Henriciella litoralis sp. nov., isolated from a tidal flat, transfer of *Maribaculum marinum* Lai *et al.* 2009 to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. and emended description of the genus *Henriciella*

Se Hee Lee,^{1†} Jae Kuk Shim,^{1†} Jeong Myeong Kim,¹ Hyung-Kyoon Choi² and Che Ok Jeon¹

Correspondence
Che Ok Jeon
cojeon@cau.ac.kr

¹Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul 156-756, Republic of Korea

²College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea

A Gram-staining-negative, strictly aerobic bacterium, designated strain SD10^T, was isolated from a tidal flat of the Yellow Sea, South Korea. Cells were non-spore-forming rods that showed catalase- and oxidase-positive reactions. Growth of strain SD10^T was observed at 15–40 °C (optimum, 25–30 °C), at pH 6.0–9.0 (optimum, pH 6.5–8.5) and in the presence of 1–10% (w/v) NaCl. Strain SD10^T contained ubiquinone-10 (Q-10) as a major isoprenoid quinone and C_{18:1}ω₇c (39.3%), C_{16:0} (20.2%), C_{17:0} (8.9%) and C_{17:1}ω₆c (8.1%) as major fatty acids. The cellular polar lipids were identified as phosphatidylglycerol, monoglycosyldiglyceride, glucuronopyranosyldiglyceride and two unidentified glycolipids. The G + C content of the genomic DNA was 55.2 mol%. Based on 16S rRNA gene sequence similarities, the strain was most closely related to *Henriciella marina* Iso4^T and *Maribaculum marinum* P38^T, with similarities of 97.8 and 97.0%, respectively. The DNA–DNA relatedness between strain SD10^T and *H. marina* Iso4^T was 12.0 ± 3.2%. A phylogenetic analysis based on 16S rRNA gene sequences showed that *M. marinum* P38^T and *H. marina* Iso4^T formed a monophyletic cluster and that their 16S rRNA gene sequence similarity was 98.1%. DNA–DNA hybridization between *H. marina* Iso4^T and *M. marinum* LMG 24711^T was 22.9 ± 2.7%, indicating that the two strains belong to separate species. On the basis of chemotaxonomic data and molecular properties, we propose that strain SD10^T represents a novel species of the genus *Henriciella*, for which the name *Henriciella litoralis* sp. nov. is proposed. The type strain is SD10^T (=KACC 13700^T =DSM 22014^T). In addition, we propose to transfer *Maribaculum marinum* Lai *et al.* 2009 to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. (type strain P38^T =CCTCC AB 208227^T =LMG 24711^T =MCCC 1A01086^T), and we present an emended description of the genus *Henriciella*.

The family *Hyphomonadaceae* was proposed by Lee *et al.* (2005a) as a member of the order *Rhodobacterales* based on 16S rRNA gene sequence analyses. Members of the family have been isolated from various marine habitats such as surface seawater, brackish water, the deep sea, Antarctic seawater, hydrothermal vents and a culture of dinoflagellates

(Moore *et al.*, 1984; Abraham *et al.*, 2002; Strömpl *et al.*, 2003; Lee *et al.*, 2007; Kang & Lee, 2009a, b). Among them, the genus *Henriciella* belonging to the family *Hyphomonadaceae* was first described by Quan *et al.* (2009) with the single species *Henriciella marina*, isolated from seawater. The coasts of the Yellow Sea around the Korean peninsula consist of vast tidal flats known as getbol, which are marine habitats that possess valuable biological resources such as micro-organisms and marine animals and play very important roles in the restoration of coastal ecosystems. Therefore, efforts have been made in our laboratory to isolate and characterize members of the bacterial community of tidal flats of the Yellow Sea (Kim *et al.*, 2010). Here, we describe the taxonomic

†These authors contributed equally to this work.

Abbreviations: GUDG, glucuronopyranosyldiglyceride; MGDG, monoglycosyldiglyceride; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SD10^T is FJ230835.

Two supplementary figures and a supplementary table are available with the online version of this paper.

characterization of a novel species belonging to the genus *Henriciella* using a polyphasic approach. Recently, *Maribaculum marinum* was described as the first member of a new genus of the family *Hyphomonadaceae* (Lai *et al.*, 2009). Phylogenetic analysis based on 16S rRNA gene sequence data showed that *M. marinum* P38^T formed a phylogenetic lineage with *H. marina* Iso4^T, with 98.1 % 16S rRNA gene sequence similarity. Therefore, we also propose the transfer of *Maribaculum marinum* to the genus *Henriciella*.

