Maribacter chungangensis sp. nov., isolated from a green seaweed, and emended descriptions of the genus *Maribacter* and *Maribacter arcticus*

Veeraya Weerawongwiwat, Hyeonji Kang, Min Young Jung and Wonyong Kim

Department of Microbiology, Chung-Ang University College of Medicine, Seoul, Republic of Korea

Correspondence
Wonyong Kim
kimwy@cau.ac.kr

A Gram-stain-negative, non-spore-forming, strictly aerobic, orange-pigmented bacterial strain, motile by gliding, designated CAU 1044^T, was isolated from a green seaweed and its taxonomic position was investigated using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CAU 1044^T formed a distinct lineage within the genus *Maribacter* and was most closely related to *Maribacter antarcticus* JCM 15445^T and *Maribacter arcticus* KOPRI 20941^T (96.3 and 95.7 % similarity, respectively). Strain CAU 1044^T contained menaquinone 6 as the only isoprenoid quinone and iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{17:0}$ 3-OH and iso-$C_{15:1}$ G as the major fatty acids. The cell wall peptidoglycan of strain CAU 1044^T contained *meso*-diaminopimelic acid and the major whole-cell sugars were glucose and ribose. The polar lipids were composed of phosphatidylethanolamine, one unidentified phospholipid, six unidentified aminolipids and four unidentified lipids. The DNA G+C content was 40.2 mol%. On the basis of phenotypic data and phylogenetic inference, strain CAU 1044^T should be classified as a representative of a novel species in the genus *Maribacter* for which the name *Maribacter chungangensis* sp. nov. is proposed. The type strain is CAU 1044^T (=KCTC 23735^T =CCUG 61948^T). Emended descriptions of the genus *Maribacter* and the species *M. arcticus* KCTC 22053^T are also proposed.

The genus *Maribacter*, a member of the family *Flavobacteriaceae* (Bernardet, 2011), was proposed by Nedashkovskaya *et al.* (2004). At the time of writing, this genus consists of ten species with validly published names: *Maribacter aquivivus*, *Maribacter orientalis*, *Maribacter sedimenticola* (type species) and *Maribacter ulvicola* (Nedashkovskaya *et al.*, 2004), *Maribacter dokdonensis* (Yoon *et al.*, 2005), *Maribacter polysiphoniae* (Nedashkovskaya *et al.*, 2007), *Maribacter arcticus* (Cho *et al.*, 2008), *Maribacter forsetii* (Barbeyron *et al.*, 2008), *Maribacter antarcticus* (Zhang *et al.*, 2009) and Maribacter stanieri (Nedashkovskaya *et al.*, 2010) (http://www.bacterio.cict.fr/). Members of the genus *Maribacter* have been isolated from various marine habitats such as green algae, red algae, seawater and marine sediment (Nedashkovskaya and Kim, 2011). In the course of the screening of bacteria with biotechnological potential from marine samples, a bacterial strain, designated CAU 1044^T, was isolated from a seaweed (*Undaria pinnatifida*) sample collected in Jeju Island (33° 22′ 47.30″ N 126° 32′ 59.32″ E) in the Republic of Korea. The purpose of the present study was to establish the taxonomic position of this bacterial strain by using polyphasic characterization.

Abbreviation: TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1044^T is JN036550.

Isolation was performed according to the protocol of Gordon & Mihm (1962) using marine agar 2216 (MA; Difco), supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). The crushed sample was diluted with sterilized saline solution. Serial dilutions were spread onto MA plates and a single colony was subcultivated under aerobic conditions at 30 °C for 3 days. Pure cultures were preserved at −70 °C in marine broth (MB; Difco) supplemented with 25 % (v/v) glycerol. *M. arcticus* KCTC 22053^T was obtained from the Korean Collection for type Cultures (KCTC; Taejon, Korea) and used as a reference strain in most analyses.

