Marinimicrobium koreense gen. nov., sp. nov. and *Marinimicrobium agarilyticum* sp. nov., novel moderately halotolerant bacteria isolated from tidal flat sediment in Korea

Jee-Min Lim,¹ Che Ok Jeon,² Jae-Chan Lee,¹ Sung-Min Song,^{1,3} Kwang-Yup Kim³ and Chang-Jin Kim¹

Correspondence
Chang-Jin Kim
changjin@kribb.re.kr

¹Korea Research Institute of Bioscience and Biotechnology, 52 Oeundong, Yusong, Daejeon 305-333, Republic of Korea

²Environmental Biotechnology National Core Research Center, PMBBRC, Division of Environmental Biotechnology, Gyeongsang National University, 660-701, Republic of Korea

³Department of Food Science and Technology, Chungbuk National University, Cheongju 361-763, Republic of Korea

Two moderately halotolerant Gram-negative bacteria were isolated from tidal flat sediment of the South Sea in Korea (the Korea Strait). The strains, designated M9^T and M18^T, were strictly aerobic, rod-shaped and non-spore-forming and motile with a flagellum and their major fatty acids were C_{16:0} and C_{19:0} cyclo ω 8c. Strains M9^T and M18^T could grow in the presence of up to 13–15% (w/v) NaCl, but their optimum salt concentrations were relatively low (0–3%, w/v). The major predominant isoprenoid quinone was Q-8 and the G + C content of the genomic DNA was 57–58 mol%. Phylogenetic analyses and comparative 16S rRNA gene sequence studies revealed that strains M9^T and M18^T formed a phylogenetic lineage distinct from the genus *Teredinibacter* within the class *Gammaproteobacteria* and were most closely related to the genera *Microbulbifer*, *Saccharophagus* and *Teredinibacter*, with less than 92.5% 16S rRNA gene sequence similarity. The level of 16S rRNA gene sequence similarity between the two strains was 96.7%. On the basis of physiological and phylogenetic properties, strains M9^T and M18^T represent separate species within a novel genus of the class *Gammaproteobacteria*, for which the names *Marinimicrobium koreense* gen. nov., sp. nov. (type species) and *Marinimicrobium agarilyticum* sp. nov. are proposed. The type strains of *Marinimicrobium koreense* and *Marinimicrobium agarilyticum* are M9^T (=KCTC 12356^T=DSM 16974^T) and M18^T (=KCTC 12357^T=DSM 16975^T), respectively.

Gram-negative bacteria belonging to the class *Gammaproteobacteria* are attracting interest because this group of bacteria has great biotechnological potential for production of hydrolytic enzymes (Howard *et al.*, 2004; Martín *et al.*, 2003) and many novel species have recently been added to the genera (Gorshkova *et al.*, 2003; Romanenko *et al.*, 2005; Satomi *et al.*, 1998, 2004; Shivaji *et al.*, 2005; Yoon *et al.*,

2004). For example, *Microbulbifer hydrolyticus* isolated from a lignin-rich pulp mill waste is capable of degrading lignin and lignin-like polymers and *Marinobacter hydrocarbonoclasticus*, with hydrocarbon-degrading activity, was isolated from an oil-producing well on an offshore platform in southern Vietnam (Huu *et al.*, 1999; González *et al.*, 1997; Gauthier *et al.*, 1992; Marquez & Ventosa, 2005). In addition, Distel *et al.* (2002) isolated a moderate halophile, *Teredinibacter turnerae* T7902^T, with cellulolytic activity from gill tissue of the shipworm. In this paper, we report the isolation of two novel moderately halotolerant, Gram-negative bacteria (strains M9^T and M18^T) from tidal flat sediment in Korea; strain M18^T showed agar-dissolving activity. We determined their phylogenetic position using a polyphasic approach as members of two different species within a new genus of the class *Gammaproteobacteria*.

Published online ahead of print on 25 November 2005 as DOI 10.1099/ij.s.0.64075-0.

The GenBank accession numbers for the 16S rRNA gene sequences of strains M9^T and M18^T are AY839869 and AY839870.

