Erythrobacter aquimaris sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea

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Three Gram-negative, non-motile, non-spore-forming, slightly halophilic rods (strains SW-110^T, SW-116 and SW-140) were isolated from sea water of a tidal flat of the Yellow Sea in Korea and subjected to a polyphasic taxonomic study. The three isolates did not produce bacteriochlorophyll *a* and were characterized chemotaxonomically by having ubiquinone-10 as the predominant respiratory lipoquinone and C_{18:1}ω7c and C_{17:1}ω6c as the major fatty acids. The DNA G + C content of the three isolates was between 62.2 and 62.9 mol%. Strains SW-110^T, SW-116 and SW-140 showed no difference in their 16S rRNA gene sequences, and their mean level of DNA–DNA relatedness was 94.8%. Phylogenetic analyses based on 16S rRNA gene sequences showed that the three strains form a distinct phylogenetic lineage within the cluster comprising *Erythrobacter* species. Similarities between the 16S rRNA gene sequences of strains SW-110^T, SW-116 and SW-140 and the type strains of *Erythrobacter* species ranged from 98.4% (with *Erythrobacter longus*) to 97.7% (with *Erythrobacter flavus*). Levels of DNA–DNA relatedness between strains SW-110^T, SW-116 and SW-140 and the type strains of all recognized *Erythrobacter* species were in the range 5.3–12.7%. On the basis of polyphasic taxonomic data, strains SW-110^T, SW-116 and SW-140 were classified as a novel *Erythrobacter* species, for which the name *Erythrobacter aquimaris* sp. nov. is proposed. The type strain is SW-110^T (=KCCM 41818^T=JCM 12189^T).

The genus *Erythrobacter* was proposed by Shiba & Simidu (1982) with a single species, *Erythrobacter longus*. Phylogenetically, the genus *Erythrobacter* belongs to the α -subclass of the *Proteobacteria* (Anzai *et al.*, 2000). The first two *Erythrobacter* species recognized, *E. longus* and *Erythrobacter litoralis* (Yurkov *et al.*, 1994), were red or orange in colour and contained bacteriochlorophyll (BChl) *a* and carotenoids (Shiba & Simidu, 1982; Yurkov *et al.*, 1994). Recently, two yellow-pigmented *Erythrobacter* species, lacking BChl *a*, have been described, *Erythrobacter citreus* and *Erythrobacter flavus* (Denner *et al.*, 2002; Yoon *et al.*, 2003). We report here on the detailed taxonomic characterization of three slightly halophilic, orange-pigmented, *Erythrobacter*-like bacterial strains, SW-110^T, SW-116 and SW-140, which were isolated from sea water of a tidal flat of the Yellow Sea in Korea.

Strains SW-110^T, SW-116 and SW-140 were isolated using the dilution plating technique on marine agar 2216 (MA;

Difco). *E. longus* DSM 6997^T, *E. litoralis* DSM 8509^T and *E. citreus* DSM 14432^T were obtained from the DSMZ. *E. flavus* KCCM 41642^T was obtained from the study of Yoon *et al.* (2003). Cell biomass of strains SW-110^T, SW-116 and SW-140 for respiratory lipoquinone analysis and for DNA extraction was obtained from cultures in marine broth 2216 (MB; Difco) at 30 °C. For fatty acid methyl ester (FAME) analysis, cell mass of strains SW-110^T, SW-116 and SW-140 was obtained from agar plates after cultivation for 5 days at 30 °C on MA. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). Presence or absence of flagella was examined using TEM with cells from exponentially growing cultures. The cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after air drying with a Philips CM-20 transmission electron microscope. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber with MA that had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated in trypticase soy broth (Difco) without NaCl. Growth at various NaCl concentrations was investigated in MB. Growth at 4–45 °C was measured on MA. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80

Abbreviations: BChl, bacteriochlorophyll; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SW-110^T, SW-116 and SW-140 are AY461441, AY461442 and AY461443, respectively.