Genomic DNA of strain CAU 1044^T was isolated by the method of Marmur (1961). The 16S rRNA gene was amplified by PCR following established procedures (Nam *et al.*, 2004). The amplified 16S rRNA gene was sequenced directly using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730; Applied Biosystems). Multiple alignments with sequences of the type strains of the ten species of the genus *Maribacter* and calculation of sequence similarity levels were carried out by using the EzTaxon server (http://

147.47.212.35:8080/) and CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distance matrices were generated by the neighbour-joining method (Jukes & Cantor, 1969). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms in the PHYLIP package (Felsenstein, 1989), and tree topology was evaluated by the bootstrap resampling method (Felsenstein, 1985) with 1000 replicates of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package. The nearly complete 16S rRNA gene sequence of strain CAU 1044^T (1340 bp) was determined and compared with the corresponding sequences of other bacterial strains in the GenBank database. Phylogenetic analysis indicated that the strain fell into the genus *Maribacter*. The neighbour-joining tree is shown in Fig. 1. The trees obtained with the two other treeing the methods showed essentially the same topology (data not shown). Pairwise analysis showed that the most closely related strains were *M. antarcticus* JCM 15445^T (96.3 % similarity), *M. arcticus* KOPRI 20941^T (95.7 %), *M. sedimenticola* KMM 3903^T (95.7 %), *M. dokdonensis* DSW-8^T (95.6 %), *M. ulvicola* KMM 3951^T (95.6 %), *M. orientalis* KMM 3947^T (95.4 %), *M. stanieri* KMM 6046^T (95.3 %), *M. forsetii* KT02ds18-6^T (95.2 %), *M. polysiphoniae* LMG 23671^T (95.0 %) and *M. aquivivus* KMM 3949^T (94.9 %).

Strain CAU 1044^T was cultivated routinely on MA at 30 °C to investigate all morphological, physiological and biochemical characteristics, except for spore formation, which was assessed on nutrient sporulation medium (Nicholson & Setlow, 1990). Cell morphology was examined by light microscopy (model DM 1000; Leica) and transmission electron microscopy (TEM) (JEM 1010; JEOL) using cells from an exponentially growing culture. For TEM, the cells

