Phaeobacter daeponensis sp. nov., isolated from a tidal flat of the Yellow Sea in Korea

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A Gram-negative, motile and egg-shaped bacterium, strain TF-218^T, was isolated from a tidal flat at Daepo Beach (Yellow Sea), Korea, and subjected to a polyphasic taxonomic analysis. Strain TF-218^T grew optimally at pH 7.0–8.0 and 37 °C in the presence of 2% (w/v) NaCl. It contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c and 11-methyl C_{18:1}ω7c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two unidentified lipids and an aminolipid. The DNA G + C content was 64.9 mol%. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain TF-218^T is phylogenetically closely related to the genera *Phaeobacter*, *Leisingera* and *Marinovum* of the *Alphaproteobacteria*. The phylogenetic and chemotaxonomic similarities suggest that strain TF-218^T represents a member of the genus *Phaeobacter*. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain TF-218^T differs from the recognized *Phaeobacter* species. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain TF-218^T represents a novel species of the genus *Phaeobacter*, for which the name *Phaeobacter daeponensis* sp. nov. is proposed. The type strain is TF-218^T (=KCTC 12794^T=JCM 13606^T).

The genus *Roseobacter* was proposed by Shiba (1991) with descriptions of two species, *Roseobacter litoralis* and *Roseobacter denitrificans*. Subsequently, two other *Roseobacter* species, *Roseobacter algicola* (Lafay *et al.*, 1995) and *Roseobacter gallaeciensis* (Ruiz-Ponte *et al.*, 1998), were described. *Roseobacter algicola* was transferred to the genus *Ruegeria* as *Ruegeria algicola*, and has recently been reclassified within a novel genus and species, namely *Marinovum algicola* (Uchino *et al.*, 1998; Martens *et al.*, 2006). *Roseobacter gallaeciensis* has recently been reclassified within a novel genus, *Phaeobacter*, as *Phaeobacter gallaeciensis* (Martens *et al.*, 2006). The genus *Leisingera* was proposed by Schaefer *et al.* (2002) with a single species, *Leisingera methylohalidivorans*. Comparative 16S rRNA gene sequence analyses have shown that the genus *Leisingera* is phylogenetically closely related to the genera *Marinovum* and *Phaeobacter* (Schaefer *et al.*, 2002; Lee *et al.*, 2005; Martens *et al.*, 2006). Here we describe a bacterial strain, TF-218^T, which is phylogenetically closely related to the genera *Leisingera*, *Marinovum* and *Phaeobacter*. The aim of the present study was to determine the exact taxonomic position of strain TF-218^T by using a polyphasic characterization that included determination of the phenotypic and chemotaxonomic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Strain TF-218^T was isolated from a tidal flat sediment of the Yellow Sea, Korea, by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). The type strains of *P. gallaeciensis*, *Phaeobacter inhibens*, *L. methylohalidivorans* and *M. algicola* were used as reference strains: *P. gallaeciensis* DSM 17395^T, *P. inhibens* DSM 16374^T, *L. methylohalidivorans* DSM 14336^T and *M. algicola* DSM 10251^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The morphological, physiological and biochemical characteristics of strain TF-218^T were investigated using routine cultivation on MA at 37 °C. The cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was determined using transmission electron microscopy with cells from exponentially growing cultures. For transmission electron microscopic observations, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined, after being air-dried, with a Philips CM-20 transmission electron microscope. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated by using trypticase soy broth prepared according to the formula of the Difco medium, except that no NaCl was used. Growth at various NaCl concentrations (0.5 and 1.0–10.0%, w/v, using increments

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain TF-218^T is DQ981486.

of 1.0 %) was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4–50 °C) was measured on MA. Catalase and oxidase activities and the hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). The hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). The hydrolysis of aesculin, gelatin and urea and the reduction of nitrate were investigated as described previously (Lanyi, 1987), with the modification that artificial seawater was used for preparation of the media. The artificial seawater contained (l⁻¹ distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described previously (Bruns *et al.*, 2001). For *in vivo* pigment-absorption spectrum analysis, two strains were cultivated aerobically in the dark at 37 °C in liquid *Erythromicrobium/Roseococcus* medium (Yurkov *et al.*, 1994; DSMZ medium no. 767) with the modification that D-glucose was used instead of acetate. The cultures were washed twice by centrifugation using a MOPS buffer (MOPS/NaOH, 0.01 M; KCl, 0.1 M; MgCl₂, 0.001 M; pH 7.5) and disrupted by sonication with a Branson 450 sonifier. After the removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer. Susceptibility to antibiotics was tested on MA plates by using antibiotic discs at the following concentrations: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Acid production from carbohydrates was tested as described by Leifson (1963). The utilization of various substrates for growth was determined as described by Baumann & Baumann (1981), using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux).

