Roseovarius halotolerans sp. nov., isolated from deep seawater

Yong-Sik Oh,¹ Hayung-Joon Lim,¹ In-Tae Cha,¹ Wan-Taek Im,²
Jae-Soo Yoo,³ Ui Gum Kang,⁴ Sung-Keun Rhee¹ and Dong-Hyun Roh¹

Correspondence

Dong-Hyun Roh

dhroh@chungbuk.ac.kr

¹Department of Microbiology and Biotechnology Research Institute, Chungbuk National University, Cheongju 361-763, Chungbuk, Republic of Korea

²Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea

³School of Electrical and Computer Engineering, Chungbuk National University, Cheongju 361-763, Chungbuk, Republic of Korea

⁴Yeongnam Agricultural Research Institute (NICS, RDA), Milyang 627-803, Republic of Korea

A Gram-reaction-negative, non-motile, aerobic bacterium, designated HJ50^T, was isolated from deep seawater of the East Sea, South Korea. Cells were ovoid to rod-shaped (0.5–0.8×1.3–3.0 µm), often with unequal ends, suggesting a budding mode of reproduction. The strain had an absolute requirement for sea salts and tolerated up to 20 % (w/v) sea salts. Propionate, DL-lactate, 2-ketogluconate, 3-hydroxybutyrate and rhamnose were used as growth substrates, but not mannitol, salicin, 4-hydroxybenzoate or acetate. The major fatty acid was summed feature 7 (C_{18:1}ω7c/ω9t/ω12t) and the DNA G+C content was 59.0±0.1 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that this strain was affiliated with the genus *Roseovarius*. Similarities between the 16S rRNA gene sequence of strain HJ50^T (1430 nt) and those of type strains of members of the genus *Roseovarius* were 94.1–96.3 %. DNA–DNA relatedness values between strain HJ50^T and the type strains of members of the genus *Roseovarius* were low (1.3–24.6 %). Physiological and biochemical differences support assignment of strain HJ50^T to the genus *Roseovarius* as a representative of a novel species. The name *Roseovarius halotolerans* sp. nov. is proposed, with HJ50^T (=KCTC 22224^T =LMG 24468^T) as the type strain.

The genus *Roseovarius* was first created by Labrenz *et al.* (1999) to accommodate species of the family *Rhodobacteraceae* that divided by budding and were Gram-negative, aerobic and peroxidase- and catalase-positive. At the time of writing, the genus comprised five recognized species, *Roseovarius tolerans* (Labrenz *et al.*, 1999), *Rva. nubinhibens* (González *et al.*, 2003), *Rva. crassostreae* (Boettcher *et al.*, 2005), *Rva. mucosus* (Biebl *et al.*, 2005) and *Rva. aestuarii* (Yoon *et al.*, 2008).

A bacterial strain, designated HJ50^T, was isolated from 200–500 m deep seawater of the East Sea of Korea using a dilution plating technique on marine agar 2216 plates (MA; Difco). Strain HJ50^T was grown routinely on marine broth 2216 (MB; Difco), MA and YTSS broth or agar. YTSS broth/agar contained (l⁻¹) 5 g yeast extract, 10 g tryptone,

20 g sea salts (Sigma) and (plates only) 15 g agar. Cultures were stored at –80 °C in MB supplemented with 25 % (v/v) glycerol. Reference strains *Rva. tolerans* DSM 11457^T, *Rva. nubinhibens* DSM 15170^T, *Rva. crassostreae* DSM 16950^T and *Rva. mucosus* DSM 17069^T were obtained from the DSMZ (Braunschweig, Germany). For analysis of lipoquinones and fatty acids, strain HJ50^T and the type strains of *Roseovarius* species were grown on MA at 30 °C for 3 days.