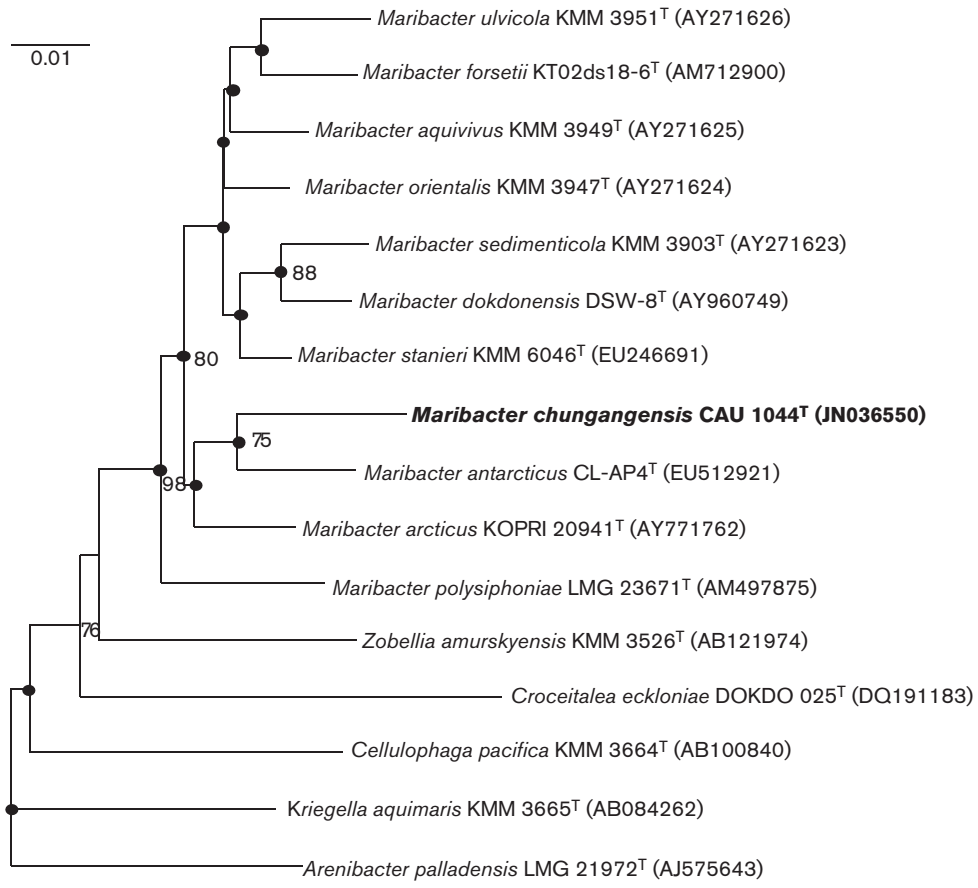

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU 1044^T, the type strains of recognized species of the genus *Maribacter* and other representatives of the family *Flavobacteriaceae*. Dots indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and least-squares algorithms. The numbers at the nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >70 % are shown. *Arenibacter palladensis* LMG 21972^T (AJ575643) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air dried. Gram staining was carried out using a bioMérieux Gram staining kit according to the manufacturer's instructions. Gliding motility was assessed on a 72 h MB culture using the hanging-drop method (Barbeyron *et al.*, 2008; Nedashkovskaya *et al.*, 2010). The presence of flexirubin-type pigments was examined as described by Bernardet *et al.* (2002). Growth in MB at 4, 10, 30, 37, 45 and 55 °C in an aerobic incubator (model MIR-253; Sanyo) and in an anaerobic chamber (Bactron; Sheldon) was evaluated by measuring the turbidity of the broth by spectrophotometry after 72 h. Growth was tested at 30 °C in MB adjusted to pH 6.0–10.0 (at increments of 0.5 pH units) by using sodium acetate/acetic acid and Na_2CO_3 buffers. Growth in the absence of NaCl and in the presence of 0–15.0 % (w/v) NaCl (at 1 % intervals) was investigated at 30 °C in MB prepared according to the formula of the Difco medium except that NaCl was excluded and that 0.45 % (w/v) $MgCl_2 . 6H_2O$ and 0.06 % (w/v) KCl were added. Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined from the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of gelatin, starch and urea were determined according to the protocols of Lányí (1987) and Smibert & Krieg (1994). Acid production from carbohydrates, enzyme activity and other physiological and biochemical features were tested using the API 50CH, API ZYM and API 20E systems at 30 °C (bioMérieux). API ZYM strips were read after 4 h at 37 °C, API 20E strips after 36 h at 30 °C and API 50CH strips after 36 h and 48 h at 30 °C. *M. arcticus* KCTC 22053^T was cultivated on MA at 30 °C to investigate physiological characteristics, namely temperature and salinity ranges for growth, nitrate reduction, hydrolysis of gelatin and starch and biochemical tests using API 50 CH, API ZYM and API 20E.

The morphological, cultural, physiological and biochemical characteristics of strain CAU 1044^T are given in Table 1 and in the species description. Overall, the results obtained in this study are in agreement with previously published data for the ten species of the genus *Maribacter* (Barbeyron *et al.*, 2008; Cho *et al.*, 2008; Nedashkovskaya *et al.*, 2004, 2007, 2010; Yoon *et al.*, 2005; Zhang *et al.*, 2009). However, strain CAU 1044^T differed from its closest relatives, *M. antarcticus* JCM 15445^T and *M. arcticus* KCTC 22053^T (Table 1) and from the type strain of the type species of the genus *Maribacter*, *M. sedimenticola* KMM 3903^T (Nedashkovskaya *et al.*, 2004), by its ability to hydrolyse gelatin and starch, to produce acid from ribose and D-tagatose, to utilize citrate and by the presence of β-galactosidase and α-glucosidase activities.