were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA with substrate concentrations as described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were studied as described by Lanyi (1987) with a modification that artificial sea water was used for preparation of media. The artificial sea water contained (per litre of distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Levring, 1946). H_2S production was tested as described by Bruns *et al.* (2001). For *in vivo* pigment-absorption spectrum analysis, three strains were cultivated aerobically in the dark at 30 °C in MB, PYGV medium (Fuerst *et al.*, 1993; DSMZ medium no. 621) and *Erythromicrobium/Roseococcus* medium (Yurkov *et al.*, 1994; DSMZ medium no. 767). *E. longus* DSM 6997^T and *E. litoralis* DSM 8509^T were used as positive controls for spectrum analysis. Cultures were washed twice by centrifugation using a MOPS buffer (MOPS/NaOH, 0.01 M; KCl, 0.1 M; MgCl_2 , 0.001 M; pH 7.5) and disrupted by sonication with a Branson Sonifier 450. After removal of cell debris, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer. Susceptibility to antibiotics was detected on MA plates by using antibiotic

discs (Advantec) (concentrations are given in Table 1). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994).

Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For quantitative analysis of cellular fatty acid composition, a loop of cell mass was harvested and FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that ribonuclease T1 was used together with ribonuclease A. The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. 16S rRNA genes were amplified by PCR using two universal primers as described by Yoon *et al.* (1998). Sequencing of the amplified 16S rRNA gene was performed as described by Yoon *et al.* (2003). Alignment of sequences was carried out with CLUSTAL W software (Thompson *et al.*, 1994). Gaps at the

Table 1. Differential phenotypic characteristics of *Erythrobacter aquimaris* sp. nov., *E. longus*, *E. litoralis*, *E. citreus* and *E. flavus*

Species: 1, *E. aquimaris* sp. nov.; 2, *E. longus* (data from Shiba & Simidu, 1982; Yurkov *et al.*, 1994); 3, *E. litoralis* (Shiba & Simidu, 1982; Yurkov *et al.*, 1994); 4, *E. citreus* (Denner *et al.*, 2002; Vybiral *et al.*, 1999); 5, *E. flavus* (Yoon *et al.*, 2003). +, Positive; −, negative; w+, weakly positive; ND, not determined; v, variable. Data in parentheses are for the type strain. All species are rod-shaped and positive for catalase and oxidase, susceptibility to chloramphenicol (100 µg per disc; 30 µg per disc for *E. citreus*) and utilization of acetate. All species are Gram-negative, non-sporulating and resistant to 100 U polymyxin B per disc (300 U per disc for *E. citreus*).

Characteristic	1	2	3	4	5
Colour of colonies	Orange	Orange	Red or orange	Yellow	Yellow
Motility	−	+	+	−	+
Presence of BChl <i>a</i>	−	+	+	−	−
Nitrate reduction	−	v (+)	−	+	−
Hydrolysis of:					
Gelatin	−	+	−	ND	−
Starch	v (+)	−	−	(−)	+
Utilization of:					
Glucose	+	+	+	v (w+)	−
Fructose	−	ND	+	(−)	−
Citrate	−	−	−	v (w+)	−
Pyruvate	+	+	+	−	+
Glutamate	−	+	+	ND	−
Succinate	+	−	+	ND	−
Lactate	−	−	+	−	v
Malate	+	−	−	−	−
Susceptibility to:					
Penicillin (20 U; 10 U for <i>E. citreus</i>)	−	−	−	v	−
Streptomycin (50 µg; 10 µg for <i>E. citreus</i>)	−	−	−	v	−
Optimum growth temperature (°C)	30–37	25–30	25–30	ND	30–37
DNA G+C content (mol%)	62.2–62.9	60–64 (60.7)	67	62.0–62.4	64.0–64.1

5' and 3' ends of the alignment were omitted from further analysis. Phylogenetic trees were inferred by using three tree-making algorithms; the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods were performed using the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated with the algorithm of Jukes & Cantor (1969) with the DNADIST program. Stability of relationships was assessed by a bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed using five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the remaining three values were used to calculate relatedness values. The DNA relatedness values quoted are the mean of the three values.

