Maritalea myrionectae gen. nov., sp. nov., isolated from a culture of the marine ciliate *Myrionecta rubra*

Chung Y. Hwang,¹ Kyung D. Cho,¹ Wonho Yih² and Byung C. Cho¹

Correspondence

Byung C. Cho
bccho@snu.ac.kr

¹School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, San 56-1, Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

²Department of Oceanography, Kunsan National University, San 68, Miryong-dong, Gunsan 573-701, Republic of Korea

A Gram-negative, rod-shaped, strictly aerobic bacterium, designated strain CL-SK30^T, was isolated from a culture of the marine ciliate *Myrionecta rubra*. 16S rRNA gene sequence analysis revealed that strain CL-SK30^T was most closely related to *Cucumibacter marinus* (92.0 % similarity) and next to the type strains of species of the genus *Devosia* (89.8–91.3 % similarities) in the family *Hyphomicrobiaceae*. Phylogenetic analyses of the 16S rRNA gene sequences showed that strain CL-SK30^T formed a robust clade together with *C. marinus*, but the sequence divergence value of 8 % between them indicated that the novel bacterium represented a distinct lineage. Strain CL-SK30^T grew optimally in the presence of 2–5 % sea salts at 30–35 °C and pH 7.2–8.0. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, two unidentified glycolipids, an unidentified phospholipid and an unidentified lipid. Ubiquinone 10 was the major quinone. The DNA G+C content was 52.7 mol%. Based on its phenotypic, chemotaxonomic and phylogenetic characteristics, strain CL-SK30^T represents a novel genus and species of the family *Hyphomicrobiaceae*, for which the name *Maritalea myrionectae* gen. nov., sp. nov. is proposed. The type strain is CL-SK30^T (=KCCM 90060^T=DSM 19524^T).

At the time of writing the family *Hyphomicrobiaceae* comprised 15 genera (List of Prokaryotic Names with Standing in Nomenclature; <http://www.bacterio.cict.fr>). The family is diverse in morphology, and many members of the family are budding and appendaged bacteria (i.e. the genera *Ancalomicrobium*, *Blastochloris*, *Dichotomicrobium*, *Filomicrobium*, *Hyphomicrobium*, *Pedomicrobium*, *Prosthecomicrobium*, *Rhodomicrobium* and *Rhodoplanes*; Garrity *et al.*, 2005). Most species in the family have been isolated from various non-marine habitats, comprising soil, freshwater, groundwater, swamps, rhizosphere of plants, nitrifying inocula, chickens and sewage (Garrity *et al.*, 2005). Only a few members of the family *Hyphomicrobiaceae* have been isolated from marine environments (e.g. *Dichotomicrobium thermohalophilum*, *Filomicrobium fusiforme* and *Hyphomicrobium aestuarii*; Garrity *et al.*, 2005). Recently, a

short prosthecae bacterium affiliated to a new genus, *Cucumibacter*, was isolated from coastal seawater (Hwang & Cho, 2008). In this study, a rod-shaped bacterium with peritrichous flagella was isolated from a culture of the marine ciliate *Myrionecta rubra* and subjected to a polyphasic taxonomic analysis.

A clonal culture of the marine phototrophic ciliate *Myrionecta rubra* MR-MAL01 was established and maintained as described by Yih *et al.* (2004). An aliquot (100 µl) of the ciliate culture in the exponential phase was taken and spread on a marine agar 2216 (MA; Difco) plate, and incubated at 20 °C for 1 week. Strain CL-SK30^T was isolated and was subsequently streaked onto fresh MA at 20 °C. The purification procedure was repeated four times. Growth of strain CL-SK30^T occurred faster at 30 °C than at 20 °C. The strain was maintained on both MA at 30 °C and in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at –80 °C.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony by using a boiling method (Englen & Kelley, 2000). The crude extracts served as a DNA template for the PCRs, which included *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using an AccuPrep PCR purification kit (Bioneer) and the direct sequence of the purified 16S rRNA gene was determined

Abbreviations: APL, aminophospholipid; DPG, diphosphatidylglycerol; GL, glycolipid; L, lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHB, poly-β-hydroxybutyrate; PL, phospholipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-SK30^T is EF988631.