An electron micrograph of a cell of strain M18^T, a photograph of colonies showing agarolytic activity and details of other properties of the novel isolates are available as supplementary material in IJSEM Online.

Two strains, M9^T and M18^T, were isolated from a tidal flat of Jeonnam province in the South Sea of Korea (the Korea Strait). For isolation, sediment sample was collected from the surface of tidal flat sediment and diluted serially in saline solution (10 % w/v). The diluted soil samples were spread on marine agar 2216 (MA) (Difco) with the addition of 8 % (w/v) NaCl (final concentration, 9.94 % w/v NaCl) and incubated for 2 days at 30 °C. The isolates were routinely cultivated on MA for 2 days at 35 °C except where indicated otherwise. Requirement for and tolerance of NaCl were determined in trypticase soy broth (Difco) medium with different amounts of NaCl after 2 days incubation at 35 °C. Anaerobic growth was determined by incubation in an anaerobic chamber at 35 °C for 5 days on MA. Optimum growth was tested at different temperatures (4–55 °C) on MA and at different pH values (5.0–10.0) in marine broth.

Gram staining was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase activity was determined by production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Nitrate reduction was assessed according to the method of Lanyi (1987). Hydrolysis of aesculin, casein, starch, Tween 20, Tween 80, hypoxanthine, tyrosine, gelatin, agar and xanthine was determined on MA according to the methods described by Cowan & Steel (1965), Lanyi (1987) and Gerhardt *et al.* (1994). The API ZYM system (bioMérieux) was used to determine the activities of some enzymes. Acid production from carbohydrates was tested as described by Leifson (1963); all suspension media were supplemented with artificial sea water containing 2 % (w/v) NaCl.

Cell morphology was studied using light microscopy and transmission electron microscopy. Motility was observed at 12 and 36 h on agar-coated wet mounts with a light microscope (Nikon E600). Agar-coated wet mounts for motility observations were prepared by placing 10 µl of a culture under a cover glass on a glass slide previously coated with a film of 0.5 % (w/v) agarose (Cambrex) in distilled water and air-dried. For investigation of flagellum type from the isolates, specimens were prepared from the exponential growth phase as described elsewhere (Lee *et al.*, 2005) and then subjected to transmission electron microscopy (JEOL JEM-1010). Whole-cell fatty acids of strains M9^T and M18^T were analysed using GC/MS following the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.) after cultivation on MA for 2 days at 35 °C. Isoprenoid quinones and polar lipids were analysed as described by Komagata & Suzuki (1987). The DNA G+C content of strains M9^T and M18^T was determined by reversed-phase HPLC, using the method of Tamaoka & Komagata (1984).

The 16S rRNA gene sequences of strains M9^T and M18^T were analysed as described previously (DeLong, 1992). The resultant 16S rRNA gene sequences of strains M9^T and M18^T for phylogenetic analysis comprised 1454 and 1455 nucleotides, respectively, and were compared with available

sequences from GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine the approximate phylogenetic affiliation. They were aligned with sequences from closely related organisms using the CLUSTAL W software (Thompson *et al.*, 1994). Unmatched regions of the 5' and 3' ends from the alignment, which were caused by the different lengths of the 16S rRNA gene sequences, were deleted. Phylogenetic trees based on 16S rRNA gene sequences were constructed using three different methods, neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Using the FASTA3 program in EBI, 16S rRNA gene sequence comparisons for similarity calculations were made between the isolated strains and other related organisms. A bootstrap analysis was performed according to the algorithm of the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package.

Cells of strains M9^T and M18^T were non-spore-forming, short rods, approximately 0.5–0.8 × 0.9–1.1 µm and 0.6–0.7 × 0.9–1.5 µm, respectively (Supplementary Fig. S1 available in IJSEM Online). They were aerobic, Gram-negative, catalase-negative and motile with a flagellum. Colonies of strains M9^T and M18^T were creamy, smooth, glistening, low-convex and circular/slightly irregular.

Table 1. Cellular fatty acid content of strains M9^T and M18^T

Data are expressed as percentages of total fatty acids. Fatty acids representing less than 0.5 % are not included.