Strain SD10^T was isolated from a tidal flat at Taeon using a previously described procedure with some modifications (Kim *et al.*, 2008). Briefly, a sediment sample was serially diluted with marine broth (MB; Difco), spread on marine agar 2216 (MA; Difco) plates and incubated at 25 °C for 5 days. Colonies were selected randomly and crude lysates containing genomic DNA from the colonies were prepared by boiling a small amount of cell material from colonies in 100 µl of 5 % Chelex 100 solution (Bio-Rad) for 10 min and centrifugation at 15 000 g for 10 min. PCR amplification of the 16S rRNA gene from the crude lysates was performed using universal primers as described previously (Lu *et al.*, 2006). The amplicons were double-digested with *Hae*III and *Hha*I. Restriction fragment length polymorphism (RFLP) patterns were analysed on 2.5 % MetaPhore agarose gels (BioWhittaker) and representative PCR products containing distinct RFLP patterns were sequenced. The resulting 16S rRNA gene sequences were analysed using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) in GenBank. From the analysis, strain SD10^T, a novel member of the genus *Henriciella*, was selected for phenotypic and phylogenetic analysis. The strain was routinely grown aerobically on MA at 25 °C for 3 days, except where indicated otherwise. The strain was stored at –80 °C in MB supplemented with 10 % (v/v) glycerol for preservation. *H. marina* Iso4^T, a gift from Dr Jin-Woo Bae, and *M. marinum* LMG 24711^T, purchased from BCCM/LMG (Belgium), were used as reference strains for DNA–DNA hybridization, phenotypic characterization and fatty acid analysis.

The 16S rRNA gene sequence (1332 nt) of strain SD10^T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program to determine an approximate phylogenetic affiliation. Sequence similarity between the isolate and related bacteria was evaluated using the Nucleotide Similarity Search program at the EzTaxon server (<http://147.47.212.35:8080>; Chun *et al.*, 2007) and aligned with sequences of closely related members by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms available in the PHYLIP software, version 3.6 (Felsenstein, 2002). The resulting tree topologies were evaluated using a bootstrap analysis based on 1000 resampled datasets with the PHYLIP package.

Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SD10^T formed a tight phyletic lineage with *H. marina* Iso4^T and *M. marinum* P38^T with a bootstrap value of 100 % (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were essentially the same as that of the neighbour-joining tree (not shown). Comparative 16S rRNA gene sequence analysis showed that strain SD10^T was closely related to *H. marina* Iso4^T and *M. marinum* P38^T, with similarities of 97.8 and 97.0 %, respectively, and the sequence similarity to other closely related taxa was less than 94.8 %. The phylogenetic analysis also showed that *M. marinum* P38^T and *H. marina* Iso4^T formed a monophyletic cluster and that their 16S rRNA gene sequence similarity was 98.1 %.

DNA–DNA hybridization was carried out to evaluate the level of DNA relatedness between strain SD10^T and *H. marina* Iso4^T and between *H. marina* Iso4^T and *M. marinum* LMG 24711^T. The concentrations of the extracted genomic DNAs were measured at 260 nm using an ELISA reader equipped with a Take3 Multi-Volume plate (SynergyMx; BioTek). The DNA samples were fragmented with *Hae*III for slot hybridization. The digested DNAs were diluted serially and, after denaturation using NaOH solution and heating (80 °C), different amounts of the fragmented DNA (0, 8, 16, 24, 32, 40, 60, 80, 120, 160 ng) were blotted onto Hybond-N+ nylon membranes (Amersham Pharmacia Biotech) in three replicates. Each DNA (4 µg) was used individually as a labelled probe for cross-hybridization (Park *et al.*, 2007). Random-primed DNA labelling with digoxigenin (DIG)–dUTP and detection of hybrids by enzyme immunoassay on nylon membrane were performed in triplicate using the DIG High Prime DNA Labelling kit (Roche Applied Science) according to the manufacturer's instructions and standard procedures (Sambrook & Russell, 2001). Hybridization signals were captured and analysed with Bio-Rad Quantity One software (version 4.62). The signal produced by hybridization of the probe to the homologous target DNA was taken to be 100 %, and signal intensities by self-hybridization of the series of dilutions were used for calculation of the levels of DNA relatedness between strain SD10^T and *H. marina* Iso4^T and between *H. marina* Iso4^T and *M. marinum* LMG 24711^T (Lee *et al.*, 2005b). Hybridization experiments were always confirmed by cross-hybridization. DNA–DNA relatedness between strain SD10^T and *H. marina* Iso4^T and between *H. marina* Iso4^T and *M. marinum* LMG 24711^T was 12.0 ± 3.2 and 22.9 ± 2.7 %, respectively, clearly below the 70 % threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001).