A transmission electron micrograph of a negatively stained cell of strain CL-SK30^T and two-dimensional thin-layer chromatograms of the polar lipids of strain CL-SK30^T and *Cucumibacter marinus* CL-GR60^T are available as supplementary material with the online version of this paper.

using an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen, Seoul, Korea. An almost-complete 16S rRNA gene sequence of strain CL-SK30^T (1352 nucleotides) was obtained and was compared with 16S rRNA gene sequences available in GenBank using BLASTN searches (Altschul *et al.*, 1990). The sequence of strain CL-SK30^T was aligned manually with those of species of the family *Hyphomicrobiaceae*, obtained from GenBank and Ribosomal Database Project II (Cole *et al.*, 2007) databases, using known 16S rRNA secondary-structure information. Phylogenetic trees were obtained by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods and 100 replications for the maximum-likelihood method. Alignment analysis was carried out using the jPHYDIT program (Jeon *et al.*, 2005), and phylogenetic analyses were performed using MEGA 4 (Tamura *et al.*, 2007) and PAUP 4.0 (Swofford, 1998). Likelihood parameters were estimated by using the hierarchical ratio tests in MODELTEST version 3.04 (Posada & Crandall, 1998).

Morphological and physiological tests were performed as follows. *Cucumibacter marinus* CL-GR60^T was used as a reference strain for all tests. Unless otherwise specified, all phenotypic characteristics of strains CL-SK30^T and *C. marinus* CL-GR60^T were based on cultures grown on MA or in MB at 30 °C. Gram-staining was performed as described by Smibert & Krieg (1994). Motility of the cells was observed by using the hanging drop method (Suzuki *et al.*, 2001). Cell morphology, bud formation and the presence and type of flagellum were observed using transmission electron microscopy (EX2; JEOL). Anaerobic growth was determined on MA and ZOF medium (Lemos *et al.*, 1985) supplemented with agar (1.5 %), using the GasPak anaerobic system (BBL) at 30 °C for 2 weeks. Poly- β -hydroxybutyrate (PHB) granules were observed by using epifluorescence microscopy (BX60; Olympus) after Nile blue A staining (Ostle & Holt, 1982). Bacteriochlorophyll *a* production was determined in 90 % acetone extracts using a spectrophotometer (Ultraspec 2000; Pharmacia Biotech) for cells that had been grown in MB or a defined medium (Biebl *et al.*, 2005), either in the light or in the dark for 7 days. The presence of photosynthetic reaction centre genes, *pufL* and *pufM*, was determined by using PCR amplification with specific primers (Allgaier *et al.*, 2003) for strains CL-SK30^T and *C. marinus* CL-GR60^T; *Porphyrobacter donghaensis* SW-132^T (=KCTC 12229^T; Yoon *et al.*, 2004) and *Escherichia coli* KACC 11930 served as a positive and a negative control, respectively. PCR product using the specific primers for *pufL* and *pufM* genes was not detected for strains CL-SK30^T, *C. marinus* CL-GR60^T or *E. coli* KACC 11930, but was detected for *P. donghaensis* SW-132^T (data not shown).

The temperature range for growth was examined on the basis of colony formation on MA incubated at temperatures ranging from 5 to 40 °C, in increments of 5 °C. The pH range (pH 4.2–11.8; pH 4.2, 5.4, 6.3, 7.2, 8.0, 8.8, 9.8, 10.2 and 11.8) for growth was determined by assessing changes in OD₆₀₀ over the incubation period (up to 7 days) in MB at 30 °C. Prior to autoclaving, the pH (4–12, in increments of 1 pH unit) was adjusted using 1 M NaOH and 1 M HCl solutions. After autoclaving and cooling, the pH values were measured for the subsamples of each medium, before the inoculation of cells. The tolerance of strain CL-SK30^T to sea salts (Sigma) was determined by assessing changes in OD₆₀₀ in synthetic ZoBell broth (l⁻¹ distilled water: Bacto peptone, 5 g; yeast extract, 1 g; ferric citrate, 0.1 g) with concentrations (0–10 % in increments of 1 %, and 15 %, w/v) of sea salts at 30 °C.

