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Ruegeria arenilitoris sp. nov., isolated from the seashore sand around a seaweed farm

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Abstract A Gram-negative, motile and rod-shaped bacterial strain, G-M8^T, which was isolated from seashore sand around a seaweed farm at Geoje island in South Korea, was characterized taxonomically. It grew optimally at 30–37 °C, at pH 7.0–8.0 and in presence of 2 % (w/v) NaCl. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences revealed that strain G-M8^T joined the cluster comprising the type strains of *Ruegeria atlantica* and *Ruegeria lacuscaerulensis*, showing 97.5 % sequence similarity, by a bootstrap resampling value of 85.8 %. It exhibited 16S rRNA gene sequence similarity values of 95.4–96.7 % to the type strains of the other *Ruegeria* species. Strain G-M8^T exhibited the highest *gyrB* sequence similarity value (88.5 %) to the type strain of *R. lacuscaerulensis*. Strain G-M8^T contained Q-10 as the predominant ubiquinone and $C_{18:1}$ $\omega 7c$ as the predominant fatty acid. The polar lipid profile of strain G-M8^T was similar to that of *R. atlantica* KCTC 12424^T. The DNA G+C content of strain G-M8^T was 64.6 mol% and its mean DNA–DNA relatedness values with *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T were 18 ± 5.3 and 10 ± 3.6 %, respectively. Differential phenotypic properties, together with the phylogenetic and genetic distinctiveness, demonstrated that strain G-M8^T is distinguished from other *Ruegeria* species. On the basis of the data presented, strain G-M8^T (=KCTC 23960^T = CCUG 62412^T) represents a novel species of the genus *Ruegeria*, for which the name *Ruegeria arenilitoris* sp. nov. is proposed.

Keywords *Ruegeria arenilitoris* · Novel species · Polyphasic taxonomy · Seashore sand

Introduction

The genus *Ruegeria*, a member of the class *Alphaproteobacteria*, was established by Uchino et al. (1998) with the reclassification of *Agrobacterium atlanticum*, *Agrobacterium gelatinovorum* and *Roseobacter algicola* as *Ruegeria atlantica*, *Ruegeria gelatinovora* and *Ruegeria algicola*, respectively. However, *Ruegeria gelatinovorans* corrig and *Ruegeria algicola* were subsequently reclassified as members of two other genera (Arahal et al. 2005; Martens et al. 2006), whilst *Silicibacter lacuscaerulensis* (Petursdottir and Kristjansson 1997) and *Silicibacter pomeroyi* (González

The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequence and *gyrB* sequence of *Ruegeria arenilitoris* G-M8^T are JQ807219 and JQ807221, respectively.

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et al. 2003) were transferred into the genus *Ruegeria* as two different species (Yi et al. 2007). It was recently shown that *Ruegeria pelagia* (Lee et al. 2007) is a later synonym of *Ruegeria mobilis* (Lai et al. 2010). The genus *Ruegeria* currently comprises seven species with validly published names: *R. atlantica* (Uchino et al. 1998), *Ruegeria lacuscaerulensis* and *Ruegeria pomeroyi* (Yi et al. 2007), *R. mobilis* (Muramatsu et al. 2007), *Ruegaria scottomollicae* (Vandecandelaere et al. 2008), *Ruegeria marina* (Huo et al. 2011) and *Ruegeria faecimaris* (Oh et al. 2011). In this study, we describe a *Ruegeria*-like bacterial strain, designated G-M8^T, which was isolated from seashore sand around a seaweed farm at Geoje island, South Korea. The aim of the present work was to determine the exact taxonomic position of strain G-M8^T by using a polyphasic approach.

Materials and methods

Bacterial strains and culture conditions

Marine sand was collected from the surface of the seashore around a seaweed farm at Geoje island in the South Sea, South Korea, and used as a source for the isolation of bacterial strains. Strain G-M8^T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Becton–Dickinson) at 25 °C and cultivated routinely on MA at 30 °C. Strain G-M8^T was maintained on MA at 4 °C for short-term preservation and as a glycerol suspension (20 %, w/v in distilled water) at −80 °C for long-term preservation. Strain G-M8^T has been deposited in the Korean Collection for Type Cultures (KCTC; South Korea) and the Culture Collection, University of Göteborg (CCUG; Sweden) as KCTC 23960^T and CCUG 62412^T, respectively. *R. atlantica* (the type species of the genus *Ruegeria*) KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T, which were used as reference strains for DNA–DNA hybridization, were obtained from the Korean Collection for Type Cultures (KCTC), Daejeon, South Korea.

Cell biomass of strain G-M8^T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids, and cell biomass of *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T for DNA extraction, were obtained from cultures grown for 3 days in marine broth 2216 (MB; Becton–Dickinson) at 30 °C. For cellular fatty acids analysis, cell mass of strain G-M8^T was harvested from MA plates after incubation for 3 days at 30 °C.