Morphology and cell size were determined by phase-contrast microscopy (Nikon 80i). Gram staining was performed with BD Gram stain kits according to the instructions of the manufacturer and by the non-staining method as described by Buck (1982). Flagellation was examined by transmission electron microscopy using cells grown on MA at 30 °C. A Formvar-coated grid was floated on a droplet of sample on Parafilm for 1 min to permit adsorption of the specimen. The grid was then transferred onto a drop of negative stain (1 % phosphotungstic acid) for 30 s, blotted with filter paper and then air-dried. The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HJ50^T is EU431217.

A TLC showing the polar lipids of strain HJ50^T is available as supplementary material with the online version of this paper.

sample was examined under a CM 20 (Philips) electron microscope. Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was assessed using oxidase reagent (bioMérieux) according to the manufacturer's instructions after cultivation for 2 days. Acid production from glucose, single-carbon-source assimilations and additional physiological characteristics were determined using the API 20NE and API ID32GN galleries according to the instructions of the manufacturer (bioMérieux) except that the culture was suspended in 3 % (w/v) sea salts. For reference, the type strains were included in this comparison and all tests were performed under the same conditions. Salt tolerance was measured on YTSS plates containing 0–25 % (w/v) sea salts (Sigma). Plates were incubated at 30 °C for 14 days. The temperature range for growth was determined on YTSS plates incubated for 14 days at 0, 4, 10, 15, 20, 25, 30, 35, 37, 40, 45 and 50 °C. Anaerobic growth was performed in Hungate anaerobic culture tubes containing YTSS broth at 25 and 35 °C with nitrogen gas. The medium was reduced with 1 mM sodium sulfide and resazurin (1 mg l⁻¹) was used to check anaerobic conditions.

Strain HJ50^T grew well on both MA and YTSS agar. Young colonies were small, white and uniformly round. Older colonies were faintly pink in the centre with a characteristic umbonate shape, circular, had entire margins and glistening surfaces and were 1.8–3.0 mm in diameter after 5 days of cultivation at 30 °C on MA. Cells were ovoid to rod-shaped and 0.5–0.8 × 1.3–3.0 µm in size, often with unequal ends (Fig. 1), suggesting a budding mode of division. Flagella in negatively stained preparations and real motility were not observed but cells occasionally showed a turning motion on the spot. Strain HJ50^T could grow in the presence of 0.5–20.0 % sea salts (Sigma) in YTSS medium, with maximum growth observed in 3–4 % sea salts. No growth occurred without addition of sea salts. For the test cultures described below, a concentration of 3 %

sea salts was used. Within the temperature range tested, the culture developed at 10–45 °C, with maximum growth at 35 °C. Growth was good at initial pH between 6.0 and 10.0, with optimum growth at pH 7.5. Strain HJ50^T was able to hydrolyse aesculin and gelatin and produce urease. Dissimilatory nitrate reduction to either nitrites or nitrogen was not found. Interestingly, strain HJ50^T could utilize propionate, 3-hydroxybutyrate and DL-lactate and produce acid from glucose, thus enabling it to be differentiated from other members of the genus *Roseovarius* (see Table 1 and the species description for further details). There was no indication of growth under anaerobic conditions for 1 month.

pufM encodes a key polypeptide in the bacterial photosynthetic reaction centre M subunit in various microorganisms (Zeng & Jiao, 2007; Allgaier *et al.*, 2003). To detect this gene in cells of strain HJ50^T, a pair of primers, *pufM*.557F and *pufM*.750R, was used to amplify the *pufM* gene (Achenbach *et al.*, 2001). PCR was run for 25 cycles with a DNA thermal cycler (MJ Mini; Bio-Rad). The following thermal profiles were used for PCR: predenaturation at 94 °C for 3 min and a repeated cycle of denaturation at 94 °C for 30 s, primer annealing at 60 °C for 30 s and extension at 72 °C for 45 s. The final cycle included an extension at 72 °C for 10 min. PCR products were separated by electrophoresis on an agarose gel (3 %, w/v) and stained with ethidium bromide. Band size was determined by comparison with a 100 bp ladder standard (Elpis Biotech). Production of bacteriochlorophyll *a* was determined by pigment absorption spectrum analysis (Lafay *et al.*, 1995). Strain HJ50^T was cultivated aerobically in the dark at 35 °C in MB. Pigments were extracted from the culture in the dark at 4 °C with 10 ml methanol. The presence of pigments was determined by visible spectrophotometry (300–900 nm) with a Biochrom Libra S21 spectrophotometer.

