Muricauda flavescens sp. nov. and *Muricauda aquimarina* sp. nov., isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea, and emended description of the genus *Muricauda*

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Four Gram-negative, non-spore-forming, slightly halophilic rods (strains SW-62^T, SW-74, SW-63^T and SW-72) with appendages were isolated from a salt lake near Hwajinpo Beach on the East Sea in Korea, and subjected to a polyphasic taxonomic analysis. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains SW-62^T, SW-74, SW-63^T and SW-72 formed a coherent cluster with *Muricauda ruestringensis*. Strains SW-62^T and SW-74 had the same 16S rRNA gene sequence, as did strains SW-63^T and SW-72. The level of 16S rRNA gene sequence similarity between strains SW-62^T and SW-63^T was 97.0%. Strains SW-62^T and SW-63^T exhibited 16S rRNA gene similarity levels of 96.5 and 98.3%, respectively, with respect to *M. ruestringensis* DSM 13258^T. The predominant isoprenoid quinone found in the four isolates and *M. ruestringensis* DSM 13258^T was MK-6. The four strains contained iso-C_{17:0} 3-OH, iso-C_{15:1} and iso-C_{15:0} as the major fatty acids. Their DNA G + C contents were 44.1–45.4 mol%. The levels of DNA–DNA relatedness indicated that strains SW-62^T and SW-74 and strains SW-63^T and SW-72 were members of two species that were different from *M. ruestringensis*. On the basis of phenotypic and phylogenetic data and genomic distinctiveness, strains SW-62^T and SW-74 and strains SW-63^T and SW-72 were placed in the genus *Muricauda* as two distinct novel species, for which the names *Muricauda flavescens* sp. nov. (type strain, SW-62^T = KCCM 41645^T = JCM 11812^T) and *Muricauda aquimarina* sp. nov. (type strain, SW-63^T = KCCM 41646^T = JCM 11811^T), respectively, are proposed.

In the course of screening for taxonomically useful micro-organisms present in a salt lake near Hwajinpo Beach of the East Sea in Korea, many halophilic or halotolerant bacteria have been isolated and characterized taxonomically. Of these isolates, four strains (SW-62^T, SW-74, SW-63^T and SW-72) were focused on in this study because they were found to have an interesting morphological property in that they possessed an appendage. The results of 16S rRNA gene

sequence comparisons indicated that the four strains are phylogenetically related to the genus *Muricauda*. The genus *Muricauda* was proposed by Bruns *et al.* (2001) with the single species *Muricauda ruestringensis*. Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Muricauda* is a member of the family *Flavobacteriaceae* within the *Cytophaga–Flavobacterium–Bacteroides* (CFB) complex (Bruns *et al.*, 2001). At the time of writing, there is only one *Muricauda* species with a validly published name (Bruns *et al.*, 2001). The aim of the present study was to investigate the possibility that strains SW-62^T, SW-74, SW-63^T and SW-72 were novel species of the genus *Muricauda*. In this work, we describe the morphological, phenotypic, phylogenetic and genomic characteristics of strains SW-62^T, SW-74, SW-63^T and SW-72.

Strains SW-62^T, SW-74, SW-63^T and SW-72 were isolated by using the standard dilution plating technique on marine agar 2216 (MA; Difco). For isoprenoid quinone analysis

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Abbreviation: CFB, *Cytophaga–Flavobacterium–Bacteroides*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SW-62^T, SW-74, SW-63^T and SW-72 are AY445073, AY445074, AY445075 and AY445076, respectively.