For fatty acid analysis, the cell mass of strain CAU 1044^T and *M. arcticus* KCTC 22053^T was harvested from tryptic soy agar (TSA; Difco) after cultivation for 3 days at 30 °C and 10 days at 22 °C, respectively. The physiological age of the biomasses was standardized by observing growth development during incubation of the two cultures and choosing the moment of harvesting according to the standard MIDI protocol (Sherlock Microbial Identification System version 6.1). Cellular fatty acid methyl esters were obtained according to the protocol of Minnikin *et al.* (1980) and separated by an automated GC system (model 6890N and 7683 autosampler; Agilent). Peaks were identified by using the Microbial Identification software package (MOORE library version 5.0; MIDI database TSBA6). The polar lipids of strain CAU 1044^T and *M. arcticus* KCTC 22053^T were identified using two-dimensional TLC by the method of Minnikin *et al.* (1984). The plates were sprayed with 10 % ethanolic molybdatophosphoric acid (for the total lipids), molybdenum blue (for phospholipids), ninhydrin (for aminolipids) and α-naphthol/sulphuric acid reagent (for glycolipids) (Sigma-Aldrich). The following analyses were performed on strain CAU 1044^T only. Isoprenoid quinones were separated by HPLC using an isocratic solvent system [methanol : isopropyl ether (3 : 1, v/v)] and a flow rate of 1 ml min^{-1} (Komagata & Suzuki, 1987). Whole-cell sugars were analysed by TLC according to the method of Komagata & Suzuki (1987). Peptidoglycan was analysed as described by Schleifer & Seidl (1985), with the modification that a cellulose paper was substituted for chromatography paper. The mol% G+C content of the genomic DNA was

Table 1. Differential properties of strain CAU 1044^T and the type strains of the most closely related species of the genus *Maribacter*

Strains: 1, CAU 1044^T (data from this study); 2, *Maribacter antarcticus* JCM 15445^T (Zhang *et al.*, 2009); 3, *Maribacter arcticus* KCTC 22053^T (data from this study except for the DNA G+C content, taken from Cho *et al.*, 2008). +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3
Temperature range for growth (°C)	4–30	4–15	4–30
Salinity range for growth (% NaCl)	0–7	2–6	1–7
Nitrate reduction	+	−	+
Hydrolysis of:			
Gelatin	+	−	−
Starch	+	−	−
Assimilation of: (API 50 CH)			
Ribose	+	−	−
Lactose	−	−	+
Xylitol	−	−	+
Turanose	−	−	+
D-Tagatose	+	−	−
Enzyme activity (API ZYM)			
Esterase (C4)	w	−	w
Trypsin	+	+	−
Naphthol-AS-BI-phosphohydrolase	+	w	+
β-Galactosidase	+	−	w
α-Glucosidase	+	−	−
Utilization of citrate (API 20E)	+	−	−
DNA G+C content (mol%)	40.2	37.1	36.0

determined using HPLC by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

The peptidoglycan of strain CAU 1044^T contained *meso*-diaminopimelic acid and TLC analysis of whole-cell hydrolysates revealed the presence of glucose and ribose. Menaquinone 6 (MK-6) was the only respiratory quinone, consistent with other species of the genus *Maribacter* and all members of the family *Flavobacteriaceae* (Bernardet, 2011). Strain CAU 1044^T contained branched-chain, saturated and unsaturated fatty acids (Table 2). The major fatty acids were iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso $C_{15:0}$ 2-OH), iso-$C_{17:0}$ 3-OH and iso-$C_{15:1}$ G and significant amounts of iso-$C_{15:0}$ 3-OH, iso-$C_{15:1}$ F, summed feature 9 (comprising iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$), $C_{16:0}$ 3-OH, $C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$ 3-OH, $C_{13:1}$, summed feature 4 ($C_{17:1}$ iso I and/or $C_{17:1}$) and $C_{14:0}$ were also present. Strain CAU 1044^T could be distinguished from *M. arcticus* KCTC 22053^T (analysed in this study) and *M. antarcticus* JCM 15445^T (Zhang *et al.*, 2009) by presence of $C_{16:0}$, $C_{13:1}$ and iso-$C_{15:1}$ F. Phosphatidylethanolamine was the only polar lipid identified in strain CAU 1044^T. The other unidentified polar lipids were one phospholipid, six aminolipids and four lipids. The polar lipids of *M. arcticus* KCTC 22053^T were phosphatidylethanolamine, five unidentified aminolipids, two unidentified aminophospholipids and five unidentified lipids (Fig. 2). The only *Maribacter* species analysed for its polar lipids so far, *M. dokdonensis* (Yoon *et al.*, 2005), also contained phosphatidylethanolamine, as well as the following unidentified polar lipids: two phospholipids, one glycolipid (identification of this spot is questionable, as glycolipids usually do not occur in members of the family *Flavobacteriaceae*) and one aminolipid. These chemotaxonomic properties support the attribution of strain CAU 1044^T to the genus *Maribacter* (Nedashkovskaya *et al.*, 2004; 2010). The genomic DNA of strain CAU 1044^T had a G+C content of 40.2 mol%, a value significantly higher than that of all species of the genus *Maribacter* except *M. polysiphoniae* (Nedashkovskaya *et al.*, 2007). These data provide sufficient evidence to recognize strain CAU 1044^T as a novel species of the genus *Maribacter*, for which the name *Maribacter chungangensis* sp. nov. is proposed. On the basis of new data obtained in this study, emended descriptions of the genus *Maribacter* and *M. arcticus* are also proposed.