By using the PCR amplification approach, a specific 229 bp product, indicative of the presence of the bacterial photosynthetic reaction centre M subunit, was clearly amplified from strains of *Rva. tolerans* and *Rva. mucosus* (Allgaier *et al.*, 2003; Biebl *et al.*, 2005). In contrast, this specific PCR product was not amplified from strain HJ50^T and bacteriochlorophyll *a* was not detected.

For cellular fatty acid analysis, strain HJ50^T and type strains of some *Roseovarius* species were cultivated for 3 days on MA. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification system (MIDI). The fatty acids were then analysed by GC (Hewlett Packard 6890) using the Microbial Identification software package (Sasser, 1990).

There were some differences between the fatty acid methyl ester composition of HJ50^T and those of type strains of members of the genus *Roseovarius*. Strain HJ50^T and other members of the genus *Roseovarius* contained predominantly C_{18:1}ω7clω9tlω12t (summed feature 7), followed by

Fig. 1. Negatively stained transmission electron micrograph of cells of strain HJ50^T. Bar, 1 µm. The dividing cell shows unequal ends, suggesting a budding mode of division.

Table 1. Phenotypic characteristics of strain HJ50^T and related *Roseovarius* species

Strains: 1, strain HJ50^T; 2, *Rva. nubinihibens* DSM 15170^T; 3, *Rva. tolerans* DSM 11457^T; 4, *Rva. crassostreae* DSM 16950^T; 5, *Rva. mucosus* DSM 17069^T; 6, *Rva. aestuarii* KCTC 22174^T (data from Yoon *et al.*, 2008). All data are from this study except where indicated. All strains were positive for catalase and oxidase, but negative for Gram staining and citrate assimilation. +, Positive; w, weakly positive; v, variable; –, negative; NA, no data available. *pufM* encodes a key polypeptide in the bacterial photosynthetic reaction centre M subunit.

Characteristic	1	2	3	4	5	6
Cell morphology	Rod to ovoid	Short rod	Short rod	Rod to ovoid	Short rods	Rod to ovoid
Flagella	–	NA	– ^{a*}	+ ^b	– ^c	+
Motility	–	–	w	+	–	+
Growth temperature (°C)						
Range	10.0–45.0	10.0–40.0 ^d	3.0–43.5 ^a	19.0–40.0 ^b	20.0–40.0 ^c	10–37
Optimum	35.0	30.0 ^d	8.0–33.5 ^a	34.0 ^b	31.0 ^c	30.0
Growth in sea salts (%)						
Range	0.5–20.0	0.5–12.5	1.0–12.5	1.0–6.0	1.0–6.0	0.5–7.0
Optimum	3.0–4.0	2.0–5.0	4.0–5.0	2.0–4.0	1.0–3.0	NA
<i>pufM</i> gene	–	–	+	–	+	NA
Urease	+	–	+	+	–	–
β-Galactosidase	–	–	+	+	–	–
Acid production from glucose	+	–	–	–	–	–
Assimilation of:						
Salicin	–	–	+	+	+	–
Propionate	+	–	–	–	–	NA
2-Ketogluconate	+	–	+	+	+	NA
3-Hydroxybutyrate	+	–	–	–	–	NA
L-Arabinose	+	v	+	+	+	–
Maltose	+	v	v	+	v	–
D-Mannose	+	–	+	+	+	–
Rhamnose	w	–	w	w	+	NA
Acetate	–	–	w	w	+	+
DNA G + C content (mol%)	59.0 ± 0.1	66.0	62.2–63.8	59.0	60.9–62.9	58.6

*Data taken from: a, Labrenz *et al.* (1999); b, Boettcher *et al.* (2005); c, Biebl *et al.* (2005); d, González *et al.* (2003).

