Ruegeria halocynthiae sp. nov., isolated from the sea squirt *Halocynthia roretzi*

Young-Ok Kim,¹ Sooyeon Park,² Bo-Hye Nam,¹ So-Jung Kang,²
Young Baek Hur,³ Sang-Jun Lee,¹ Tae-Kwang Oh²
and Jung-Hoon Yoon⁴

Correspondence

Jung-Hoon Yoon
jhyoon69@skku.edu

¹Biotechnology Research Division, National Fisheries Research and Development Institute (NFRDI), Gijang, Busan 619-705, Republic of Korea

²Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Daejeon, Republic of Korea

³South East Sea Fisheries Research Institute, Tongyoung 650-943, Republic of Korea

⁴Department of Food Science and Biotechnology, Sungkyunkwan University, Jangan-gu, Suwon, Republic of Korea

A Gram-negative, non-motile, rod-shaped bacterial strain, designated MA1-6^T, was isolated from a sea squirt (*Halocynthia roretzi*) collected from the South Sea, Korea, and was characterized to determine its taxonomic position. Strain MA1-6^T grew optimally at pH 7.0–8.0, at 30 °C and in the presence of 2–3 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MA1-6^T fell within the clade comprising *Ruegeria* species and exhibited 95.3–96.5 % similarity to the type strains of recognized *Ruegeria* species. Strain MA1-6^T contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c as the major fatty acid, which is consistent with data for *Ruegeria* species. The major polar lipids detected in strain MA1-6^T and *Ruegeria atlantica* KCTC 12424^T were phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and an unidentified lipid. The DNA G + C content of strain MA1-6^T was 58.6 mol%. Differential phenotypic properties, together with phylogenetic distinctiveness, demonstrated that strain MA1-6^T can be distinguished from recognized *Ruegeria* species. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain MA1-6^T is considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria halocynthiae* sp. nov. is proposed; the type strain is MA1-6^T (=KCTC 23463^T=CCUG 60744^T).

The genus *Ruegeria* was proposed by Uchino *et al.* (1998) based on the reclassification of *Agrobacterium atlanticum*, *Agrobacterium gelatinovorum* and *Roseobacter algicola* as *Ruegeria atlantica*, *Ruegeria gelatinovora* and *Ruegeria algicola*, respectively. However, *Ruegeria gelatinovorans* corrig. was subsequently reclassified as *Thalassobius gelatinovorans* (Arahal *et al.*, 2005) and *Ruegeria algicola* was moved to a new genus, *Marinovum*, as *Marinovum algicola* (Martens *et al.*, 2006). Recently, *Silicibacter lacuscaerulensis* (Petursdottir & Kristjansson, 1997) and *Silicibacter pomeroyi* (González *et al.*, 2003) were transferred to the genus *Ruegeria* as two different species (Yi *et al.*, 2007), and five more *Ruegeria* species, *Ruegeria mobilis* (Muramatsu *et al.*, 2007), *Ruegeria pelagia* (Lee *et al.*, 2007), *Ruegeria scottomollicae* (Vandecastelaere *et al.*, 2008), *Ruegeria marina* (Huo *et al.*, 2011) and *Ruegeria faecimaris* (Oh *et al.*, 2011), have been described. However, it

was recently shown that *Ruegeria pelagia* is a later synonym of *Ruegeria mobilis* (Lai *et al.*, 2010). At the time of writing, the genus *Ruegeria* thus comprised seven recognized species. In this study, we describe a *Ruegeria*-like bacterial strain, designated MA1-6^T, which was isolated from a sea squirt (*Halocynthia roretzi*) collected from the South Sea, Korea, and establish its exact taxonomic position by using a polyphasic characterization that included determination of phenotypic properties and a detailed phylogenetic investigation based on 16S rRNA gene sequences.