Oxidase and catalase tests were performed according to the protocols described by Smibert & Krieg (1994). Hydrolysis of gelatin, starch and Tween 80 was determined according to Hansen & Sørheim (1991). In addition, other enzyme activities were assayed using the API ZYM kit (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared using artificial seawater (ASW; l⁻¹ distilled water: NaCl, 24 g; MgCl₂·6H₂O, 10.9 g; Na₂SO₄, 4 g; CaCl₂·2H₂O, 1.5 g; KCl, 0.7 g; NaHCO₃, 0.2 g; KBr, 0.1 g; H₃BO₃, 0.027 g; SrCl₂·6H₂O, 0.03 g; NaF, 0.003 g; Lyman & Fleming, 1940). Carbon utilization was tested on basal agar medium [Tris/HCl, 50 mM, pH 7.5; NH₄Cl, 19 mM; K₂HPO₄·3H₂O, 0.33 mM; FeSO₄·7H₂O, 0.1 mM; 1.5 % Bacto agar (Difco) in ASW (NaCl, 400 mM; MgSO₄·7H₂O, 100 mM; KCl, 20 mM; CaCl₂·2H₂O, 20 mM)] containing 0.2 % of the carbon source as described by Baumann & Baumann (1981), but with a slight modification that full-strength ASW instead of half-strength ASW was used. Strain CL-SK30^T was incubated for 2 weeks at 30 °C and carbon utilization was scored as negative when growth was equal to or less than that in the negative control with no carbon source. Resistance to antibiotics was determined by using the disc-diffusion plate method (Bauer *et al.*, 1966).

Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and identified by using two-dimensional TLC followed by spraying with the appropriate detection reagents (Komagata & Suzuki, 1987). The fatty acid methyl esters of whole cells of strain CL-SK30^T grown on tryptic soy agar (TSA; Difco), supplemented with 2.5 % (w/v) NaCl, at 25 °C for 7 days were analysed using gas chromatography, according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Micro-organisms (KCCM) in Seoul, Korea. The quinone system was determined according to Minnikin *et al.* (1984) and analysed by using HPLC as described by Collins (1985). The DNA G+C content was analysed using HPLC (HP 100; Hewlett Packard) analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), after DNA extraction using the method of Marmur (1961). Lambda phage DNA was used as a standard.

Table 1. Selected characteristics that differentiate strain CL-SK30^T (*Maritalea myrionectae* gen. nov., sp. nov.) from related genera in the family Hyphomicrobiaceae

Taxa: 1, strain CL-SK30^T (data from this study); 2, *C. marinus* CL-GR60^T (Hwang & Cho, 2008); 3, *Devosia* (for type strains: Nakagawa *et al.*, 1996; Rivas *et al.*, 2003; Yoo *et al.*, 2006; Lee, 2007; Yoon *et al.*, 2007; Ryu *et al.*, 2008). +, Positive; –, negative; v, variable; Q, ubiquinone.

Characteristic	1*	2*	3†
Source of isolation	Culture of a marine ciliate, <i>Myrionecta rubra</i>	Seawater	Soil, rhizosphere, nitrifying inoculum, beach sediment
Motility	+	+	v
Hydrolysis of gelatin	+	+	–
API ZYM test			
Acid phosphatase	–	+	v
α -Chymotrypsin	+	–	–
Cystine arylamidase	+	–	–
α -Glucosidase	+	–	v
Lipase (C14)	–	+	–
Trypsin	+	–	v
Valine arylamidase	+	–	v
Major quinone	Q-10	Q-10	Q-10, Q-11
DNA G+C content (mol%)	52.7	62.9	59.1–66.2

*Phenotypic tests were based on cultures grown on MA or in MB at 30 °C.

†Phenotypic tests were based on cultures grown on TSA, R2A, LB, PY or YED media, supplemented with either no NaCl or 0.5 % NaCl at 25–35 °C except for *Devosia subaequoris* (grown on MA at 30 °C; Lee, 2007).

Cells of strain CL-SK30^T were Gram-negative, rod-shaped and approximately 0.4–0.5 µm wide and 1.0–2.0 µm long. Cells were motile by means of peritrichous flagella (see Supplementary Fig. S1, available in IJSEM Online). Strain CL-SK30^T was strictly aerobic. PHB and bacteriochlorophyll *a* were not present in strain CL-SK30^T or *C. marinus* CL-GR60^T. Other phenotypic characteristics of strain CL-SK30^T are given in the genus and species descriptions, and in Table 1 and Supplementary Fig. S2 (available in IJSEM Online).