C_{16:0} (Table 2). In addition, C_{19:0} cyclo ω8c, an unknown fatty acid (ECL 18.079), C_{12:0} and C_{12:0} 3-OH also occurred in the novel isolate. Strain HJ50^T differed from the other *Roseovarius* species in that it lacked iso-C_{18:0}, which is found in *Rva. tolerans* and *Rva. mucosus*, and summed feature 3, which is found in *Rva. nubinihibens* and *Rva. crassostreae*.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by TLC on Merck silica gel 60 F₂₅₄ plates (20 × 20 cm, 0.5 cm thick) using a mixture of petroleum benzene and diethyl ether (85:15, v/v) as the developing solvent. Spots were scraped off and then analysed by HPLC as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). Polar lipids were extracted and examined by two-dimensional TLC according to the method of Tindall (1990) (see Supplementary Fig. S1 in IJSEM Online). The major isoprenoid quinone was ubiquinone-10, as observed in *Rva. tolerans* (Labrenz *et al.*, 1999) and *Rva. mucosus* (Biebl *et al.*, 2005). Strain HJ50^T contained diphosphatidylglycerol as well as phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine, as shown previously in *Rva. tolerans* (Labrenz

et al., 1999) and *Rva. mucosus* (Biebl *et al.*, 2005). In addition, cells of strain HJ50^T contained unidentified lipids. These results show clear differences in polar lipid composition compared with other members of the *Rhodobacteraceae*, such as *Marinovum algicola*, *Leisingera methylohalidivorans*, *Ruegeria atlantica*, *Roseobacter litoralis* and *Phaeobacter inhibens* (Labrenz *et al.*, 1998; Martens *et al.*, 2006). Taken together, the major fatty acids and polar lipid data indicate that strain HJ50^T belongs to the genus *Roseovarius*.

For phylogenetic analysis of strain HJ50^T, DNA was extracted using a commercial genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer set 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') and purified PCR products were sequenced by Solgent, Daejeon, South Korea (Park *et al.*, 2006). Full 16S rRNA gene sequences were compiled using SEQMAN software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997). Gaps

Table 2. Fatty acid compositions of strain HJ50^T and the type strains of *Roseovarius* species

Strains: 1, strain HJ50^T; 2, *Rva. nubinhibens* DSM 15170^T; 3, *Rva. tolerans* DSM 11457^T; 4, *Rva. crassostreae* DSM 16950^T; 5, *Rva. mucosus* DSM 17069^T; 6, *Rva. aestuarii* KCTC 22174^T (data from Yoon *et al.*, 2008). Data are from this study unless indicated. Values are percentages of total fatty acids; values less than 0.5 % are not shown. Equivalent chain lengths (ECL) are given for unknown fatty acids.

Fatty acids	1	2	3	4	5	6
Saturated						
C _{12:0}	5.85	6.94			6.11	5.10
C _{16:0}	10.35	12.46	14.29	10.46	8.12	16.20
C _{18:0}	2.90	1.10	2.32	1.58	1.20	1.00
Branched-chain						
iso-C _{18:0}			2.08		3.34	
Hydroxy						
C _{12:0} 2-OH					1.10	
C _{16:0} 2-OH	0.95		1.59	2.81	5.42	
C _{10:0} 3-OH	0.67			3.45	1.57	
C _{12:0} 3-OH	5.55	4.97	3.39			5.30
C _{12:1} 3-OH	2.73				3.80	
C _{16:0} 3-OH				0.63		
Cyclo						
C _{19:0} cyclo ω 8c	9.18	17.43			9.52	1.50
Summed features*						
3		1.13		9.32		
4				2.07	0.90	0.60
7	52.60	47.98	69.79	68.92	57.11	68.00
9					0.93	
Methyl ester						
C _{19:0} 10-methyl				0.75		
Unknown						
11.798	0.91	1.24			0.86	
15.283		6.75				
18.079	8.32		6.54			