Strain MA1-6^T was isolated by the standard dilution plating technique at 20 °C on marine agar 2216 (MA; Difco). The type strain (KCTC 12424^T) of *Ruegeria atlantica*, the type species of the genus *Ruegeria*, which was used as a reference strain for polar lipid analysis, was obtained from the Korean Collection for Type Cultures (KCTC), Daejeon, South Korea. The morphological, physiological and biochemical characteristics of strain MA1-6^T were investigated by using routine incubation on MA at 30 °C. Cell morphology was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MA1-6^T is HQ852038.

examined by light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). The presence of flagella was examined by using transmission electron microscopy on cells from an exponentially growing culture. For this, cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. Growth at 4, 10, 15, 20, 25, 30, 37 and 40 °C was measured on MA. The Gram reaction was investigated by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. The pH range for growth was determined in marine broth 2216 (MB; Difco) adjusted to pH 4.0–10.5 (increments of 0.5 pH units) by the addition of HCl and Na₂CO₃; pH was verified after autoclaving. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0% (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded and that 0.45% (w/v) MgCl₂ · 6H₂O or 0.06% (w/v) KCl was added. Growth in the presence of 2.0–10.0% NaCl (increments of 1.0%) was investigated in MB. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA with the substrate concentrations described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin, Tweens 20, 40, 60 and 80, and urea were investigated as described by Lányi (1987) with the modification that artificial seawater was used for the preparation of media. The artificial seawater contained (per litre 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). Acid production from carbohydrates was investigated as described by Leifson (1963). Utilization of various substrates for growth was tested according to Baumann & Baumann (1981), after supplementation with 1% (v/v) vitamin solution (Staley, 1968) and 2% (v/v) Hutner's mineral salts (Cohen-Bazire *et al.*, 1957). Susceptibility to various antibiotics was determined on MA plates by using discs containing 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) or carbenicillin (100 µg). Enzyme activities and other physiological properties were determined by using the API ZYM and API 20E systems (bioMérieux).

Cell biomass of strain MA1-6^T for chromosomal DNA extraction and for analyses of isoprenoid quinones and polar lipids, and cell biomass of *Ruegeria atlantica* KCTC 12424^T for polar lipid analysis were obtained from cultures grown for 3 days in MB at 30 °C. Chromosomal DNA was extracted and purified according to the method described by Yoon *et al.* (1996), with the modification that RNase T1 was used in combination with RNase A to minimize

contamination of RNA. The 16S rRNA gene was amplified by PCR as described previously (Yoon *et al.*, 1998) by using two universal primers (5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3'). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The DNA G + C content was determined according to the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987), by using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For analysis of cellular fatty acids, cell mass of strain MA1-6^T was harvested from MA plates after incubation for 3 days at 30 °C. Fatty acids were saponified, methylated and extracted by using the standard MIDI protocol (Sherlock Microbial Identification System, version 4.0). Fatty acids were analysed by GC (Hewlett Packard 6890) and were identified by using the TSBA40 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with molybdophosphoric acid, molybdenum blue, ninhydrin and α-naphthol reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987), and Dragendorff's reagent (Sigma).

The morphological, cultural, physiological and biochemical characteristics of strain MA1-6^T are given in the species description below and in Table 1. The almost-complete 16S rRNA gene sequence of strain MA1-6^T comprised 1386 nt. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain MA1-6^T formed a distinct evolutionary lineage within the cluster comprising *Ruegeria* species (Fig. 1). Strain MA1-6^T exhibited 16S rRNA gene sequence similarity of 95.3–96.5% to the type strains of recognized *Ruegeria* species and less than 95.1% to the type strains of other species used in this study. The predominant isoprenoid quinone detected in strain MA1-6^T was ubiquinone-10 (Q-10), as with members of the genus *Ruegeria* (Yi *et al.*, 2007; Muramatsu *et al.*, 2007). The fatty acid profile of strain MA1-6^T is shown in Table 2, together with those of the type strains of *Ruegeria* species analysed under identical conditions and using the same methods (Oh *et al.*, 2011). The major fatty acid found in strain MA1-6^T was C_{18:1ω7c}, which is consistent with data for *Ruegeria* species and the vast majority of members of the *Alphaproteobacteria*. The fatty acid profile of strain MA1-6^T was similar to those of the type strains of *Ruegeria* species, although there were differences in the proportions of some components (Table 2). The polar lipid profile of strain MA1-6^T was compared with that of *Ruegeria atlantica* KCTC 12424^T, which was grown and analysed under identical conditions (Fig. 2). The polar lipid profile of strain MA1-6^T was similar to that of *Ruegeria atlantica* KCTC 12424^T in that phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and an unidentified lipid are major components and minor amounts of diphosphatidylglycerol and unidentified lipids are present