Transmission electron micrographs showing the appendages found on most cells of strains SW-62^T, SW-74, SW-63^T and SW-72 are available as supplementary material in IJSEM Online.

and DNA extraction, cell biomass of the four isolates and *M. ruestringensis* DSM 13258^T was obtained after cultivation in marine broth 2216 (MB; Difco) for 2 days at 30 °C. For fatty acid methyl ester analysis, cell mass of the four isolates and *M. ruestringensis* DSM 13258^T was obtained from agar plates after incubation for 3 days at 30 °C on MA. *M. ruestringensis* DSM 13258^T was obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Cell morphology was examined by using light microscopy (E600 apparatus; Nikon) and transmission electron microscopy (CM-20 apparatus; Philips). Gram reactions were determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated in trypticase/soy broth (Difco) lacking NaCl. Growth at various NaCl concentrations was investigated for 14 days at 30 °C in MB. Growth at various temperatures was measured on MA at 4–50 °C. Catalase and oxidase activities and hydrolysis of casein and starch were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin, urea and gelatin and nitrate reduction were studied as described previously (Lanyi, 1987) with the modification that artificial sea water was used. The artificial sea water contained the following (per litre of 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). Hydrolysis of Tweens 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965) or tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA plates with the substrate concentrations described previously (Cowan & Steel, 1965). Hydrolysis of birchwood xylan (Sigma) was tested on solid marine salts basal medium (Baumann & Baumann, 1981) supplemented with 0.5 % (w/v) xylan as the sole carbon source. H₂S production was tested as described previously (Bruns *et al.*, 2001). 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).

Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass was harvested and fatty acid methyl esters 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 previously (Yoon *et al.*, 1996), with the exception that ribonuclease T1 was used 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. The 16S rRNA gene was amplified by a PCR using two universal primers, 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). 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 using five replications for each sample. Of the values obtained, the highest and lowest for each sample were excluded. DNA–DNA relatedness values are the means of the remaining three values.

The colonies of strains SW-62^T SW-74, SW-63^T and SW-72 were golden yellow in colour on MA, whereas those of *M. ruestringensis* DSM 13258^T were yellow on MA. Acid production from D-melezitose was observed only for strains SW-62^T and SW-74. Other morphological, cultural, physiological and biochemical characteristics are shown in Table 1 or are given in the species descriptions (see below). Strains SW-62^T SW-74, SW-63^T and SW-72 contained unsaturated menaquinone with six isoprene units (MK-6) as the predominant isoprenoid quinone. In this study, the predominant isoprenoid quinone of *M. ruestringensis* DSM 13258^T was also analysed and found to be MK-6. Strains SW-62^T SW-74, SW-63^T and SW-72 had cellular fatty acid profiles containing large amounts of straight-chain, branched and hydroxy fatty acids; the major fatty acids were iso-C_{17:0} 3-OH, iso-C_{15:1} and iso-C_{15:0} (Table 2). These fatty acid profiles were similar to that of *M. ruestringensis* DSM 13258^T (analysed in this study; see Table 2). For some fatty acids, particularly C_{15:0}, the proportions present in *M. ruestringensis* DSM 13258^T differed between this study and the study of Bruns *et al.* (2001), perhaps because of differences in cultivation conditions. The DNA G + C contents of strains SW-62^T, SW-74, SW-63^T and SW-72 were 45.2, 45.4, 44.1 and 44.2 mol%, respectively.

The 16S rRNA gene sequences of strains SW-62^T, SW-74, SW-63^T and SW-72 determined in this study each comprised 1481 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA sequence. The results of 16S rRNA gene sequence comparisons showed that the sequences of strains SW-62^T and SW-74 were identical to each other; so also were strains SW-63^T and SW-72. The level of 16S rRNA gene sequence similarity between strain SW-62^T and strain SW-63^T was 97.0 %. Phylogenetic trees based on 16S rRNA gene sequences demonstrated that the four strains form the radiation of the cluster with *M. ruestringensis*. In the phylogenetic tree based on the neighbour-joining algorithm (Yoon *et al.*, 2003), strains SW-63^T and SW-72 formed a coherent cluster with *M. ruestringensis* DSM 13258^T at a bootstrap resampling value of 96.4 %; this cluster joined strains SW-62^T and SW-74 at a bootstrap resampling value of 100 % (Fig. 1). Strains SW-62^T and SW-74 and strains SW-63^T and SW-72 exhibited 16S rRNA gene sequence similarity levels of 96.5 and 98.3 %, respectively.