Physiological characteristics of strain SD10^T were examined by growing the isolate at 5–40 °C (at 5 °C intervals) on MA and at pH 4.0–9.5 (at 0.5 pH unit intervals) in MB. The pH was adjusted prior to sterilization by the addition of HCl or NaOH and measured again after sterilization. Gram staining was performed using a bioMérieux Gram stain kit according to the instructions of the manufacturer.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic relationships of strain SD10^T, *M. marinum* P38^T and related taxa. Bootstrap values are shown at branch nodes based on 1000 resamplings; only values over 50% are given. The sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

Cell morphology, the formation of flagella and hyphae and motility of strain SD10^T were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described previously (Jeon *et al.*, 2004). NaCl requirements and tolerance were determined in nutrient broth supplemented with modified artificial seawater [0–15% (w/v) NaCl (in increments of 1%), 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 0.64 g KCl and 1.3 g CaCl₂ per litre]. Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution. Nitrate reduction was tested according to the method of Lányi (1987). Anaerobic growth was assessed on MA under anaerobic conditions (with 4–10% CO₂) using the GasPak Plus system (BBL) at 25 °C for 20 days. Additional enzyme activities and biochemical features and the utilization of carbon sources of strain SD10^T and reference strains were determined by using API 20 NE and API ZYM strips (bioMérieux) and the GN2 MicroPlate system (Biolog) at 25 °C based on the manufacturers' instructions.

When tested on MA, growth of strain SD10^T was observed at 15–40 °C (optimum, 25–30 °C), at pH 6.0–9.0 (optimum pH 6.5–8.5) and in the presence of 1–10% (w/v) NaCl (optimum, 2–5%). Bacterial cells were Gram-staining-negative, strictly aerobic, motile rods (0.4–0.6 µm wide and 0.8–2.8 µm long) with a single polar flagellum (Supplementary Fig. S1, available in IJSEM Online). Some of these properties are in accordance with the characteristics of members of the genus *Henriciella*, whereas others allow the differentiation of strain SD10^T from closely related *Henriciella* species (Table 1, Supplementary Table S1).

Isoprenoid quinones were analysed using an HPLC (model LC-20A; Shimadzu) equipped with a diode array detector

(SPD-M20A; Shimadzu) and a reversed-phase column (250 × 4.6 mm Kromasil; Akzo Nobel) as described by Komagata & Suzuki (1987). For analysis of fatty acid methyl esters, cells of strain SD10^T were harvested from MA plates after incubation at 25 °C for 3 days. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.). Polar lipids were determined by TLC as described by Minnikin *et al.* (1977). The DNA G + C content of strain SD10^T was determined using an HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The major respiratory lipoquinone of strain SD10^T was ubiquinone 10 (Q-10). The cellular polar lipids were identified as phosphatidylglycerol (PG), monoglycosyldiglyceride (MGDG), glucuronopyranosyldiglyceride (GUDG) and two unidentified glycolipids (GL1 and GL2) (Supplementary Fig. S2). Among these polar lipids, the presence of MGDG and GUDG appears to be a characteristic signature for members of the family *Hyphomonadaceae*, together with the absence of phosphatidylethanolamine and phosphatidylcholine (Alain *et al.*, 2008; Kang & Lee, 2009a). The major cellular fatty acids (>8% of the total fatty acids) were C_{18:1}ω7c (39.3%), C_{16:0} (20.2%), C_{17:0} (8.9%) and C_{17:1}ω6c (8.1%) (Table 2). This profile is similar to those of the phylogenetically related strains *H. marina* Iso4^T and *M. marinum* LMG 24711^T, but differs in the proportions of some components. For example, fatty acid C_{12:0} 3-OH was not detected from strain SD10^T, whereas it was detected from *H. marina* Iso4^T and *M. marinum* LMG 24711^T. The G + C content of strain SD10^T was 55.2 mol%. The major lipoquinone, major fatty acids and G + C content were in accordance with those of members of the genus *Henriciella* (Quan *et al.*, 2009).