*Summed features represent groups of two of three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3, C_{14:0} 3-OH/iso-C_{16:1} I (double bond position indicated by a capital letter is unknown); summed feature 4, C_{16:1} ω 7c/iso-C_{15:0} 2-OH; summed feature 7, C_{18:1} ω 7c/ ω 9t/ ω 12t; summed feature 9, unknown fatty acids ECL 18.846/18.858.

were edited using BIOEDIT (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1980). The phylogenetic tree was constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods in MEGA3 (Kumar *et al.*, 2004) or the PHYLIP package (Felsenstein, 1993) with bootstrap values based on 1000 replications (Felsenstein, 1985).

To determine the phylogenetic position of strain HJ50^T, its 16S rRNA gene sequence (1430 nt) was compared with those of type strains of members of the *Alphaproteobacteria* retrieved from GenBank. Phylogenetic analyses based on neighbour-joining, maximum-parsimony and maximum-likelihood analysis showed that strain HJ50^T could be placed within the evolutionary radiation enclosed by the genus *Roseovarius* (Fig. 2). Analysis of specific signature nucleotides at several positions in the secondary structure

of the 16S rRNA gene can be used to differentiate the *Rva. nubinhibens*/*Rva. crassostreae* cluster from the *Rva. tolerans*/*Rva. mucosus* cluster (Biebl *et al.*, 2005). The helical regions of strain HJ50^T at positions 240–242/284–286 and 113–115/312–314 were characterized as UAG/CUA and GUU/AAC, respectively, which were the same as in *Rva. crassostreae*, *Rva. nubinhibens* and *Rva. aestuarii*. However, nucleotides at positions 577–580/761–764 were GCAC/GUGC, which were the same as in *Rva. tolerans* and *Rva. mucosus*. 16S rRNA gene sequence similarities between strain HJ50^T and type strains of members of the genus *Roseovarius* ranged from 94.1 to 96.3 %.

For the measurement of the chromosomal DNA G + C content, the extracted genomic DNA was degraded enzymically into nucleosides and the G + C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. The DNA G + C content of HJ50^T was 59.0 ± 0.1 mol% (*n* = 3).

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequence comparisons showing the phylogenetic relationships of strain HJ50^T within the *Alphaproteobacteria*. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are given at nodes. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. The sequence of *Burkholderia cepacia* ATCC 25416^T was used as an outgroup and GenBank accession numbers are given in parentheses. Bar, 2 substitutions per 100 nucleotide positions.

DNA–DNA hybridization experiments were performed between HJ50^T and four type strains of members of the genus *Roseovarius* using photobiotin-labelled DNA probes and microdilution wells as described by Ezaki *et al.* (1989). 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 are quoted as DNA relatedness values.

DNA–DNA relatedness values of strain HJ50^T were 1.3, 3.3, 5.3 and 24.6% with *Rva. tolerans* DSM 11457^T, *Rva. crassostreae* DSM 16950^T, *Rva. nubinihibens* DSM 15170^T and *Rva. mucosus* DSM 17069^T, respectively.

Therefore, based on data from this polyphasic study, strain HJ50^T should be classified in the genus *Roseovarius* as a representative of a novel species, for which the name *Roseovarius halotolerans* sp. nov. is proposed.

Description of *Roseovarius halotolerans* sp. nov.

Roseovarius halotolerans (ha.lo.to'le.rans. Gr. n. *hals* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations).

Cells are Gram-reaction-negative, non-motile, aerobic and oval- to rod-shaped (0.5–0.8 µm in diameter and 1.3–3.0 µm long), frequently with unequal ends before and after division. Colonies are opaque, white to faintly pink in the centre, circular and glistening. Optimal growth temperature is 35 °C. Optimal pH for growth is 7.5.