The major polar lipids were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), two unidentified glycolipids (GL1 and GL2), an unidentified phospholipid (PL1) and an unidentified lipid (L; Supplementary Fig. S2, in IJSEM Online). Phosphatidylethanolamine (PE) and an unidentified aminophospholipid (APL) were detected only in minor amounts (Supplementary Fig. S2). The polar lipids PG, DPG, GL1, GL2, PL1, PE and APL were commonly detected in strain CL-SK30^T and *C. marinus* CL-GR60^T (Supplementary Fig. S2). The dominant fatty acid for strain CL-SK30^T was C_{18:1}ω7c (82.7 %), which is a feature of the vast majority of species within the *Alphaproteobacteria* (Martens *et al.*, 2006), followed by 11-methyl C_{18:1}ω7c (5.0 %), C_{18:0} (3.5 %), C_{16:0} (3.0 %), C_{10:0} (2.4 %) and C_{16:1}ω7c and/or C_{15:0} iso 2-OH (1.1 %). The fatty acids C_{17:0}, iso-C_{19:0}, C_{17:1}ω8c, unknown fatty acid [equivalent chain-length (ECL) 18.814] and C_{19:1}ω6c and/or C_{17:0} cyclo and/or unknown fatty acid (ECL 18.846) were present in trace amounts (<1 %). The major isoprenoid quinone was ubiquinone 10 (Q-10). The DNA G+C content was 52.7 mol%.

Analysis of the 16S rRNA gene sequence revealed a clear affiliation with the family Hyphomicrobiaceae (Fig. 1). In all the phylogenetic trees, strain CL-SK30^T formed a robust clade together with *C. marinus*, but the sequence divergence value of 8 % between them indicated that the novel bacterium represented a distinct lineage. The clade containing strain CL-SK30^T and the genus *Cucumibacter* was clearly separated from the nearest clade containing the genera *Devosia* and ‘*Candidatus Devosia euplotis*’, supported by high bootstrap values (100, 98 and 85 % in the neighbour-joining, maximum-parsimony and maximum-likelihood trees, respectively). The sequence similarity between strain CL-SK30^T and *C. marinus* CL-GR60^T was 92.0 %; sequence similarities between strain CL-SK30^T and members of the genera *Devosia*, *Hyphomicrobium* and *Rhodomicrobium* in the family Hyphomicrobiaceae were 89.8–91.3, 86.1–88.4 and 88.3 %, respectively. The low levels of sequence similarity with other bacteria and the distinct phylogenetic position indicated that strain CL-SK30^T should be assigned as representing a novel genus and species.

In addition, strain CL-SK30^T could be differentiated from the phylogenetically closest genus *Cucumibacter* based on chemotaxonomic and phenotypic characteristics. The difference in G+C contents between strain CL-SK30^T and *C. marinus* was >10 mol% (Table 1), showing a distinct genetic difference at the genus level (Stackebrandt & Liesack, 1993; Goodfellow *et al.*, 1997). A major unidentified polar lipid in strain CL-SK30^T (i.e. L; Supplementary Fig. S2, in IJSEM Online) apparently had

Fig. 1. Neighbour-joining tree derived from 16S rRNA gene sequences for strain CL-SK30^T and related members of the family Hyphomicrobiaceae with *Escherichia coli* as an outgroup. Only bootstrap values greater than 70 % are shown (1000 resamplings) at nodes. Solid circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. Bar, 0.02 nucleotide substitutions per site.

a different position in the polar lipid profile compared with that of a major unidentified lipid of *C. marinus* (i.e. L1; Supplementary Fig. S2). In addition, the absence of minor polar lipids (i.e. PC, PL2 and L2–4; Supplementary Fig. S2) differentiated strain CL-SK30^T from *C. marinus*. Strain CL-SK30^T could also be distinguished from *C. marinus* by

certain phenotypic traits: the ability to produce α -chymotrypsin, cystine arylamidase, α -glucosidase, trypsin and valine arylamidase, and the inability to produce acid phosphatase and lipase (C14) (Table 1). Strain CL-SK30^T could also be differentiated from the phylogenetically related genus *Devosia* in the family Hyphomicrobiaceae

based on chemotaxonomic and phenotypic characteristics. The presence of the sole major respiratory quinone (Q-10) also differentiated strain CL-SK30^T from the genus *Devosia* (Table 1). In addition, the presence of certain polar lipids (i.e. GL1, GL2, PL1, L, PE and APL; Supplementary Fig. S2) clearly distinguished strain CL-SK30^T from *Devosia geojensis* (Ryu *et al.*, 2008). Strain CL-SK30^T could also be differentiated from the genus *Devosia* by certain phenotypic traits: the ability to hydrolyse gelatin and to produce α -chymotrypsin and cystine arylamidase (Table 1).