The physiological, biochemical and phylogenetic properties of strain SD10^T support its assignment to a novel species within the genus *Henriciella*, for which the name

Table 1. Phenotypic characteristics of strain SD10^T and type strains of related species

Strains: 1, strain SD10^T (data from this study); 2, *H. marina* Iso4^T (unless indicated, data from Quan *et al.*, 2009); 3, *M. marinum* LMG 24711^T (unless indicated, data from Lai *et al.*, 2009). All strains are positive for the following characteristics: division by binary fission, oxidase and catalase activities, the presence of flagella, alkaline phosphatase*, esterase (C4)*, esterase lipase (C8)*, lipase (C14)*, leucine arylamidase*, valine arylamidase*, cystine arylamidase*, trypsin*, α -chymotrypsin* and acid phosphatase* (API ZYM). All strains are negative for the following characteristics: Gram staining, prostheca formation, nitrate reduction, indole production, glucose acidification*, arginine dihydrolase*, sporulation and growth at 4 °C. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3
Isolation source	Tidal flat	Seawater	Seawater
Colony colour†	Y	TWH	G
Cell shape‡	O/R	O/R	O/SR
Hypha formation	–	+	ND
Growth at/in:			
40 °C	+	–	+
15 % NaCl	–	+	–
Hydrolysis of aesculin	+	–*	–*
Enzyme activities (API ZYM)			
Naphthol-AS-BI phosphohydrolase	w	+	+
α -Glucosidase	w	+	+
β -Glucosidase	w	–*	–*
Carbon utilization (Biolog GN2)			
Tween 80	–	+	–*
L-Arabinose	+	+	–*
D-Fructose	+	–*	+
α -D-Glucose	–	–*	+
Raffinose	+	–*	–*
Sucrose	–	+	+
Glycerol	+	–*	–*
DNA G + C content (mol%)	55.2	56.2	61.0

*Data from this study (25 °C).

†G, Grey; TWH, translucent white; Y, yellow.

‡O, Ovoid; R, rod; SR, short rod.

Henriciella litoralis sp. nov. is proposed. In addition, we propose the transfer of *Maribaculum marinum* to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. and we provide an emended description of the genus *Henriciella*.

Description of *Henriciella litoralis* sp. nov.

Henriciella litoralis (li.to.ra'lis. L. fem. adj. *litoralis* of or belonging to the seashore).

Cells are Gram-negative, strictly aerobic, motile rods (0.4–0.6 μ m wide and 0.8–2.8 μ m long) with a polar flagellum. Colonies on MA are yellow, convex and round with entire margins. Growth occurs at 15–40 °C (optimum, 25–30 °C), at pH 6.0–9.0 (optimum, pH 6.5–8.5) and in the

presence of 1–10 % (w/v) NaCl (optimum, 2–5 %). Division mode is binary fission. Catalase- and oxidase-positive. In the API 20NE kit, negative for nitrate reduction, indole production, glucose acidification and arginine dihydrolase activity and for assimilation of D-glucose, L-arabinose, D-mannose, maltose, D-mannitol, malic acid, potassium gluconate, N-acetylglucosamine, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Aesculin is hydrolysed. Urea and gelatin are not hydrolysed. In the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin and acid phosphatase activities are present and weak enzymic activities are observed for naphthol-AS-BI-phosphohydrolase, α -glucosidase and β -glucosidase, but β -glucuronidase, N-acetyl- β -glucosaminidase, β -galactosidase, α -fucosidase, α -galactosidase, β -glucuronidase and α -mannosidase are absent. Positive for utilization of Biolog GN2 MicroPlate system substrates dextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, D-fructose, L-fucose, D-galactose, myo-inositol, D-mannose, methyl β -D-glucoside, raffinose, L-rhamnose, turanose, acetic acid, cis-aconitic acid, citric acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, quinic acid, D-saccharic acid, succinic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-ornithine, L-serine, L-threonine, inosine, uridine, phenyl-ethylamine, glycerol, DL- α -glycerol phosphate and D-glucose 6-phosphate. Other organic substrates included in the Biolog GN2 microplate are not oxidized. The major isoprenoid quinone is Q-10. Contains PG, MGDG, GUDG and two unidentified glycolipids as polar lipids. The major cellular fatty acids are C_{18:1} ω 7c, C_{16:0}, C_{17:0} and C_{17:1} ω 6c. The DNA G + C content of the type strain is 55.2 mol% (HPLC).

