	-	-	+	-
Phenylacetic acid	+	-	-	+
Propionate	+	-	-	+
Valerate	-	-	-	+
DNA G + C content (mol%)	57.9	55-57	ND	54.9

8 *Data from API ZYM tests.

9

1 **Fig. 1.** Neighbour-joining tree showing the phylogenetic positions of AR11^T and the nearest

2 neighbours based on 16S rRNA gene sequences. Scale bar represents 2 substitutions per 100

3 nucleotide position. Bootstrap values expressed as percentages of 500 replications. Bootstrap

4 percentages >50 % are shown.

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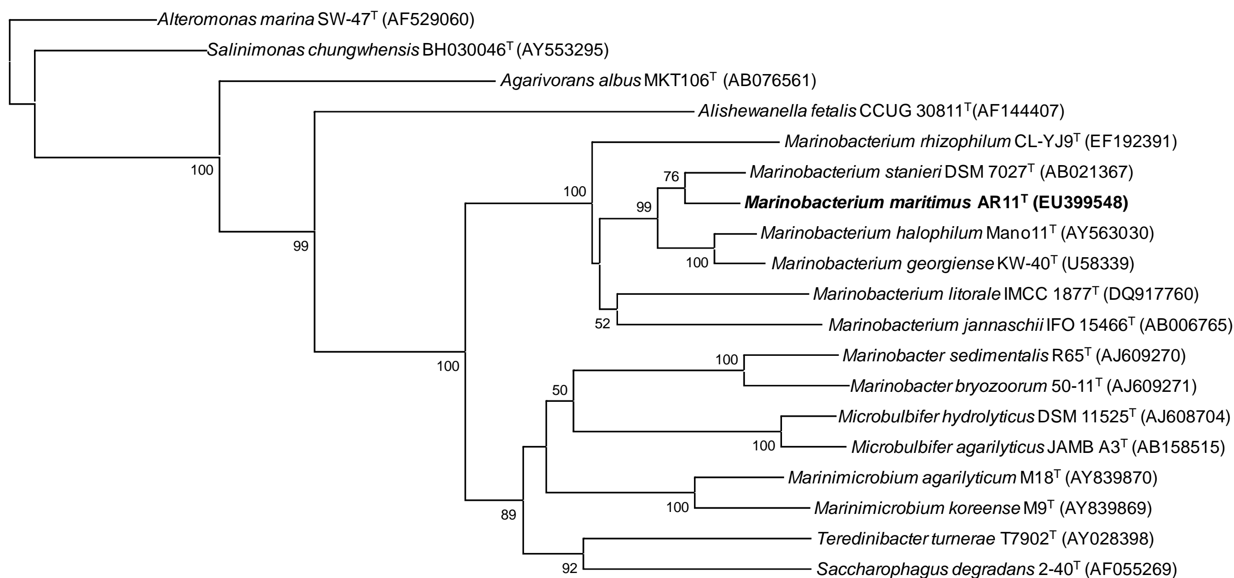

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1 **Fig. 2.** Negatively stained transmission electron micrograph. Bars: 0.5 μm.

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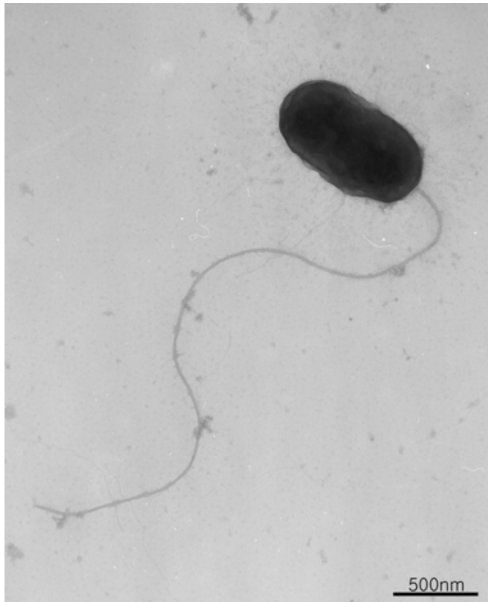

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sFig 1. Phylogenic relationships of isolates AR11^T and some related microorganism species on the basis of 16S rRNA gene sequences. Genbank accession numbers are given in parentheses. The percentage that supported topology in the maximum likelihood tree is indicated. Bootstrap percentages (based on 500 replications) >50 % are shown. Bar, 0.001 accumulated changes per nucleotide.