Fatty acid	Strain M9 ^T	Strain M18 ^T
Saturated		
C _{9:0}	0.68	0.15
C _{10:0}	3.34	7.65
C _{10:0} 3-OH	5.31	5.79
C _{11:0}	1.20	0.41
C _{11:0} 3-OH	0.58	0.14
C _{12:0}	1.54	0.55
C _{14:0}	3.08	0.92
C _{15:0}	5.71	1.10
C _{16:0}	24.46	26.02
C _{17:0}	4.24	2.26
C _{17:0} cyclo	1.09	0.58
C _{18:0}	2.64	3.59
C _{19:0} cyclo ω8c	22.38	22.99
Unsaturated		
C _{17:1} ω8c	0.70	0.26
C _{18:1} ω7c	3.46	5.30
11-Methyl C _{18:1} ω7c	1.01	0.58
Summed features		
Summed feature 3*	15.55	18.05

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1} ω7c and/or iso C_{15:0} 2-OH.

Growth of strains M9^T and M18^T was not observed under anaerobic conditions. Strains M9^T and M18^T showed optimum growth at low NaCl concentrations of 1–3 and 0–1 % (w/v), respectively. However, they could grow in the presence of up to 13–15 % (w/v) NaCl, meaning that they are moderately halotolerant. Strains M9^T and M18^T grew in the range of pH 6.0–10.5 in marine broth; optimal growth was observed at pH 7.0–7.5 and 7.0–8.0, respectively. The isolates displayed mesophilic growth, with optimum growth at 35–40 °C.

The predominant isoprenoid quinone of strains M9^T and M18^T was ubiquinone-8 (Q-8). The genomic DNA G+C contents of strains M9^T and M18^T were respectively 57 and 58 mol%. Strains M9^T and M18^T contained C_{16:0}, C_{19:0} cyclo ω 8c and summed feature 3 (C_{16:1} ω 7c and/or iso C_{15:0} 2-OH) as the major cellular fatty acids on MA, but their fatty acid profiles were slightly different (Table 1). Most cultural and biochemical characteristics of strains M9^T and M18^T were similar, but there were some differences. For example, cells of strain M9^T showed an oxidase-negative reaction, but those of strain M18^T were oxidase-positive. Strain M9^T contained large amounts of phosphatidylethanolamine and

diphosphatidylglycerol and small amounts of two unknown phospholipids (PL1, PL2) as the polar lipids, while strain M18^T contained only a large amount of phosphatidylethanolamine and a small amount of diphosphatidylglycerol (data not shown). Strain M18^T also hydrolysed agar, whereas strain M9^T did not (Supplementary Fig. S2). In enzyme production, such as α - and β -galactosidase and α -glucosidase activities, they also had a few different characteristics. Further physiological and biochemical characteristics of strains M9^T and M18^T are compared in detail in the species descriptions and in Supplementary Table S1. Other morphological, physiological and biochemical characteristics of strains M9^T and M18^T are compared with those of phylogenetically related type strains in Table 2.

Almost complete 16S rRNA gene sequences of strains M9^T and M18^T were obtained and used for initial BLAST searches in GenBank and further phylogenetic analyses. The tree constructed by the NJ method using 16S rRNA gene sequences showed that strains M9^T and M18^T formed a phylogenetic lineage distinct from the genera *Microbulbifer*, *Saccharophagus* and *Teredinibacter* within the class *Gammaproteobacteria*, with a bootstrap value of 64 % (Fig. 1). The topologies of

Table 2. Differential phenotypic characteristics of strains M9^T and M18^T and related type strains