The type strain is SD10^T (=KACC 13700^T =DSM 22014^T), which was isolated from a tidal flat of the Taean coast in South Korea.

Description of *Henriciella aquimarina* (Lai *et al.* 2009) nom. nov.

Henriciella aquimarina (a.qui.ma.ri'na. L. fem. n. *aqua* water; L. fem. adj. *marina* of or belonging to the sea; N.L. fem. adj. *aquimarina* from seawater).

Basonym: *Maribaculum marinum* Lai *et al.* 2009.

The description is as given for *Maribaculum marinum* by Lai *et al.* (2009). The type strain is P38^T (=CCTCC AB 208227^T =LMG 24711^T =MCCC 1A01086^T).

Emended description of the genus *Henriciella* Quan *et al.* 2009

The description of the genus *Henriciella* is as given by Quan *et al.* (2009) with the following amendments. The

Table 2. Cellular fatty acid compositions of strain SD10^T, *H. marina* Iso4^T and *M. marinum* LMG 24711^T

Strains: 1, strain SD10^T; 2, *H. marina* Iso4^T; 3, *M. marinum* LMG 24711^T. All data were obtained in this study after growth on MA at 25 °C for 3 days. Data are expressed as percentages of total fatty acids; fatty acids amounting to less than 0.5 % in all strains are not shown. tr, Trace amount (<0.5 %); –, not detected.

Fatty acid	1	2	3
Saturated			
C _{15:0}	2.1	4.5	1.4
C _{16:0}	20.2	9.3	20.6
C _{17:0}	8.9	4.5	4.6
C _{18:0}	2.1	tr	2.7
C _{20:0}	1.7	1.2	3.3
Hydroxy			
C _{12:1} 3-OH	–	0.8	–
C _{12:0} 3-OH	–	2.5	4.6
C _{18:1} 2-OH	–	–	2.0
Branched			
iso-C _{17:0}	tr	0.7	–
iso-C _{17:0} 3-OH	–	1.5	–
Unsaturated			
C _{15:1} ω8c	–	0.7	–
C _{16:1} ω5c	6.2	3.3	6.4
C _{17:1} ω6c	8.1	8.9	3.3
C _{17:1} ω8c	5.0	10.6	3.5
C _{18:1} ω5c	2.1	1.2	0.9
C _{18:1} ω7c	39.3	45.0	36.5
11-Methyl C _{18:1} ω7c	2.9	0.6	8.3
Cyclopropane acids			
C _{19:0} cyclo ω8c	–	–	1.3
Summed features*			
Summed feature 3	tr	2.2	0.7

*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.

major fatty acid is C_{18:1} ω7c. The DNA G+C content is 55.2–62.0 mol%.

Acknowledgements

This research was supported by a Chung-Ang University Research Scholarship Grant in 2009.

References

- Abraham, W. R., Strömpl, C., Bennasar, A., Vancanneyt, M., Snauwaert, C., Swings, J., Smit, J. & Moore, E. R. B. (2002). Phylogeny of *Maricaulis* Abraham *et al.* 1999 and proposal of *Maricaulis virginensis* sp. nov., *M. parjimensis* sp. nov., *M. washingtonensis* sp. nov. and *M. salignorans* sp. nov. *Int J Syst Evol Microbiol* 52, 2191–2201.
- Alain, K., Tindall, B. J., Intertaglia, L., Catala, P. & Lebaron, P. (2008). *Hellea balneolensis* gen. nov., sp. nov., a prosthecate alphaproteobacterium from the Mediterranean Sea. *Int J Syst Evol Microbiol* 58, 2511–2519.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57, 2259–2261.

Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Jeon, C. O., Park, W., Ghiorse, W. C. & Madsen, E. L. (2004). *Polaromonas naphthalenivorans* sp. nov., a naphthalene-degrading bacterium from naphthalene-contaminated sediment. *Int J Syst Evol Microbiol* 54, 93–97.

Kang, H. S. & Lee, S. D. (2009a). *Hirschia maritima* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 59, 2264–2268.

Kang, H. S. & Lee, S. D. (2009b). *Ponticaulis koreensis* gen. nov., sp. nov., a new member of the family Hyphomonadaceae isolated from seawater. *Int J Syst Evol Microbiol* 59, 2951–2955.

Kim, J. M., Le, N. T., Chung, B. S., Park, J. H., Bae, J.-W., Madsen, E. L. & Jeon, C. O. (2008). Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and o-, m-, and p-xylenes by the newly isolated bacterium *Pseudoxanthomonas spadix* BD-a59. *Appl Environ Microbiol* 74, 7313–7320.

Kim, J. M., Lee, S. H., Jung, J. Y. & Jeon, C. O. (2010). *Marinobacterium lutimaris* sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol* 60, 1828–1831.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–207.

Lai, Q., Yuan, J. & Shao, Z. (2009). *Maribaculum marinum* gen. nov., sp. nov., isolated from deep seawater. *Int J Syst Evol Microbiol* 59, 3083–3087.

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

Lee, K. B., Liu, C. T., Anzai, Y., Kim, H., Aono, T. & Oyaizu, H. (2005a). The hierarchical system of the ‘Alphaproteobacteria’: description of Hyphomonadaceae fam. nov., Xanthobacteraceae fam. nov. and Erythrobacteraceae fam. nov. *Int J Syst Evol Microbiol* 55, 1907–1919.

Lee, J. C., Jeon, C. O., Lim, J. M., Lee, S. M., Lee, J. M., Song, S. M., Park, D. J., Li, W. J. & Kim, C. J. (2005b). *Halomonas taeanensis* sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea. *Int J Syst Evol Microbiol* 55, 2027–2032.

Lee, K., Lee, H. K., Choi, T. H. & Cho, J. C. (2007). *Robiginotomaculum antarcticum* gen. nov., sp. nov., a member of the family Hyphomonadaceae, from Antarctic seawater. *Int J Syst Evol Microbiol* 57, 2595–2599.

Lu, S., Park, M., Ro, H.-S., Lee, D. S., Park, W. & Jeon, C. O. (2006). Analysis of microbial communities using culture-dependent and culture-independent approaches in an anaerobic/aerobic SBR reactor. *J Microbiol* 44, 155–161.

Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 27, 104–117.

Moore, R. L., Weiner, R. M. & Gebbers, R. (1984). Genus *Hyphomonas* Pongratz 1957 nom. rev. emend., *Hyphomonas polymorpha* Pongratz 1957 nom. rev. emend., and *Hyphomonas neptunium* (Leifson 1964) comb. nov. emend. (*Hyphomicrobium neptunium*). *Int J Syst Bacteriol* 34, 71–73.

Park, M., Ryu, S. H., Vu, T. H., Ro, H. S., Yun, P. Y. & Jeon, C. O. (2007). *Flavobacterium defluvii* sp. nov., isolated from activated sludge. *Int J Syst Evol Microbiol* 57, 233–237.

Quan, Z.-X., Zeng, D.-N., Xiao, Y.-P., Roh, S. W., Nam, Y.-D., Chang, H.-W., Yoon, J.-H., Oh, H.-M. & Bae, J.-W. (2009). *Henriciella marina*

gen. nov., sp. nov., a novel member of the family *Hyphomonadaceae* isolated from the East Sea. *J Microbiol* **47**, 156–161.

Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Strömpl, C., Hold, G. L., Lünsdorf, H., Graham, J., Gallacher, S., Abraham, W.-R., Moore, E. R. B. & Timmis, K. N. (2003). *Oceanicaulis alexandrii* gen. nov., sp. nov., a novel stalked bacterium isolated from

a culture of the dinoflagellate *Alexandrium tamarense* (Lebour) Balech. *Int J Syst Evol Microbiol* **53**, 1901–1906.

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.