Strains SW-110^T, SW-116 and SW-140 were similar in most phenotypic characteristics. After 5 days of cultivation at 30 °C on MA, cells were rods, approximately 0.6–0.9 µm wide by 2.0–4.0 µm long. No flagella were located by TEM. Colonies were 1.0–2.0 mm in diameter, smooth, glistening, circular, convex and orange-pigmented after 5 days of incubation at 30 °C on MA. Strain SW-110^T was slightly darker pigmented than strains SW-116 and SW-140. The strains grew optimally at 30–37 °C; growth was observed at 10 but not at 4 °C. The maximum growth temperature of strains SW-110^T and SW-116 was 40 °C, and that of strain SW-140 was 41 °C. Optimal pH for growth was between 6.5 and 7.5; no growth was observed at pH 5.0. All strains were slightly halophilic; optimal growth occurred at 2–3 % (w/v) NaCl, and there was no growth in the absence of NaCl. Strains SW-110^T and SW-116 did not tolerate > 10 % (w/v) NaCl, and strain SW-140 did not tolerate > 9 % (w/v) NaCl. The strains did not grow on MA under anaerobic conditions. Starch was hydrolysed by strains SW-110^T and SW-140, but not by strain SW-116. The sonicated cell extracts of strains SW-110^T, SW-116 and SW-140 did not display absorption maxima characteristic for BChl *a* in three different media. Other physiological and biochemical characteristics are shown in Table 1 or are given in the species description below. Phenotypic characteristics that differentiate strains SW-110^T, SW-116 and SW-140 from *Erythrobacter* species are summarized in Table 1.

Strains SW-110^T, SW-116 and SW-140 had ubiquinone with ten isoprene units (Q-10) as the predominant respiratory lipoquinone at a peak ratio of approximately 90–93 %. The fatty acid profiles of the orange-pigmented isolates were characterized by a common core of unsaturated fatty acids C_{18:1}ω7c and C_{17:1}ω6c and straight-chain fatty acid C_{16:0} in varying amounts; hydroxy fatty acids were also present. The general fatty acid profile was similar between the isolates and *Erythrobacter* species. However, strains SW-110^T,

SW-116 and SW-140 differed from *Erythrobacter* species in the total number of fatty acids and relative abundance of shared components (Table 2). The DNA G + C contents of strains SW-110^T, SW-116 and SW-140 were 62.2, 62.9 and 62.7 mol%, respectively.

The 16S rRNA gene sequences of the three strains determined in this study each comprised 1444 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Strains SW-110^T, SW-116 and SW-140 showed no difference in their 16S rRNA gene sequences. Comparative 16S rRNA gene sequence analyses showed that the three strains are phylogenetically closely related to *Erythrobacter* species of the *Sphingomonadaceae*. Similarities between the 16S rRNA gene sequences of strains SW-110^T, SW-116 and SW-140 with *Erythrobacter* species ranged from 98.4 % (with *E. longus*) to 97.7 % (with *E. flavus*). Fig. 1 shows a

Table 2. Percentage cellular fatty acid composition of strains SW-110^T, SW-116 and SW-140 (*E. aquimaris* sp. nov.) and type strains of *Erythrobacter* species

Strains: 1, *E. aquimaris* SW-110^T; 2, *E. aquimaris* SW-116; 3, *E. aquimaris* SW-140; 4, *E. longus* DSM 6997^T (data from Yoon *et al.*, 2003); 5, *E. litoralis* DSM 8509^T (Yoon *et al.*, 2003); 6, *E. flavus* KCCM 41642^T (Yoon *et al.*, 2003); 7, *E. citreus* RE35F/1^T (Denner *et al.*, 2002). –, Not detected. Fatty acids representing less than 0.5 % in all strains were omitted.