Table 1. Differential phenotypic characteristics of strain MA1-6^T and the type strains of related *Ruegeria* species

Strains: 1, MA1-6^T; 2, *Ruegeria atlantica* KCTC 12424^T (data from Rüger & Höfle, 1992; Oh *et al.*, 2011); 3, *Ruegeria lacuscaerulensis* KCTC 2953^T (Petersdottir & Kristjansson, 1997; Oh *et al.*, 2011); 4, *Ruegeria mobilis* CIP 109181^T (Muramatsu *et al.*, 2007; Oh *et al.*, 2011); 5, *Ruegeria pomeroyi* DSM 15171^T (González *et al.*, 2003; Oh *et al.*, 2011); 6, *Ruegeria scottomollicae* CCUG 55858^T (Vandecastelaere *et al.*, 2008; Oh *et al.*, 2011). All strains are positive for activity of oxidase and catalase, utilization of* D-glucose, acetate and L-malate, acid production from* D-ribose, and activity of* alkaline phosphatase. All strains are weakly positive for activity of* esterase (C4). All strains are negative for Gram staining, utilization of* sucrose, trehalose, maltose, benzoate, salicin, formate and L-glutamate, acid production from* melezitose, lactose and raffinose, and activity of* lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. w, Weakly positive.

Characteristic	1	2	3	4	5	6
Optimum growth temperature (°C)	30	20–30	45	25–30	30	20
Growth at:						
4 °C	–	–	–	–	–	+
40 °C	–	–	+	–	+	w
Motility	–	–	–	+	+	+
Anaerobic growth	–/++†	–	–	++‡	–	–‡
Nitrate reduction	+	+	+	–	–	–
Hydrolysis of:						
Aesculin	–	+	–	+	–	+
Xanthine	+	+	+	–*	+	–*
Tween 80	–	–	–	–	–	+
Utilization of:*						
L-Arabinose	–	–	–	+	–	–
Cellobiose	+	+	+	+	–	+
D-Fructose	–	+	+	+	–	+
D-Galactose	+	+	+	+	–	+
D-Mannose	+	+	+	+	–	+
D-Xylose	–	+	–	+	+	+
Citrate	w	+	+	–	+	+
Pyruvate	–	+	+	+	+	+
Succinate	+	+	+	+	–	+
Acid production from:*						
L-Arabinose	–	+	–	+	–	+
Cellobiose	–	+	–	+	–	+
D-Fructose	–	–	w	+	–	+
D-Galactose	+	+	–	–	–	+
D-Glucose	+	–	–	w	–	+
myo-Inositol	–	–	–	+	–	+
Maltose	–	+	–	w	–	+
D-Mannitol	–	–	–	+	–	+
D-Mannose	+	–	–	w	–	+
Melibiose	–	–	–	–	–	+
L-Rhamnose	–	–	–	–	–	–
D-Sorbitol	–	–	–	–	–	+
Sucrose	–	–	–	w	–	+
Trehalose	–	+	–	w	–	+
D-Xylose	–	+	–	w	+	+
Enzyme activity (API ZYM)*						
Esterase lipase (C8)	+	–	w	–	w	–
Leucine arylamidase	+	w	w	w	+	w
Acid phosphatase	+	–	–	–	–	–
β -Galactosidase	–	–	+	–	–	–
α -Glucosidase	+	–	–	–	–	–
DNA G + C content (mol%)	58.6	55	66.2	58.5	67.9–68.1	61

*Data for reference strains are from Oh *et al.* (2011) and were obtained using the same methods and under the same conditions as the novel isolate.

†Determined without/with nitrate.

‡Data from Oh *et al.* (2011); the result without and with nitrate.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain MA1-6^T among recognized *Ruegeria* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points; only values >50% are shown. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain MA1-6^T and the type strains of related *Ruegeria* species

Strains: 1, MA1-6^T; 2, *Ruegeria atlantica* KCTC 12424^T; 3, *Ruegeria lacuscaerulensis* KCTC 2953^T; 4, *Ruegeria mobilis* CIP 109181^T; 5, *Ruegeria pomeroyi* DSM 15171^T; 6, *Ruegeria scottomollicae* CCUG 55858^T. Data for the reference strains are from Oh *et al.* (2011) and were obtained under the same conditions and using the same methods as the novel isolate. Fatty acids that represent <0.5% in all strains were omitted. —, Not detected.