Strains: 1, *Microbulbifer maritimus* TF-17^T (data from Yoon *et al.*, 2004); 2, *Microbulbifer hydrolyticus* DSM 11525^T (Gonzalez *et al.*, 1997); 3, *Microbulbifer elongatus* DSM 6810^T (Yoon *et al.*, 2003b); 4, *Microbulbifer salpaludis* SM-1^T (Yoon *et al.*, 2003a); 5, *Teredinibacter turnerae* T7902^T (Distel *et al.*, 2002); 6, *Saccharophagus degradans* 2-40^T (Ekborg *et al.*, 2005). +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristic	M9 ^T	M18 ^T	1	2	3	4	5	6
Morphology	Short rod	Short rod	Rod	Rod	Coccus or rod	Rod	Rod	Pleomorphic rod
Flagella	Single	Single	Non-motile	Non-motile	Motile	Non-motile	Single, polar	Motile
Catalase	–	–	ND	+	+	+	+	+
Oxidase	–	+	+	+	ND	+	+	+
Nitrate reduction	–	–	–	–	–	ND	ND	+
Hydrolysis of:								
Agar	–	+	–	–	+	W	–	ND
Gelatin	–	–	+	+	+	ND	ND	ND
Tween 80	–	+	+	+	ND	+	ND	ND
Aesculin	+	+	–	+	ND	+	ND	ND
Casein	–	–	+	+	ND	W	ND	ND
Tyrosine	–	–	+	ND	ND	W	ND	+
Acid production from:								
Glucose	+	+	–	–	–	+	+	ND
Xylose	+	+	–	+	+	–	+	ND
Fructose	+	+	ND	ND	ND	–	+	ND
Lactose	+	+	ND	ND	ND	–	ND	ND
Mannitol	–	–	–	ND	ND	–	ND	ND
Mannose	+	+	ND	ND	ND	–	ND	ND
Sucrose	+	+	–	–	+	–	+	ND
Major fatty acid(s)	C _{16:0} , C _{19:0} cyclo ω 8c	C _{16:0} , C _{19:0} cyclo ω 8c	iso-C _{15:0}	iso-C _{15:0} , iso-C _{17:1} ω 9c	iso-C _{15:0}	iso-C _{15:0} , C _{16:0}	ND	iso-C _{16:0}
DNA G+C content (mol%)	57	58	59.9	57.7	ND	59	49–51	45.8

phylogenetic trees built using the ML and MP algorithms also supported the conclusion that strains M9^T and M18^T formed a phylogenetic line distinct from these genera (data not shown). Comparative 16S rRNA gene sequence analyses showed that strains M9^T and M18^T were most closely related to members of the genera *Microbulbifer*, *Saccharophagus* and *Teredinibacter*, with no more than 92.5 % 16S rRNA gene sequence similarity. The level of 16S rRNA gene sequence similarity between strains M9^T and M18^T was 96.7 %, which suggests that the strains should be classified within the same genus and different species (Rossello-Mora & Amann, 2001; Stackebrandt *et al.*, 2002). Therefore, on the basis of physiological and molecular properties, strains M9^T and M18^T represent a novel genus, *Marinimicrobium* gen. nov., and two different species within the class *Gammaproteobacteria*, for which the names *Marinimicrobium koreense* gen. nov., sp. nov. and *Marinimicrobium agarilyticum* sp. nov. are proposed.

Description of *Marinimicrobium* gen. nov.

Marinimicrobium (Ma.ri.ni.mi.cro'bi.um. L. adj. *marinus* of the sea; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Marinimicrobium* microbe living in the sea).

Strictly aerobic, chemoheterotrophic and moderately halotolerant. Colonies are creamy, smooth, glistening and circular/slightly irregular. Cells are Gram-negative,

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships based on 16S rRNA gene sequences of strains M9^T and M18^T and other related taxa. Bootstrap values are shown as percentages of 1000 replicates, when more than 50 %. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.1 changes per nucleotide position.

non-spore-forming, short rods, approximately 0.5–0.8 µm wide and 0.9–1.5 µm long. Nitrate is not reduced to nitrite. Cells are motile by means of a flagellum. Catalase-negative. The predominant isoprenoid quinone is Q-8. The major fatty acids are C_{16:0}, C_{19:0} cyclo ω8c and summed feature 3 (C_{16:1} ω7c and/or iso C_{15:0} 2-OH). The G + C content of the genomic DNA is 57–58 mol% (HPLC). Phylogenetically, the genus belongs to the class *Gammaproteobacteria*. The type species is *Marinimicrobium koreense*.