Table 1. Phenotypic characteristics of *Muricauda* species

1, *M. flavescens*; 2, *M. aquimarina*; 3, *M. ruestringensis*. All species are rod-shaped. The following tests are positive for all species: oxidase (data from this study); hydrolysis of aesculin (this study), tyrosine (this study), Tweens 20, 40, 60 and 80 (all this study); growth on fructose, succinate (this study), raffinose, cellobiose, sucrose, lactose and mannose; and acid production from D-fructose, melibiose, D-glucose, D-mannose, D-cellobiose, lactose, sucrose, maltose, D-trehalose and D-raffinose. The following tests are negative for all species: Gram stain; spore formation; growth in 0% NaCl and at 4 °C; nitrate reduction; H₂S production; hydrolysis of casein (this study), gelatin, hypoxanthine (this study), starch, urea (this study) and xanthine (this study); growth on acetate, pyruvate, glutamate (this study), butyrate (this study), citrate (this study), lactate (this study), formate (this study), methanol (this study), ethanol, benzoate (this study), serine, mannitol and hexadecane; and acid production from D-sorbitol, *myo*-inositol, D-ribose, D-mannitol, adonitol and L-rhamnose. Symbols: +, positive; –, negative.

Characteristic	1	2	3*
Catalase	+	+	–†
Facultative anaerobe	–	–	+
Optimal temp. for growth (°C)	30–37	30–37	20–30
Growth on:			
Glucose	+	+	–
Malate	–	–	+
Alanine	–	–	+
Arginine	–	–	+
Acid production from:‡			
D-Xylose	–	–	+
L-Arabinose	–	–	+
D-Melezitose	+	–	+
D-Galactose	–	–	+
DNA G+C content (mol%)	45.2–45.4	44.1–44.2	41

*Data from Bruns *et al.* (2001).

†Positive in this study.

‡Data for *M. ruestringensis* from this study.

respectively, with respect to the type strain of *M. ruestringensis* (Fig. 1). The four isolates exhibited levels of 16S rRNA gene sequence similarity of less than 91.6% (between strains SW-63^T and SW-72 and *Zobellia uliginosa* ATCC 14397^T) to other species used in the phylogenetic analysis (Fig. 1). Levels of DNA–DNA relatedness between strains SW-62^T and SW-74 and between strains SW-63^T and SW-72 were 90.7 and 93.6%, respectively. The mean level of DNA–DNA relatedness between strains SW-62^T and SW-74 and strains SW-63^T and SW-72 was 9.2%. Strains SW-62^T and SW-63^T exhibited DNA–DNA relatedness levels of 4.9 and 7.0%, respectively, with respect to *M. ruestringensis* DSM 13258^T.

Preliminary studies on micro-organisms present in a salt lake near Hwajinpo Beach on the East Sea in Korea have

Table 2. Cellular fatty acid composition (%) of strains SW-62^T, SW-74, SW-63^T and SW-72 and *M. ruestringensis* DSM 13258^T on MA

1, SW-62^T; 2, SW-74; 3, SW-63^T; 4, SW-72; 5, DSM 13258^T. Fatty acids representing less than 0.5% in all strains were omitted. –, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain fatty acids					
C _{15:0}	12.4	11.9	5.9	5.1	13.2
C _{16:0}	0.6	0.6	0.4	0.2	0.4
Branched fatty acids					
iso-C _{14:0}	–	–	0.6	0.7	–
iso-C _{15:0}	16.4	15.7	23.7	23.8	14.7
iso-C _{15:1} G*	19.9	19.5	21.6	21.1	20.5
anteiso-C _{15:0}	2.1	2.0	2.0	2.5	1.1
iso-C _{16:0}	0.6	0.6	0.5	0.5	–
iso-C _{17:1} ω9c	1.3	1.3	1.5	1.4	1.4
Unsaturated fatty acids					
C _{15:1} ω6c	0.9	0.9	0.9	0.8	0.9
C _{17:1} ω6c	0.5	0.7	0.5	0.4	1.0
C _{17:1} ω8c	0.4	0.4	–	0.2	0.7
Hydroxy fatty acids					
C _{15:0} 2-OH	0.6	0.6	0.2	0.3	0.5
C _{15:0} 3-OH	1.0	1.0	1.4	1.6	1.8
iso-C _{15:0} 3-OH	4.7	5.0	5.2	5.5	4.6
C _{16:0} 3-OH	0.8	0.9	0.6	0.7	0.4
iso-C _{16:0} 3-OH	2.9	3.0	4.0	4.6	1.7
C _{17:0} 2-OH	1.3	1.4	0.7	0.9	0.7
C _{17:0} 3-OH	0.7	0.8	0.3	0.4	1.3
iso-C _{17:0} 3-OH	19.9	20.4	17.3	17.7	20.9
Summed feature 3†	4.1	4.2	2.3	2.7	4.2
Unknown fatty acids‡					
11:543 (ECL)	–	0.6	0.8	0.8	0.6
13:565 (ECL)	5.4	5.8	4.8	4.9	6.5
16:582 (ECL)	1.6	1.5	1.3	1.4	1.7