Table 2. Cellular fatty acid composition (%) of strain CAU 1044^T and the type strains of the most closely related species of the genus *Maribacter*

Strains: 1, CAU 1044^T (data from this study); 2, *Maribacter antarcticus* JCM 15445^T (Zhang *et al.*, 2009); 3, *Maribacter arcticus* KCTC 22053^T (data from this study). Fatty acids amounting to <1 % of the total fatty acids in all strains are not shown. TR, Trace (<1 %); −, not detected.

Fatty acids	1	2	3
Saturated			
$C_{14:0}$	1.2	TR	1.2
$C_{15:0}$	−	3.4	−
$C_{16:0}$	3.4	TR	TR
Unsaturated			
$C_{13:1}$	1.5	−	−
$C_{15:1}\omega6c$	−	1.4	−
Branched-chain			
anteiso-$C_{15:0}$	2.8	1.5	4.6
anteiso-$C_{15:1}$ A	TR	−	4.1
iso-$C_{15:0}$	17.9	17.2	8.4
iso-$C_{15:1}$ G	14.9	16.8	16.7
iso-$C_{15:1}$ F	4.5	−	−
iso-$C_{16:0}$	TR	−	2.2
iso-$C_{17:1}\omega9c$	−	4.9	−
Hydroxy			
$C_{15:0}$ 2-OH	TR	TR	1.6
$C_{16:0}$ 3-OH	3.9	1.2	2.4
$C_{16:1}$ 2-OH	TR	2.4	1.3
$C_{17:0}$ 2-OH	TR	−	2.3
iso-$C_{15:0}$ 3-OH	5.6	12.6	5.5
iso-$C_{16:0}$ 3-OH	2.6	2.1	2.4
iso-$C_{17:0}$ 3-OH	15.0	14.9	13.7
Summed features*			
3	15.3	13.1	19.6
4	1.3	1.7	1.4
9	4.3	−	7.0

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 4 comprises iso-$C_{17:1}$ I and/or $C_{17:1}$. Summed feature 9 comprises iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

Emended description of the genus *Maribacter* Nedashkovskaya *et al.* 2004, emend. Barbeyron *et al.* 2008, emend. Nedashkovskaya *et al.* 2010

This description is as given by Nedashkovskaya *et al.* (2004) and emended by Barbeyron *et al.* (2008) and Nedashkovskaya *et al.* (2010) with the following amendments. The major polar lipid of the strains analysed is phosphatidylethanolamine. Several unidentified phospholipids, aminolipids, aminophospholipids and lipids also occur. Nitrate reduction and hydrolysis of gelatin and starch are species-dependent.

Emended description of the species *Maribacter arcticus* Cho *et al.* 2008

This description is as given by Cho *et al.* (2008) with the following amendments. The major polar lipid is phosphatidylethanolamine. Five unidentified aminolipids, two unidentified aminophospholipids and five unknown lipids also occur.