Fatty acid	1	2	3	4	5	6
Straight-chain						
C _{10:0}	3.0	2.6	6.3	—	2.8	—
C _{12:0}	2.9	3.1	4.5	—	1.2	0.1
C _{16:0}	4.6	3.6	1.8	5.3	8.2	2.5
C _{18:0}	1.1	0.6	2.0	1.5	2.0	0.6
Unsaturated						
C _{17:1ω7c}	0.4	—	—	0.5	0.4	1.0
C _{18:1ω7c}	54.4	63.5	69.8	74.1	66.7	79.1
C _{20:1ω7c}	—	0.3	0.8	0.3	0.4	0.3
Hydroxy						
C _{10:0} 3-OH	—	0.5	3.3	3.2	0.6	2.8
C _{12:0} 3-OH	4.8	5.2	7.5	0.8	6.1	0.3
C _{16:0} 2-OH	11.1	6.9	—	5.9	5.2	4.3
C _{18:1} 2-OH	0.5	1.2	—	1.1	0.2	3.0
11-Methyl-C _{18:1ω7c}	16.1	11.3	1.8	4.2	5.4	2.5
Cyclo C _{19:0ω8c}	—	—	0.6	—	—	—
Unknown ECL 11.799*	0.8	0.2	1.2	3.0	0.1	2.5

*ECL, equivalent chain-length.

(Fig. 2). The DNA G+C content of strain MA1-6^T was 58.6 mol%. The results obtained from the chemotaxonomic analyses are sufficient to support the result of the phylogenetic analysis based on 16S rRNA gene sequences, suggesting that strain MA1-6^T may be a member of the genus *Ruegeria*.

Strain MA1-6^T was clearly distinguishable from recognized *Ruegeria* species based on differences in several phenotypic characteristics, most of which were determined under the same conditions and methods in our laboratory (Table 1). The phylogenetic distinctiveness and differential phenotypic properties of strain MA1-6^T were sufficient to show that it represents a species that is separate from recognized species of the genus *Ruegeria* (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain MA1-6^T is considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria halocynthiae* sp. nov. is proposed.

Description of *Ruegeria halocynthiae* sp. nov.

Ruegeria halocynthiae (ha.lo.cyn.thi'a.e. N.L. gen. n. *halocynthiae* of *Halocynthia*, named after the generic name of the sea squirt *Halocynthia roretzi*, from which the type strain was isolated).

Cells are Gram-negative, non-motile rods, 0.2–0.6 × 1.0–10.0 µm in size; a few cells longer than 10.0 µm may occur. Colonies on MA are circular to slightly irregular, flat, glistening, smooth, cream-coloured and 2.0–3.0 mm in diameter after incubation for 3 days at 30 °C. Anaerobic growth does not occur on MA but does occur on MA supplemented with nitrate. Optimal growth occurs at 30 °C; growth occurs at 10 and 37 °C but not at 4 or 40 °C. Optimal pH for growth is between 7.0 and 8.0; growth

Fig. 2. TLCs of polar lipids of strain MA1-6^T (a) and *Ruegeria atlantica* KCTC 12424^T (b). PC, Phosphatidylcholine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; AL, unidentified aminolipid; L1–3, unidentified lipids.

occurs at pH 5.5 but not at pH 5.0. Optimal growth occurs in the presence of 2–3 % (w/v) NaCl; growth occurs in the presence of 6.0 % (w/v) NaCl but not in the absence of NaCl or in the presence of more than 7.0 % (w/v) NaCl. Nitrate reduction is positive. Hydrolyses hypoxanthine, L-tyrosine and xanthine, but not aesculin, casein, gelatin, starch, Tween 80 or urea. Utilizes cellobiose, D-galactose, D-glucose, D-mannose, acetate, citrate (weakly), L-malate and succinate as carbon and energy sources, but not L-arabinose, D-fructose, maltose, sucrose, trehalose, D-xylose, benzoate, formate, pyruvate, salicin or L-glutamate. Acid is produced from D-galactose, D-glucose, D-mannose and D-ribose, but not from L-arabinose, cellobiose, D-fructose, *myo*-inositol, lactose, maltose, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose or D-xylose. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, neomycin, penicillin G and polymyxin B, but not to kanamycin, lincomycin, novobiocin, oleandomycin, streptomycin or tetracycline. In assays with the API ZYM system, positive for alkaline phosphatase, esterase lipase

(C8), leucine arylamidase, acid phosphatase and α -glucosidase, weakly positive for esterase (C4), but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The predominant ubiquinone is Q-10. The major fatty acid (>10 % of the total) is C_{18:1} ω 7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and an unidentified lipid.