*The double bond positions indicated by upper-case letter are unknown.

†Summed feature 3 represents iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, which could not be separated by GLC with the MIDI system.

‡ECL, equivalent chain length.

shown that one of the major groups of bacterial isolates belongs to the CFB complex. The CFB complex is known to be predominant in marine environments, including sea water, intertidal sediments and Antarctic sea ice among others (Bowman *et al.*, 1997; Pinhassi *et al.*, 1997; Llobet-Brossa *et al.*, 1998; Glöckner *et al.*, 1999; Barbeyron *et al.*, 2001; Kirchman, 2002). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strains SW-62^T, SW-74, SW-63^T and SW-72 are most closely related to the genus *Muricauda*, a member of the CFB complex. Their morphological properties are similar to those of *M. ruestringensis*; in particular, all possess an appendage not found in the phylogenetically related genus *Zobellia*

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of strains SW-62^T, SW-74, SW-63^T and SW-72 and representatives of the family *Flavobacteriaceae*. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. *Bacteroides fragilis* ATCC 25285^T was used as the outgroup. Solid circles indicate nodes that were also found in the maximum-likelihood tree. Bar, 0.01 substitution per nucleotide position.

(Barbeyron *et al.*, 2001; Bruns *et al.*, 2001). The results of chemotaxonomic analyses are consistent with the results of phylogenetic and morphological analyses. The chemotaxonomic properties of the four strains are similar to those of *M. ruestringensis* (Bruns *et al.*, 2001). The fatty acid profiles of the genus *Zobellia*, the four strains and *M. ruestringensis* DSM 13258^T are similar, but there are differences in the proportions of some fatty acids, particularly iso-C_{15:1} (Barbeyron *et al.*, 2001).

Strains SW-62^T, SW-74, SW-63^T and SW-72 were found to have almost identical cultural and physiological characteristics (Table 1). There are no sequence differences in 16S rRNA gene sequences between strains SW-62^T and SW-74 or between strains SW-63^T and SW-72. DNA–DNA relatedness values indicate that strains SW-62^T and SW-74 and strains SW-63^T and SW-72 are members of two different genomic species (Wayne *et al.*, 1987). Strains SW-62^T and SW-74 and strains SW-63^T and SW-72 are similar to *M. ruestringensis* in most physiological characteristics, except growth on some substrates and acid production from some sugars (Table 1). While *M. ruestringensis* grows in anaerobic conditions, the four isolates do not grow in anaerobic conditions on MA or on MA supplemented with nitrate. Strains SW-62^T and SW-74 and strains SW-63^T and SW-72 are phylogenetically and genetically distinguishable from *M. ruestringensis* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented above, strains SW-62^T and SW-74 and strains SW-63^T and SW-72 should be placed in the genus *Muricauda* as two distinct novel species, for which the names *Muricauda flavesceus* sp. nov. and *Muricauda aquimarina* sp. nov., respectively, are proposed.

Emended description of the genus *Muricauda* Bruns *et al.* 2001

The description is as given previously (Bruns *et al.*, 2001), with the following amendments. Facultatively anaerobic or strictly aerobic. Optimal growth occurs between 20 and 30 °C or between 30 and 37 °C. The fatty acid profile is characterized by large amounts of branched and straight-chain fatty acids. The DNA G + C content is 41–45 mol%.