Description of *Marinimicrobium koreense* sp. nov.

Marinimicrobium koreense (ko.re.en'se. N.L. neut. adj. *koreense* pertaining to Korea).

Growth of cells occurs at salinities in the range 0–15 % (w/v) NaCl (optimum 1–3 % w/v). Oxidase-negative. Grows at between 10 and 45 °C (optimum 35–40 °C) and from pH 6.0 to 10.5 (optimum pH 7.0–7.5). API ZYM kit gives positive results for alkaline phosphatase, esterase (C4), esterase lipase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase and *N*-acetyl-β-glucosaminidase and negative results for lipase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. Aesculin, starch and Tween 20 are hydrolysed. Casein, gelatin, L-tyrosine, xanthine and Tween 80 are not hydrolysed. Acids are produced from D-glucose, D-fructose, D-ribose, D-xylose, α-D-lactose, maltose, D-trehalose, L-arabinose, D-melibiose, D-mannose and sucrose, but not from D-mannitol, adonitol, raffinose, glycerol or inositol. Shows no agarolytic activity. Contains large amounts of phosphatidylethanolamine and diphosphatidylglycerol and small amounts of two unknown phospholipids (PL1, PL2) as the polar lipids. The DNA G + C content is 57 mol%.

The type strain is strain M9^T (=KCTC 12356^T = DSM 16974^T), isolated from tidal flat sediment in Korea.

Description of *Marinimicrobium agarilyticum* sp. nov.

Marinimicrobium agarilyticum (a.ga.ri.ly'ti.cum. N.L. n. *agarum* agar; N.L. adj. *lyticus* from Gr. adj. *lutikos* dissolving; N.L. neut. adj. *agarilyticum* agar-dissolving).

Cells grow at salinities in the range 0–13 % (w/v) NaCl (optimum 0–1 % w/v). Oxidase-positive. Grows at between 12 and 40 °C (optimum 35 °C) and from pH 6.0 to 10.5 (optimum pH 7.0–8.0). API ZYM kit gives positive results for alkaline phosphatase, esterase (C4), esterase lipase, valine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and *N*-acetyl-β-glucosaminidase and negative results for lipase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, β-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. Agar, aesculin, starch, Tween 80 and Tween 20 are hydrolysed. Casein, gelatin, L-tyrosine and

xanthine are not hydrolysed. Acids are produced from D-glucose, D-fructose, D-ribose, D-xylose, α -D-lactose, maltose, D-trehalose, L-arabinose, raffinose, rhamnose, D-melibiose, D-mannose and sucrose, but not from D-mannitol, adonitol, glycerol or inositol. Shows agarolytic activity. Contains a large amount of phosphatidylethanolamine and a small amount of diphosphatidylglycerol as the polar lipids. The DNA G+C content is 58 mol%.

The type strain is strain M18^T (=KCTC 12357^T=DSM 16975^T), isolated from tidal flat sediment in Korea.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology (grant MG05-0101-1-0), Republic of Korea.