Fatty acid	1	2	3	4	5	6	7
Straight-chain:							
C _{15:0}	2.7	1.6	2.4	2.8	0.5	3.0	0.3
C _{16:0}	10.3	5.5	7.5	11.4	2.7	6.4	8.2
C _{17:0}	3.2	1.2	1.4	10.1	–	7.3	–
C _{18:0}	0.4	–	–	1.0	–	0.5	–
Branched:							
Anteiso-C _{15:0}	–	–	–	–	–	–	0.6
Anteiso-C _{16:0}	–	–	–	–	–	–	0.7
Anteiso-C _{17:0}	–	–	–	–	–	–	1.2
Unsaturated:							
C _{16:1} ω5c	1.3	1.1	1.4	–	0.7	–	3.6
C _{17:1} ω6c	27.6	25.0	23.6	3.9	28.5	6.2	5.6
C _{17:1} ω8c	2.5	4.5	2.3	4.8	4.3	8.1	0.7
C _{18:1} ω5c	1.5	1.3	1.3	–	2.2	–	4.2
C _{18:1} ω7c	26.8	32.0	30.7	50.9	46.9	46.2	49.3
11-Methyl-C _{18:1} ω7c	4.4	8.3	6.6	–	3.7	5.5	4.4
Hydroxy:							
C _{14:0} 2-OH	3.9	5.2	6.3	5.0	2.9	0.9	0.9
C _{15:0} 2-OH	7.4	6.7	6.8	8.6	2.6	9.5	–
C _{16:0} 2-OH	2.2	2.0	1.9	1.5	1.5	1.4	–
C _{18:1} 2-OH	0.5	0.9	0.6	–	–	0.3	2.0
Iso-C _{16:0} 3-OH	–	–	–	–	1.1	–	1.0
Summed feature 3*	4.6	4.8	6.0	–	2.3	4.6	14.9

*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 iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence data showing the phylogenetic position of strains SW-110^T, SW-116 and SW-140 within the *Sphingomonadaceae*. Bootstrap values (1000 replications) are shown as percentages at each node only if they are 50% or greater. Bar, 0.01 substitutions per nucleotide position. *Rhodospirillum rubrum* ATCC 11170^T was used as the outgroup.

dendrogram of estimated phylogenetic relationships, inferred from comparisons of 16S rRNA gene sequences between the three isolates and all recognized *Erythrobacter* species and with some other species of the α -*Proteobacteria*. A 16S rRNA gene sequence similarity of >97% clearly indicates that the three strains are closely related phylogenetically to *Erythrobacter* species but does not allow us to evaluate confidently whether the three isolates should be considered to represent a separate species. Sequence similarities to all other species of the *Sphingomonadaceae* included in the phylogenetic analysis were lower than 96.6% (Fig. 1). Levels of DNA–DNA relatedness observed between strains SW-110^T, SW-116 and SW-140 were 88.5–102.1%, suggesting that the strains belong to the same species (Wayne *et al.*, 1987). Levels of DNA–DNA relatedness to reference type strains of recognized *Erythrobacter* species were between 5.3 and 12.7%. This genomic evidence demonstrates that the three strains represent a separate species within the genus *Erythrobacter* (Wayne *et al.*, 1987).

16S rRNA gene sequence comparison showed that strains SW-110^T, SW-116 and SW-140 exhibited the closest phylogenetic affinities to *Erythrobacter* species (Fig. 1). The relationship between the cluster comprising the three strains and *Erythrobacter* species and the cluster comprising the genera *Porphyrobacter* and *Erythromicrobium* was supported by a high bootstrap resampling value of 99.9% (Fig. 1). The results obtained in the morphological and chemotaxonomic analyses are consistent with the result of the monothetic classification by 16S rRNA gene sequence analysis, although clear chemotaxonomic differentiation is not necessarily possible between the genus *Erythrobacter* and the two phylogenetically related genera *Porphyrobacter* and *Erythromicrobium* (Fuerst *et al.*, 1993; Rainey *et al.*, 2003; Yurkov *et al.*, 1994). Strains SW-110^T, SW-116 and SW-140 show nearly identical phenotypic characteristics (Table 1). The three strains are also phylogenetically and genetically similar. In view of these combined data, strains SW-110^T, SW-116 and SW-140 should be considered as

members of the same species. There are some physiological differences between strains SW-110^T, SW-116 and SW-140 and other *Erythrobacter* species, including the ability to utilize some substrates (Table 1). Therefore, on the basis of phenotypic, chemotaxonomic and phylogenetic data and genomic distinctiveness, strains SW-110^T, SW-116 and SW-140 should be placed in the genus *Erythrobacter* as a novel species, for which the name *Erythrobacter aquimaris* sp. nov. is proposed.