Cell biomass of strain TF-218^T for DNA extraction and for isoprenoid quinone and polar lipid analyses was obtained from cultures grown in MB at 37 °C. Cell biomass of *P. gallaeciensis* DSM 17395^T and *P. inhibens* DSM 16374^T for polar lipid analyses was obtained from cultures grown in MB at 25 and 28 °C, respectively. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize the contamination of RNA. The 16S rRNA gene was amplified by using a PCR with two universal primers, 5'-GAGTTTG-ATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCC-AGCC-3', as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were analysed as described by

Komagata & Suzuki (1987), using reversed-phase HPLC. For cellular fatty acid analysis, cell mass of strain TF-218^T was harvested from MA plates after cultivation for 3 days at 37 °C; cell mass of *P. gallaeciensis* DSM 17395^T, *P. inhibens* DSM 16374^T, *L. methylohalidivorans* DSM 14336^T and *M. algicola* DSM 10251^T was harvested from MA plates after cultivation for 3 days at 25, 28, 25 and 28 °C, respectively. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by using two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The presence of phosphatidylcholine was identified by spraying Dragendorff's reagent (Sigma). The DNA G + C content was determined by using the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by using reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample: the highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain TF-218^T are given in the species description (see below) or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain TF-218^T determined in this study comprised 1419 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analysis showed that strain TF-218^T was most closely related phylogenetically to members of the genera *Phaeobacter*, *Leisingera* and *Marinovum* within the *Alphaproteobacteria*. In the phylogenetic tree obtained using the neighbour-joining algorithm, strain TF-218^T formed part of the clade comprising two *Phaeobacter* species, with a bootstrap resampling value of 58.4 %, and this cluster was part of the lineage of *L. methylohalidivorans*, with a bootstrap resampling value of 88.9 % (Fig. 1). These phylogenetic relationships were also maintained in trees generated with the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain TF-218^T exhibited 16S rRNA gene sequence similarity values of 97.5, 97.7, 97.3 and 96.3 % with respect to the type strains of *P. gallaeciensis*, *P. inhibens*, *L. methylohalidivorans* and *M. algicola*, respectively. The 16S rRNA gene sequence similarity values between strain TF-218^T and other species used in the phylogenetic analysis were below 95.2 %. The predominant isoprenoid quinone detected in strain TF-218^T was Q-10 (at a peak area ratio of approximately 94 %). The fatty acid profile of strain TF-218^T comprised major amounts of unsaturated, straight-chain and hydroxy fatty acids and 11-methyl C_{18:1}ω7c; major fatty acids (> 10 % of total fatty acids) were C_{18:1}ω7c (57.7 %) and 11-methyl-C_{18:1}ω7c

Table 1. Differential phenotypic characteristics of strain TF-218^T and members of the genera *Phaeobacter*, *Leisingera* and *Marinovum*

Taxa: 1, strain TF-218^T; 2, *P. gallaeciensis* (data from Ruiz-Ponte *et al.*, 1998; Martens *et al.*, 2006); 3, *P. inhibens* (Martens *et al.*, 2006); 4, *L. methylohalidivorans* (Schaefer *et al.*, 2002; Martens *et al.*, 2006); 5, *M. algicola* (Lafay *et al.*, 1995; Ruiz-Ponte *et al.*, 1998; Martens *et al.*, 2006). +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, not determined or not described; v, variable reaction. Data in parentheses are for type strains. All of the taxa shown are positive for motility, catalase and oxidase and all are negative for Gram-staining, hydrolysis of Tween 80 and the presence of bacteriochlorophyll *a*.

Characteristic	1	2	3	4	5
Colony colour	No pigment	Brown	Brown	No pigment	Beige/pinkish beige
Growth at:					
4 °C	+	–	+	+	–
37 °C	+	+	–	–	+
Nitrate reduction	+	–	–	–	–
β-Galactosidase	–	–	ND	ND	+
Hydrolysis of:					
Starch	–	–	–	+	w
Gelatin	–	–	–	–	+
Utilization of:					
L-Arabinose	–	ND	–	–	+*
D-Cellobiose	–	+	+	–	–
D-Fructose	–	+	+	–	+
Fucose	–	ND	–	–	–/+*
D-Galactose	–	+	+	–	–
D-Glucose	+	+	+	–	+
Glycerol	+	+	+	–	–
Maltose	–	+	+	–†	+
D-Mannose	–	+	+	–†	–
Sorbitol	–	+	+	–	–/+*
Sucrose	–	+	+	–†	+
Trehalose	–	+	+	–	+
D-Xylose	–	+	+	–†	–
L-Glutamate	–	+	+	–†	+
Leucine	+	+	+	–	–*
Serine	+	–	+	–	ND
Acetate	+	v(+)	+	–	v(+)
Butyrate	–	+	+	–	–
Citrate	+	–	+	–	+
Succinate	+	+	+	–	–
Susceptibility to:					
Penicillin G	+	–	+	+	+
Kanamycin	+	+	+†	w†	w
Gentamicin	+	+	+†	+†	w†
Streptomycin	+	+	+	+†	–
Chloramphenicol	+	v(+)	+	+†	+
Novobiocin	–	–†	–†	–†	–
Neomycin	+	+†	+†	w†	–
Polar lipids‡	PC, PG, PE, L, AL, PL	PC, PG, PE, L, AL, PL	PC, PG, PE, L, AL, PL	PG, PE, PL, L, AL	PC, PG, PE, L, AL, PL
DNA G + C content (mol%)	64.9	57.6–58.0	55.7	60.5	(60)