Description of *Muricauda flavesceus* sp. nov.

Muricauda flavesceus (fla.ves'cens. L. v. *flavesco* to become golden yellow; L. part. adj. *flavesceus* becoming golden yellow).

Cells are non-motile, non-spore-forming rods that are 0.2–0.5 µm wide by 2.5–6.0 µm long. Gram-negative. Appendages are found on most cells (see Supplementary Figure in IJSEM Online). On MA, colonies are golden yellow in colour, glistening, circular, slightly convex and 0.8–1.2 mm in diameter after 3 days incubation at 30 °C. Optimal growth temperature is 30–37 °C. Growth occurs at 10 °C, but not at 4 °C. Maximum growth temperature is 44 °C. Optimal growth pH is around 7.0. Growth occurs weakly at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2% (w/v) NaCl. Growth occurs in the presence of 9% (w/v) NaCl, but not without NaCl or in the presence of more than 10% (w/v) NaCl. No growth occurs under anaerobic conditions on MA. The predominant respiratory lipoquinone is MK-6. The major fatty acids are iso-C_{17:0} 3-OH, iso-C_{15:1}, iso-C_{15:0} and C_{15:0}. The DNA G + C content (determined by HPLC) is 45.2–45.4 mol%. Other phenotypic properties are given in Table 1.

The type strain, SW-62^T (=KCCM 41645^T = JCM 11812^T), was isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea. The reference strain is SW-74.

Description of *Muricauda aquimarina* sp. nov.

Muricauda aquimarina (a.qui.ma.ri'na. L. n. *aqua* water; L. adj. *marinus* of the sea; N.L. adj. *aquimarina* pertaining to sea water).

Cells are non-motile, non-spore-forming rods that are 0.2–0.5 µm wide by 2.5–6.0 µm long. Gram-negative. Appendages are found on most cells (see Supplementary Figure in IJSEM Online). On MA, colonies are golden yellow in colour, glistening, circular, slightly convex and 0.8–1.2 mm in diameter after 3 days incubation at 30 °C. Optimal growth temperature is 30–37 °C. Growth occurs at 10 °C, but not at 4 °C. Maximum growth temperature is 44 °C. Optimal growth pH is around 7.0. Growth occurs weakly at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2 % (w/v) NaCl. Growth occurs in the presence of 9 % (w/v) NaCl, but not without NaCl or in the presence of more than 10 % (w/v) NaCl. No growth occurs under anaerobic conditions on MA. The predominant respiratory lipoquinone is MK-6. The major fatty acids are iso-C_{15:0}, iso-C_{15:1} and iso-C_{17:0} 3-OH. The DNA G + C content (determined by HPLC) is 44.1–44.2 mol%. Other phenotypic properties are given in Table 1.

The type strain, SW-63^T (=KCCM 41646^T = JCM 11811^T), was isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea. The reference strain is SW-72.

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References

- Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B. & Potin, P. (2001). *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 985–997.
- Baumann, L. & Baumann, P. (1981). The marine Gram-negative eubacteria; genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria*, pp. 1302–1330. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- 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.
- 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.
- Glöckner, F. O., Fuchs, B. M. & Amann, R. (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl Environ Microbiol* **65**, 3721–3726.
- Kirchman, D. L. (2002). The ecology of *Cytophaga-Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* **39**, 91–100.
- 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.
- Llobet-Brossa, E., Rosselló-Mora, R. & Amann, R. (1998). Microbial community composition of Wadden Sea sediments as revealed by fluorescence *in situ* hybridization. *Appl Environ Microbiol* **64**, 2691–2696.
- Pinhassi, J., Zweifel, U. L. & Hagström, Å. (1997). Dominant marine bacterioplankton species found among colony-forming bacteria. *Appl Environ Microbiol* **63**, 3359–3366.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. Newark, DE: MIDI.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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 *Nocardioideis* and related taxa based on 16S rRNA gene 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.