The phylogenetic, chemotaxonomic and physiological data obtained in this study indicate that strain CL-SK30^T should be assigned as representing a novel genus and species in the family *Hyphomicrobiaceae*, for which the name *Maritalea myrionectae* gen. nov., sp. nov. is proposed.

Description of *Maritalea* gen. nov.

Maritalea (Ma.ri.ta'le.a. L. neut. n. *mare* the sea; L. fem. n. *talea* a staff, rod; N.L. fem. n. *Maritalea* rod inhabiting marine environments).

Cells are Gram-negative and rod-shaped. Motile by means of peritrichous flagella. Growth is strictly aerobic. Oxidase and catalase-positive. Major polar lipids are PG, DPG, two unidentified glycolipids, an unidentified phospholipid and an unidentified lipid. Major cellular fatty acids are C_{18:1} ω 7c and 11-methyl C_{18:1} ω 7c. The isoprenoid quinone is Q-10. Phylogenetically, the genus is a member of the family *Hyphomicrobiaceae*. The type species is *Maritalea myrionectae*.

Description of *Maritalea myrionectae* sp. nov.

Maritalea myrionectae (my.ri'o.nec.tae. N.L. gen. n. *myrionectae* of *Myrionecta rubra*, the generic name of the ciliate from which the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. After 3 days on MA plates at 30 °C, colonies are circular, convex, creamy and approximately 1 mm in diameter. Cells are approximately 0.4–0.5 μ m wide and 1.0–2.0 μ m long. Grows at 10–40 °C (optimum, 30–35 °C) and pH 6.3–9.8 (optimum, pH 7.2–8.0). Growth occurs at sea salts concentrations of 1–10 % (w/v) (optimum, 2–5 %). PHB and bacteriochlorophyll *a* are absent. Bud formation is observed. Gelatin, starch and Tween 80 are hydrolysed. According to the API ZYM system, alkaline phosphatase, α -chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), β -galactosidase, α - and β -glucosidases, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase are positive, whereas *N*-acetyl- β -glucosaminidase, acid phosphatase, α -fucosidase, α -galactosidase, β -glucuronidase, lipase (C14) and α -mannosidase are negative. Utilizes L-arabinose, L-arginine, L-asparagine, betaine, D-fructose, D-galactose, D-glucose, glutamic acid, glycerol, lactose, D-leucine, L-leucine, D-mannitol, mannose, pyruvate, sucrose and trehalose, but not DL-cysteine, dimethylsulfide, glycine, *p*-hydroxyphenylacetate,

maleic acid, succinate or urea as a sole carbon. Cells are sensitive to (μ g per disc) cephalixin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), penicillin G (10) and vancomycin (30), but resistant to streptomycin (10). In addition to the polar lipids listed in the genus description, minor or trace amounts of phosphatidylethanolamine and an unidentified aminophospholipid can be detected. The G+C content of DNA of the type strain is 52.7 mol%.

The type strain, CL-SK30^T (=KCCM 90060^T=DSM 19524^T), was isolated from a culture of the marine ciliate *Myrionecta rubra*.

Acknowledgements

We acknowledge the expert technical support of Ms In-Sung Lee (electron microscopy) of the National Center for Inter-university Research Facilities at Seoul National University. This work was supported in part by the Ministry of Maritime Affairs and Fisheries (the Korea EAST-1 program) to B.C.C., the BK21 project of the Korean Government to B.C.C. & C.Y.H. and a NRL grant from MOST & KOSEF (M1-0302-00-0068) to W.Y.

References

- Allgaier, M., Uphoff, H., Felske, A. & Wagner-Döbler, I. (2003). Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**, 5051–5059.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.
- Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes*, pp. 1302–1330. Edited by M. P. Starr, H. G. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Biebl, H., Allgaier, M., Tindall, B. J., Kobizek, M., Lünsdorf, H., Pukall, R. & Wagner-Döbler, I. (2005). *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. *Int J Syst Evol Microbiol* **55**, 1089–1096.
- Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & other authors (2007). The ribosomal database project (RDP-II): introducing *myrDP* space and quality controlled public data. *Nucleic Acids Res* **35**, D169–D172.
- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- Englen, M. D. & Kelley, L. C. (2000). A rapid DNA isolation procedure for the identification of *Campylobacter jejuni* by the polymerase chain reaction. *Lett Appl Microbiol* **31**, 421–426.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Garrity, G. M., Bell, J. A. & Lilburn, T. (2005). Family VIII. *Hyphomicrobiaceae* Babudieri 1950, 589. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, *The Proteobacteria*, part C, *The Alpha-*