*Data from Labrenz *et al.* (1999) for the type strain.

†Data from this study.

‡AL, aminolipid; L, unidentified lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain TF-218^T and related taxa. Bootstrap percentages (of 1000 replications) greater than 50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with maximum-likelihood and maximum-parsimony algorithms. *Stappia stellulata* IAM 12621^T (GenBank accession no. D88525) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

(16.6%) (Table 2). This fatty acid profile was similar to those of the type strains of *P. gallaeciensis*, *P. inhibens*, *L. methylohalidivorans* and *M. algicola*, although there were

differences in the proportions of some fatty acids (Table 2). The major polar lipids detected in strain TF-218^T were phosphatidylcholine, phosphatidylglycerol, phosphatidyl-

Table 2. Cellular fatty acid compositions (%) of strain TF-218^T and members of the genera *Phaeobacter*, *Leisingera* and *Marinovum*

Strains: 1, strain TF-218^T; 2, *P. gallaeciensis* DSM 17395^T; 3, *P. inhibens* DSM 16374^T; 4, *L. methylohalidivorans* DSM 14336^T; 5, *M. algicola* DSM 10251^T. Data are from this study. Fatty acids that represented <0.5% in all strains were omitted. —, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain fatty acids					
C _{12:0}	1.2	0.4	0.4	0.6	—
C _{16:0}	8.6	6.3	5.2	3.2	2.5
C _{17:0}	0.6	0.2	0.2	0.1	0.3
C _{18:0}	2.4	1.3	2.0	0.5	2.5
Unsaturated fatty acids					
C _{17:1} ω _{8c}	—	—	—	0.2	0.8
C _{18:1} ω _{7c}	57.7	74.5	70.8	77.5	81.0
Hydroxy fatty acids					
C _{10:0} 3-OH	1.7	1.9	1.8	1.8	1.1
C _{12:0} 3-OH	2.6	1.6	2.0	2.1	1.2
C _{16:0} 2-OH	5.6	2.7	2.8	4.7	—
C _{18:1} 2-OH	—	—	—	0.6	—
11-Methyl C _{18:1} ω _{7c}	16.6	7.8	11.8	4.7	9.6
Summed feature 3*	0.5	0.3	0.2	0.5	0.2
Unknown fatty acid (equivalent chain-length, 11.799)	2.3	2.6	2.7	3.1	—

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1}ω_{7c} and/or iso-C_{15:0} 2-OH.

Fig. 2. TLC chromatograms of polar lipids of *P. gallaeciensis* DSM 17395^T (a), *P. inhibens* DSM 16374^T (b) and strain TF-218^T (c). Abbreviations: AL, aminolipid; L, unidentified lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid.

ethanolamine, two unidentified lipids and an aminolipid (Fig. 2). The DNA G+C content of strain TF-218^T was 64.9 mol%.

Strain TF-218^T is phylogenetically closely related to the two recognized *Phaeobacter* species. It was similar to the two *Phaeobacter* species in terms of chemotaxonomic properties. Strain TF-218^T differed from *L. methylohalidivorans* in containing phosphatidylcholine and in terms of nitrate reduction, growth at 37 °C and the utilization of several substrates (Martens *et al.*, 2006; Table 1). Strain TF-218^T can also be differentiated from *M. algicola* in view of the phylogenetic relationships among strain TF-218^T and the genera *Phaeobacter*, *Leisingera* and *Marinovum* (Fig. 1). Accordingly, it was concluded that strain TF-218^T is more closely affiliated to the members of the genus *Phaeobacter* than it is to those of the genera *Leisingera* and *Marinovum* or to a novel genus, on the basis of phylogenetic and chemotaxonomic relatedness (Martens *et al.*, 2006; Fig. 1). Strain TF-218^T exhibited mean DNA–DNA relatedness values of 15 and 18 % with respect to *P. gallaeciensis* DSM 17395^T and *P. inhibens* DSM 16374^T, respectively. These values indicate that strain TF-218^T represents a genomic species that is distinct from the two *Phaeobacter* species (Wayne *et al.*, 1987). Strain TF-218^T can be clearly distinguished from the two recognized *Phaeobacter* species on the basis of differences in phenotypic properties, including colony pigmentation, nitrate reduction, utilization of several substrates and DNA G+C content (Table 1). On the basis of the data presented, strain TF-218^T should be placed in the genus *Phaeobacter* as representing a novel species, for which the name *Phaeobacter daeponensis* sp. nov. is proposed.