Description of *Maribacter chungangensis* sp. nov.

Maribacter chungangensis (chung.ang.en′sis. N.L. masc. adj. *chungangensis* of or belonging to Chung-Ang

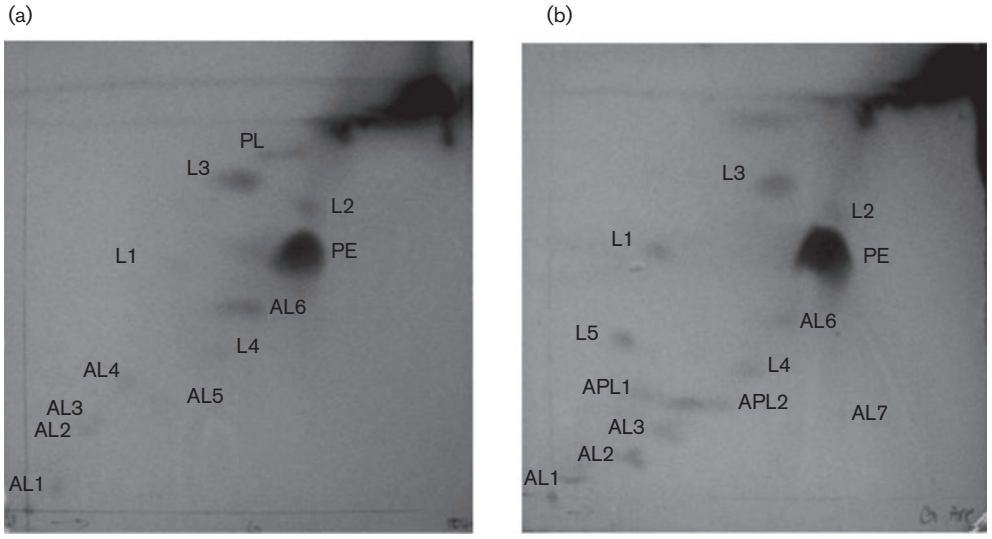

Fig. 2. Thin layer chromatograms of the total polar lipids of strain CAU 1044^T (a) and *M. arcticus* KCTC 22053^T (b) after staining with 10 % ethanolic molybdatophosphoric acid. PE, Phosphatidylethanolamine; PL, unidentified phospholipid; AL1–7, unidentified aminolipids; APL1–2, unidentified aminophospholipids; L1–5, unidentified lipids.

University where the taxonomic studies on this species were performed).

Cells are Gram-stain-negative, strictly aerobic rods approximately 0.3–0.4 μm in diameter and 1.4–2.8 μm in length, motile by gliding. Endospores are not observed. Colonies on MA are orange, circular, convex with entire margins after 3 days of incubation at 30 °C. Flexirubin-type pigments are absent (KOH-test-negative). Growth occurs at 4–30 °C (optimum, 30 °C) and at pH 6.0–10.0 (optimum, pH 8.5). NaCl is not required for growth but up to 7.0 % (w/v) NaCl is tolerated. Catalase and oxidase activities are positive. Gelatin and starch are hydrolysed. Urea is not hydrolysed. According to the API 20E strip, citrate utilization and nitrate reduction are positive, but the results for β-galactosidase (ONPG), arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities; H_2S, indole and acetoin production; and fermentation/oxidation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose are negative. In the API 50CH gallery, ribose, D-tagatose and 5-ketogluconate are assimilated; all other substrates are not assimilated. In the API ZYM system, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-D-glucosaminidase and α-mannosidase activities are present. Weak esterase (C4), cystine arylamidase and α-galactosidase activities are also present. Lipase (C14), chymotrypsin, β-glucuronidase and α-fucosidase activities are absent. The cell wall peptidoglycan contains *meso*-diaminopimelic acid. The only isoprenoid quinone is MK-6. The whole-cell hydrolysate contains glucose and ribose. The polar lipid pattern consists of phosphatidylethanolamine, one unidentified phospholipid, six unidentified aminolipids and four unidentified lipids. The predominant cellular fatty acids (>10 % of the total fatty acids) are iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or $C_{15:0}$ iso 2-OH), iso-$C_{17:0}$ 3-OH and iso-$C_{15:1}$ G.