References

- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.
- Distel, D. L., Morrill, W., MacLaren-Toussaint, N., Franks, D. & Waterbury, J. (2002). *Teredinibacter turnerae* gen. nov., sp. nov., a dinitrogen-fixing, cellulolytic, endosymbiotic γ -proteobacterium isolated from the gills of wood-boring molluscs (Bivalvia: Teredinidae). *Int J Syst Evol Microbiol* **52**, 2261–2269.
- Ekborg, N. A., Gonzalez, J. M., Howard, M. B., Taylor, L. E., Hutcheson, S. W. & Weiner, R. M. (2005). *Saccharophagus degradans* gen. nov., sp. nov., a versatile marine degrader of complex polysaccharides. *Int J Syst Evol Microbiol* **55**, 1545–1549.
- Felsenstein, J. (2002). PHYLIP – phylogeny inference package, version 3.6a. Distributed by the author. University of Washington, Seattle, USA.
- Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P. & Bertrand, J.-C. (1992). *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**, 568–576.
- Gerhardt, P., Murray, R. G. M., Wood, W. A. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.
- González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Microbulbifer hydrolyticus* gen. nov., sp. nov. and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst Bacteriol* **47**, 369–376.
- Gorshkova, N. M., Ivanova, E. P., Sergeev, A. F., Zhukova, N. V., Alexeeva, Y., Wright, J. P., Nicolau, D. V., Mikhailov, V. V. & Christen, R. (2003). *Marinobacter excellens* sp. nov., isolated from sediments of the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 2073–2078.
- Howard, M. B., Ekborg, N. A., Taylor, L. E., II, Weiner, R. M. & Hutcheson, S. W. (2004). Chitinase B of “*Microbulbifer degradans*” 2-40 contains two catalytic domains with different chitinolytic activities. *J Bacteriol* **186**, 1297–1303.
- Huu, N. B., Denner, E. B. M., Ha, D. T. C., Wanner, G. & Stan-Lotter, H. (1999). *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. *Int J Syst Bacteriol* **49**, 367–375.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–208.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lee, E. M., Jeon, C. O., Choi, I., Chang, K.-S. & Kim, C.-J. (2005). *Silanimonas lenta* gen. nov., sp. nov., a slightly thermophilic and alkaliphilic gammaproteobacterium isolated from a hot spring. *Int J Syst Evol Microbiol* **55**, 385–389.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Marquez, M. C. & Ventosa, A. (2005). *Marinobacter hydrocarbonoclasticus* Gauthier et al. 1992 and *Marinobacter aquaeolei* Nguyen et al. 1999 are heterotypic synonyms. *Int J Syst Evol Microbiol* **55**, 1349–1351.
- Martin, S., Márquez, M. C., Sánchez-Porro, C., Mellado, E., Arahal, D. R. & Ventosa, A. (2003). *Marinobacter lipolyticus* sp. nov., a novel moderate halophile with lipolytic activity. *Int J Syst Evol Microbiol* **53**, 1383–1387.
- Romanenko, L. A., Schumann, P., Rohde, M., Zhukova, N. V., Mikhailov, V. V. & Stackebrandt, E. (2005). *Marinobacter bryozoorum* sp. nov. and *Marinobacter sediminum* sp. nov., novel bacteria from the marine environment. *Int J Syst Evol Microbiol* **55**, 143–148.
- Rossello-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.
- Satomi, M., Kimura, B., Hayashi, M., Shouzen, Y., Okuzumi, M. & Fujii, T. (1998). *Marinospirillum* gen. nov., with descriptions of *Marinospirillum megaterium* sp. nov., isolated from kusaya gravity, and transfer of *Oceanospirillum minutulum* to *Marinospirillum minutulum* comb. nov. *Int J Syst Bacteriol* **48**, 1341–1348.
- Satomi, M., Kimura, B., Hayashi, M., Okuzumi, M. & Fujii, T. (2004). *Marinospirillum insulare* sp. nov., a novel halophilic helical bacterium isolated from kusaya gravity. *Int J Syst Evol Microbiol* **54**, 163–167.
- Shivaji, S., Gupta, P., Chaturvedi, P., Suresh, K. & Delille, D. (2005). *Marinobacter maritimus* sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands. *Int J Syst Evol Microbiol* **55**, 1453–1456.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M. & 10 other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- 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., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Yoon, J. H., Kim, I. G., Shin, D. Y., Kang, K. H. & Park, Y. H. (2003a). *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* **53**, 53–57.
- Yoon, J. H., Kim, H., Kang, K. H., Oh, T.-K. & Park, Y. H. (2003b). Transfer of *Pseudomonas elongata* Humm 1946 to the genus *Microbulbifer* as *Microbulbifer elongatus* comb. nov. *Int J Syst Evol Microbiol* **53**, 1357–1361.
- Yoon, J. H., Kim, I. G., Oh, T. K. & Park, Y. H. (2004). *Microbulbifer maritimus* sp. nov., isolated from an intertidal sediment from the Yellow Sea, Korea. *Int J Syst Evol Microbiol* **54**, 1111–1116.