- Beta-, Delta-, and Epsilonproteobacteria*, pp. 476–566. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Goodfellow, M., Manfio, G. P. & Chun, J. (1997).** Towards a practical species concept for cultivable bacteria. In *Species: the Units of Biodiversity*, pp. 25–59. Edited by M. F. Claridge, H. A. Dawah & M. R. Wilson. London: Chapman Hall.
- Hansen, G. H. & Sørheim, R. (1991).** Improved method for phenotypical characterization of marine bacteria. *J Microbiol Methods* **13**, 231–241.
- Hwang, C. Y. & Cho, B. C. (2008).** *Cucumibacter marinus* gen. nov., sp. nov., a novel marine bacterium in the family Hyphomicrobiaceae. *Int J Syst Evol Microbiol* **58**, 1591–1597.
- Jeon, Y.-S., Chung, H., Park, S., Hur, I., Lee, J.-H. & Chun, J. (2005).** jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.
- Jukes, T. H. & Cantor, C. R. (1969).** Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Komagata, K. & Suzuki, K. (1987).** Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lee, S. D. (2007).** *Devosia subaequoris* sp. nov., isolated from beach sediment. *Int J Syst Evol Microbiol* **57**, 2212–2215.
- Lemos, M. L., Toranzo, A. E. & Barja, J. L. (1985).** Modified medium for the oxidation-fermentation test in the identification of marine bacteria. *Appl Environ Microbiol* **49**, 1541–1543.
- Lyman, J. & Fleming, R. H. (1940).** Composition of sea water. *J Mar Res* **3**, 134–146.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Martens, T., Heidorn, T., Pukall, R., Simon, M., Tindall, B. J. & Brinkhoff, T. (2006).** Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte et al. 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay et al. 1995) Uchino et al. 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. *Int J Syst Evol Microbiol* **56**, 1293–1304.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- 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.
- Nakagawa, Y., Sakane, T. & Yokota, A. (1996).** Transfer of “*Pseudomonas riboflavina*” (Foster 1944), a Gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov., sp. nov., nom. rev. *Int J Syst Bacteriol* **46**, 16–22.
- Ostle, A. G. & Holt, J. G. (1982).** Nile blue A as fluorescent stain for poly- β -hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.
- Posada, D. & Crandall, K. A. (1998).** MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Rivas, R., Willems, A., Subba-Rao, N. S., Mateos, P. F., Dazzo, F. B., Kroppenstedt, R. M., Martinez-Molina, E., Gillis, M. & Velázquez, E. (2003).** Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans*, an aquatic legume from India. *Syst Appl Microbiol* **26**, 47–53.
- Ryu, S. H., Chung, B. S., Le, N. T., Jang, H. H., Yun, P.-Y., Park, W. & Jeon, C. O. (2008).** *Devosia geojensis* sp. nov., isolated from diesel-contaminated soil in Korea. *Int J Syst Evol Microbiol* **58**, 633–636.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- 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.
- Stackebrandt, E. & Liesack, W. (1993).** Nucleic acids and classification. In *Handbook of New Bacterial Systematics*, pp. 151–194. Edited by M. Goodfellow & A. G. O'Donnell. London: Academic Press.
- Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (2001).** Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovoliticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1639–1652.
- Swofford, D. L. (1998).** PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Yih, W., Kim, H. S., Jeong, H. J., Myung, G. & Kim, Y. G. (2004).** Ingestion of cryptophyte cells by the marine photosynthetic ciliate *Mesodinium rubrum*. *Aquat Microb Ecol* **36**, 165–170.
- Yoo, S.-H., Weon, H.-Y., Kim, B.-Y., Hong, S.-B., Kwon, S.-W., Cho, Y.-H., Go, S.-J. & Stackebrandt, E. (2006).** *Devosia soli* sp. nov., isolated from greenhouse soil in Korea. *Int J Syst Evol Microbiol* **56**, 2689–2692.
- Yoon, J.-H., Lee, M.-H. & Oh, T.-K. (2004).** *Porphyrobacter donghaensis* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **54**, 2231–2235.
- Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007).** *Devosia insulae* sp. nov., isolated from soil, and emended description of the genus *Devosia*. *Int J Syst Evol Microbiol* **57**, 1310–1314.