The type strain, MA1-6^T (=KCTC 23463^T=CCUG 60744^T), was isolated from a sea squirt (*Halocynthia roretzi*) collected in the South Sea, Korea. The DNA G + C content of the type strain is 58.6 mol% (determined by HPLC).

Acknowledgements

This work was supported by a grant from the National Fisheries Research and Development Institute (NFRDI) and the 21C Frontier Program of Microbial Genomics and Applications (grant 11-2008-00-002-00) from the Ministry of Education, Science & Technology (MEST) of the Republic of Korea.

References

- Arahal, D. R., Macián, M. C., Garay, E. & Pujalte, M. J. (2005). *Thalassobius mediterraneus* gen. nov., sp. nov., and reclassification of *Ruegeria gelatinovorans* as *Thalassobius gelatinovorus* comb. nov. *Int J Syst Evol Microbiol* **55**, 2371–2376.
- 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. G. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- 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.
- 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.
- 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.
- Huo, Y.-Y., Xu, X.-W., Li, X., Liu, C., Cui, H.-L., Wang, C.-S. & Wu, M. (2011). *Ruegeria marina* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **61**, 347–350.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lai, Q., Yuan, J., Li, F., Zheng, T. & Shao, Z. (2010). *Ruegeria pelagia* is a later heterotypic synonym of *Ruegeria mobilis*. *Int J Syst Evol Microbiol* **60**, 1918–1920.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

- Lee, K., Choo, Y.-J., Giovannoni, S. J. & Cho, J.-C. (2007). *Ruegeria pelagia* sp. nov., isolated from the Sargasso Sea, Atlantic Ocean. *Int J Syst Evol Microbiol* **57**, 1815–1818.
- 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.* 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.
- 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.
- Muramatsu, Y., Uchino, Y., Kasai, H., Suzuki, K. & Nakagawa, Y. (2007). *Ruegeria mobilis* sp. nov., a member of the Alphaproteobacteria isolated in Japan and Palau. *Int J Syst Evol Microbiol* **57**, 1304–1309.
- Oh, K.-H., Jung, Y.-T., Oh, T.-K. & Yoon, J.-H. (2011). *Ruegeria faecimaris* sp. nov., isolated from a tidal flat sediment. *Int J Syst Evol Microbiol* **61**, 1182–1188.
- Petursdottir, S. K. & Kristjansson, J. K. (1997). *Silicibacter lacuscaerulensis* gen. nov., sp. nov., a mesophilic moderately halophilic bacterium characteristic of the Blue Lagoon geothermal lake in Iceland. *Extremophiles* **1**, 94–99.
- Rüger, H.-J. & Höfle, M. G. (1992). Marine star-shaped-aggregate-forming bacteria: *Agrobacterium atlanticum* sp. nov.; *Agrobacterium meteori* sp. nov.; *Agrobacterium ferrugineum* sp. nov., nom. rev.; *Agrobacterium gelatinovorum* sp. nov., nom. rev.; and *Agrobacterium stellulatum* sp. nov., nom. rev. *Int J Syst Bacteriol* **42**, 133–143.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- 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.
- 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 kieliense* gen. nov., sp. nov., nom. rev. *J Gen Appl Microbiol* **44**, 201–210.
- Vandecastelaere, I., Nercessian, O., Segaeert, E., Achouak, W., Faimali, M. & Vandamme, P. (2008). *Ruegeria scottomollicae* sp. nov., isolated from a marine electroactive biofilm. *Int J Syst Evol Microbiol* **58**, 2726–2733.
- Yi, H., Lim, Y. W. & Chun, J. (2007). Taxonomic evaluation of the genera *Ruegeria* and *Silicibacter*: a proposal to transfer the genus *Silicibacter* Petursdottir and Kristjansson 1999 to the genus *Ruegeria* Uchino *et al.* 1999. *Int J Syst Evol Microbiol* **57**, 815–819.
- 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 rDNA 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.