Description of *Erythrobacter aquimaris* sp. nov.

Erythrobacter aquimaris (a. qui. ma' ris. L. n. *aqua* water; L. gen. n. *maris* of the sea; N.L. gen. n. *aquimaris* of the water of the sea).

Cells are rod-shaped (0.6–0.9 × 2.0–4.0 µm), non-spore-forming and non-motile. Colonies on MA (5 days, 30 °C) are 1.0–2.0 mm in diameter, smooth, glistening, circular, convex and orange-pigmented. Growth occurs at 10 and 40 °C, but not at 4 or above 42 °C. Optimal pH for growth is 6.5–7.5; growth is not observed at pH 5.0. Optimal growth occurs in the presence of 2–3% (w/v) NaCl; growth does not occur in the absence of NaCl or in the presence of more than 10% NaCl. Growth does not occur under anaerobic conditions on MA. Urease-negative. Aesculin, Tweens 20, 40, 60 and 80 and tyrosine are hydrolysed. Casein, hypoxanthine and xanthine are not hydrolysed. H₂S is not produced. Acid is not produced from adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, stachyose, sucrose, D-trehalose or D-xylose. Butyrate is utilized; formate, methanol, ethanol and benzoate are not. The predominant respiratory lipoquinone is Q-10. The predominant fatty acids are C_{18:1}ω7c and C_{17:1}ω6c. The DNA G+C content is 62.2–62.9 mol% (62.2% for the type strain) (determined by HPLC).

The type strain, SW-110^T (= KCCM 41818^T = JCM 12189^T),

and reference strains SW-116 (=KCCM 41819=JCM 12190) and SW-140 (=KCCM 41820=JCM 12191) were isolated from sea water of a tidal flat of the Yellow Sea in Korea.

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References

- Anzai, Y., Kim, H., Park, J.-Y., Wakabayashi, H. & Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50**, 1563–1589.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Denner, E. B. M., Vybiral, D., Koblížek, M., Kämpfer, P., Busse, H.-J. & Velimirov, B. (2002). *Erythrobacter citreus* sp. nov., a yellow-pigmented bacterium that lacks bacteriochlorophyll *a*, isolated from the western Mediterranean Sea. *Int J Syst Evol Microbiol* **52**, 1655–1661.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution 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. (1993). PHYLIP: Phylogenetic Inference Package, version 3.5. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Fuerst, J. A., Hawkins, J. A., Holmes, A., Sly, L. I., Moore, C. J. & Stackebrandt, E. (1993). *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. *Int J Syst Bacteriol* **43**, 125–134.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Levring, T. (1946). Some culture experiments with *Ulva* and artificial seawater. *K Fysiogr Sallsk Lund Forh* **16**, 45–56.
- Rainey, F. A., Silva, J., Nobre, M. F., Silva, M. T. & da Costa, M. S. (2003). *Porphyrobacter cryptus* sp. nov., a novel slightly thermophilic, aerobic, bacteriochlorophyll *a*-containing species. *Int J Syst Evol Microbiol* **53**, 35–41.
- 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*. Newark, DE: MIDI Inc.
- Shiba, T. & Simidu, U. (1982). *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll *a*. *Int J Syst Bacteriol* **32**, 211–217.
- 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.
- Vybiral, D., Denner, E. B. M., Haller, C. M., Busse, H.-J., Witte, A., Höfle, M. G. & Velimirov, B. (1999). Polyphasic classification of 0.2 µm filterable bacteria from the Western Mediterranean Sea. *Syst Appl Microbiol* **22**, 635–646.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). 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 intra-specific phylogenetic analysis of the genus *Nocardioide*s and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kim, H., Kim, I.-G., Kang, K. H. & Park, Y.-H. (2003). *Erythrobacter flavus* sp. nov., a slight halophile from the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 1169–1174.
- Yurkov, V., Stackebrandt, E., Holmes, A. & 7 other authors (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.