The type strain is CAU 1044^T (=KCTC 23735^T=CCUG 61948^T), isolated from a green seaweed collected from Jeju Island in the Republic of Korea. The DNA G+C content is 40.2 mol%.

Acknowledgements

This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A085138).

References

Barbeyron, T., Carpentier, F., L'haridon, S., Schüler, M., Michel, G. & Amann, R. (2008). Description of *Maribacter forsetii* sp. nov., a marine *Flavobacteriaceae* isolated from North Sea water, and emended description of the genus *Maribacter*. *Int J Syst Evol Microbiol* **58**, 790–797.

Bernardet, J.-F. (2011). Family I. *Flavobacteriaceae* Reichenbach 1992. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4, pp. 106–111. Edited by W. Whitman. Baltimore: Williams & Wilkins.

Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of Flavobacterium and Cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin/Cummings.

Cho, K. H., Hong, S. G., Cho, H. H., Lee, Y. K., Chun, J. & Lee, H. K. (2008). *Maribacter arcticus* sp. nov., isolated from Arctic marine sediment. *Int J Syst Evol Microbiol* **58**, 1300–1303.

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

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.

Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. H. Munro. New York: Academic Press.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–208.

Lányí, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Nam, S. W., Kim, W., Chun, J. & Goodfellow, M. (2004). *Tsukamurella pseudospumae* sp. nov., a novel actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **54**, 1209–1212.

Nedashkovskaya, O. I. & Kim, S. B. (2011). Genus XXXII. *Maribacter* Nedashkovskaya, Kim, Han, Lysenko, Rohde, Rhee, Frolova, Falsen, Mikhailov and Bae 2004b, 1021^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4, pp. 235–238. Edited by W. Whitman. Baltimore: The Williams & Wilkins Co.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Rohde, M., Rhee, M. S., Frolova, G. M., Falsen, E., Mikhailov, V. V. & Bae, K. S. (2004). *Maribacter* gen. nov., a new member of the family *Flavobacteriaceae*, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov., *Maribacter aquivivus* sp. nov., *Maribacter orientalis* sp. nov. and *Maribacter ulvicola* sp. nov. *Int J Syst Evol Microbiol* **54**, 1017–1023.

Nedashkovskaya, O. I., Vancanneyt, M., De Vos, P., Kim, S. B., Lee, M. S. & Mikhailov, V. V. (2007). *Maribacter polysiphoniae* sp. nov., isolated from a red alga. *Int J Syst Evol Microbiol* **57**, 2840–2843.

Nedashkovskaya, O. I., Kim, S. B. & Mikhailov, V. V. (2010). *Maribacter stanieri* sp. nov., a marine bacterium of the family *Flavobacteriaceae. Int J Syst Evol Microbiol* **60**, 214–218.

Nicholson, W. L. & Setlow, P. (1990). Sporulation, germination and outgrowth. In *Molecular Biological Methods for Bacillus*, pp. 391–450. Edited by C. R. Harwood & S. M. Cutting. Chichester: Wiley.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Schleifer, K. H. & Seidl, P. H. (1985). Chemical composition and structure of murein. In *Chemical Methods in Bacterial Systematics*, pp. 201–219. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Yoon, J. H., Kang, S. J., Lee, S. Y., Lee, C. H. & Oh, T. K. (2005). *Maribacter dokdonensis* sp. nov., isolated from sea water off a Korean island, Dokdo. *Int J Syst Evol Microbiol* **55**, 2051–2055.

Zhang, G. I., Hwang, C. Y., Kang, S.-H. & Cho, B. C. (2009). *Maribacter antarcticus* sp. nov., a psychrophilic bacterium isolated from a culture of the Antarctic green alga *Pyramimonas gelidicola. Int J Syst Evol Microbiol* **59**, 1455–1459.