Emended description of the genus *Phaeobacter* Martens *et al.* 2006

The description is as given by Martens *et al.* (2006), with the following amendments. On MA, colonies are brownish to dark brown or yellowish white; a diffusible brownish pigment is produced or not produced. Nitrate reduction is

variable. The DNA G+C contents are in the range 55.7–64.9 mol%.

Description of *Phaeobacter daeponensis* sp. nov.

Phaeobacter daeponensis (dae.po.nen'sis. N.L. masc. adj. *daeponensis* of Daepo, Korea, where the type strain was isolated).

Cells are Gram-negative and oval (0.4–0.9 × 0.7–2.0 µm). Motile by means of single polar flagella. Colonies on MA are circular, slightly convex, smooth, glistening, yellowish white in colour and 1.5–2.5 mm in diameter after 3 days incubation at 37 °C. Growth occurs at 4 and 42 °C, but not at 43 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Growth occurs in the presence of 8 % (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 9 % (w/v) NaCl. Anaerobic growth does not occur on MA, but occurs on MA supplemented with nitrate. Catalase- and oxidase-positive. Bacteriochlorophyll *a* is absent. Hypoxanthine and L-tyrosine are hydrolysed, but aesculin, casein, xanthine and Tweens 20, 40 and 60 are not. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. L-Malate and pyruvate are utilized as carbon and energy sources, but benzoate, formate and salicin are not. Acid is produced from D-ribose, but not from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, sucrose, D-trehalose, D-xylose, myo-inositol, D-mannitol or D-sorbitol. Susceptible to ampicillin, carbenicillin, cephalothin, oleandomycin and polymyxin B, but not to lincomycin or tetracycline. The

predominant ubiquinone is Q-10. The major fatty acids (>10% of total fatty acids) are C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two unidentified lipids and an aminolipid. The DNA G + C content of the type strain is 64.9 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, TF-218^T (=KCTC 12794^T=JCM 13606^T), was isolated from a tidal flat at Daepo Beach (Yellow Sea), Korea.

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References

- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes*, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestrinensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* 51, 1997–2006.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by nonsulfur purple bacteria. *J Cell Comp Physiol* 49, 25–68.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–207.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Schumann, P. & Hirsch, P. (1999). *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. *Int J Syst Bacteriol* 49, 137–147.
- Lafay, B., Ruimy, R., de Traubenberg, C. R., Breittmayer, V., Gauthier, M. J. & Christen, R. (1995). *Roseobacter algicola* sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate *Prorocentrum lima*. *Int J Syst Bacteriol* 45, 290–296.
- Lanyi, 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. (2005). 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.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* 85, 1183–1184.
- 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. 1998 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.
- 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.
- Ruiz-Ponte, C., Cilia, V., Lambert, C. & Nicolas, J. L. (1998). *Roseobacter gallaeciensis* sp. nov., a new marine bacterium isolated from rearings and collectors of the scallop *Pecten maximus*. *Int J Syst Bacteriol* 48, 537–542.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schaefer, J. K., Goodwin, K. D., McDonald, I. R., Murrell, J. C. & Oremland, R. S. (2002). *Leisingera methylohalidivorans* gen. nov., sp. nov., a marine methylotroph that grows on methyl bromide. *Int J Syst Evol Microbiol* 52, 851–859.
- Shiba, T. (1991). *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll *a*. *Syst Appl Microbiol* 14, 140–145.
- Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* 95, 1921–1942.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 25, 125–128.
- Uchino, Y., Hirata, A., Yokota, A. & Sugiyama, J. (1998). Reclassification of marine *Agrobacterium* species: proposals of *Stappia stellulata* gen. nov., comb. nov., *Stappia aggregata* sp. nov., nom. rev., *Ruegeria atlantica* gen. nov., comb. nov., *Ruegeria gelatinovora* comb. nov., *Ruegeria algicola* comb. nov., and *Ahrensia kielienensis* gen. nov., sp. nov., nom. rev. *J Gen Appl Microbiol* 44, 201–210.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* 46, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rRNA gene sequences. *Int J Syst Bacteriol* 48, 187–194.
- Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2003). *Psychrobacter jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* 53, 449–454.
- Yurkov, V., Stackebrandt, E., Holmes, A., Fuerst, J. A., Hugenholtz, P., Golecki, J., Gad'on, N., Gorlenko, V. M., Kompantseva, E. I. & Drews, G. (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* 44, 427–434.