Optimum growth occurs in the presence of 3–4% sea salts; tolerates up to 20% (w/v) sea salts. β-Galactosidase and arginine dihydrolase are not detected. Utilizes D-glucose, L-arabinose, L-rhamnose, sucrose, D-mannose, maltose, D-sorbitol, propionate, 2-ketogluconate, 3-hydroxybutyrate and DL-lactate, but not D-ribose, melibiose, L-fucose, D-mannitol, inositol, caprate, valerate, citrate, 4-hydroxybenzoate, itaconate, suberate, malonate, acetate, 5-ketogluconate, 3-hydroxybenzoate, phenylacetate, malate, adipate, salicin, glycogen, N-acetylglucosamine, L-histidine, L-alanine or L-serine. Other characteristics are given in Table 1. Does not have *pufM* or produce bacteriochlorophyll *a*. Phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and diphosphatidylglycerol are present. The principal fatty acid is summed feature 7 (C_{18:1}ω7c/ω9t/ω12t). The DNA G+C content of the type strain is 59.0 ± 0.1 mol%.

The type strain is HJ50^T (=KCTC 22224^T =LMG 24468^T), isolated from deep water of the East Sea, South Korea.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (The Regional Research Universities Program/Chungbuk BIT Research-Oriented University Consortium).

References

- Achenbach, L. A., Carey, J. & Madigan, M. T. (2001). Photosynthetic and phylogenetic primers for detection of anoxygenic phototrophs in natural environments. *Appl Environ Microbiol* **67**, 2922–2926.
- 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.
- Biebl, H., Allgaier, M., Lunsdorf, H., Pukall, R., Tindall, B. J. & Wagner-Döbler, I. (2005). *Roseovarius mucosus* sp. nov., a member of the *Roseobacter* clade with trace amounts of bacteriochlorophyll *a*. *Int J Syst Evol Microbiol* **55**, 2377–2383.
- Boettcher, K. J., Geaghan, K. K., Maloy, A. P. & Barber, B. J. (2005). *Roseovarius crassostreae* sp. nov., a member of the *Roseobacter* clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. *Int J Syst Evol Microbiol* **55**, 1531–1537.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol Rev* **45**, 316–354.
- 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.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

- Felsenstein, J. (1993).** PHYLIP (phylogeny inference package), version 3.5. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- González, J. M., Covert, J. S., Whitman, W. B., Henriksen, J. R., Mayer, F., Scharf, B., Schmitt, R., Buchan, A., Fuhrman, J. A. & other authors (2003).** *Silicibacter pomeroyi* sp. nov. and *Roseovarius nubinihibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. *Int J Syst Evol Microbiol* **53**, 1261–1269.
- Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- 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.
- Kluge, A. G. & Farris, J. S. (1969).** Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Braker, G. & Hirsch, P. (1998).** *Antarctobacter heliothermus* gen. nov., sp. nov., a budding bacterium from hypersaline and heliothermal Ekho Lake. *Int J Syst Bacteriol* **48**, 1363–1372.
- 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., Rausch de Traubenber, C., 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.
- 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.
- Park, J. W., Oh, Y. S., Lim, J. Y. & Roh, D. H. (2006).** Isolation and characterization of cold-adapted strains producing β -galactosidase. *J Microbiol* **44**, 396–402.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shin, Y. K., Lee, J.-S., Chun, C. O., Kim, H.-J. & Park, Y.-H. (1996).** Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J Microbiol Biotechnol* **6**, 68–69.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990).** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2008).** *Roseovarius aestuarii* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **58**, 1198–1202.
- Zeng, Y. & Jiao, N. (2007).** Source environment feature related phylogenetic distribution pattern of anoxygenic photosynthetic bacteria as revealed by *pufM* analysis. *J Microbiol* **45**, 205–212.