Morphological, physiological and biochemical characterization

The cell morphology and flagellation were examined by using light microscopy (BX51; Olympus) and transmission electron microscopy (CM-20; Philips), respectively. To assess the flagellation on cells from an exponentially growing MA culture, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (1029; Forma, N_2:CO_2:H_2, 86:7:7 %) on MA which had been prepared anaerobically under nitrogen atmosphere. Strain G-M8^T was cultured at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40, 45 and 50 °C) to measure optimal temperature and temperature range for growth. 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 by using trypticase soy broth prepared according to the formula of the Becton–Dickinson medium except that NaCl was excluded and that 0.45 % (w/v) $MgCl_2 \cdot 6H_2O$ or 0.06 % (w/v) KCl was added. Growth at various NaCl concentrations (2.0–10.0 %, w/v, at increments of 1.0 %) was investigated in MB. The pH range for growth was determined in MB adjusted to pH 4.5–9.5 (using increments of 0.5 pH units) by using sodium acetate/acetic acid and Na_2CO_3 buffers. The pH values were verified after autoclaving. Catalase and oxidase activities were determined as described by Cowan and Steel (1965). Hydrolysis of casein, hypoxanthine, L-tyrosine and xanthine was tested on MA, using the substrate concentrations described by Cowan and Steel (1965). Nitrate reduction and hydrolysis of aesculin and Tweens 20, 40, 60 and 80 were investigated as described previously (Lányí 1987) with the modification that artificial seawater was used for the preparation of media. Hydrolysis of gelatin and urea were investigated by using Nutrient gelatin and Urea agar base media (Becton–Dickinson), respectively, with the modification that artificial seawater was used for the preparation of media. The artificial seawater

contained (l^{-1} distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2 \cdot 6H_2O$, 5.94 g $MgSO_4 \cdot 7H_2O$ and 1.3 g $CaCl_2 \cdot 2H_2O$ (Bruns et al. 2001). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was tested according to Baumann and Baumann (1981), supplemented with 1 % (v/v) vitamin solution (Staley 1968) and 2 % (v/v) Hutner's mineral salts (Cohen-Bazire et al. 1957). Susceptibility to antibiotics was investigated on MA plates using antibiotic discs (Advantec) containing the following (μg per disc unless otherwise stated): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Enzyme activities were determined, after incubation for 8 h at 30 °C, by using the API ZYM system (bioMérieux).

Molecular studies

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) using two universal primers, 9F (5′-GAGTTTGATCCTGG CTCAG-3′) and 1512R (5′-ACGGTTACCTTGT TACGACTT-3′), and the PCR products were purified by using a QIAquick PCR purification kit (Qiagen). 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 5′ and 3′ ends of the alignment were omitted from further analysis. Phylogenetic analysis was performed as described by Yoon et al. (2012). The amplification of the DNA gyrase B subunit gene (*gyrB*) was performed by using two primers, UP-1 and UP-2r, according to the method described previously (Yamamoto and Harayama 1995) and the PCR products were purified with the QIAquick PCR purification kit (Qiagen). The amplified *gyrB* was cloned into pGEM T-easy vector (Promega) according to the manufacturer's instructions. Sequence of the *gyrB* was determined for both strands by extension from vector-specific priming sites (T7 and SP-6 primers from pGEM T-easy vector). Phylogenetic analysis of the *gyrB* sequence was performed as described by Yoon et al. (2007). 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 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–DNA relatedness values. The DNAs of strain G-M8^T, *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T were used individually as labelled DNA probes for reciprocal hybridization.

Chemotaxonomic characterization

Isoprenoid quinones were extracted according to the method of Komagata and Suzuki (1987) and analyzed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The isoprenoid quinones were eluted by a mixture of methanol/isopropanol (2:1, v/v), using a flow rate of 1 ml min^{-1} at room temperature and detected by UV absorbance at 275 nm. Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI (Sherlock Microbial Identification System, version 4.0). The fatty acids were analysed by GC (Hewlett-Packard 6890) and 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 separated by two-dimensional TLC using chloroform/methanol/water (65:25:3.8, v/v/v) for the first dimension and chloroform/methanol/acetic acid/water (40:7.5:6:1.8, v/v/v/v) for the second dimension as described by Minnikin et al. (1977). Individual polar lipids were identified by spraying with the ethanolic molybdophosphoric acid, molybdenum blue, ninhydrin and α-naphthol reagents (Minnikin et al. 1984; Komagata and Suzuki 1987) and with Dragendorff's reagent (Sigma). The DNA G+C content was determined by the method of Tamaoka and Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC equipped with a YMC ODS-A (250 × 4.6 mm) column. The nucleotides were eluted by a mixture of 0.55 M $NH_4H_2PO_4$ (pH 4.0) and acetonitrile (40:1, v/v), using flow rate of 1 ml min^{-1}

at room temperature and detected by UV absorbance at 270 nm. *Escherichia coli* DNA was used a standard.

Results and discussion

Morphological, cultural, physiological, and biochemical characteristics

Strain G-M8^T was found to be a facultatively aerobic, Gram-negative, non-spore-forming rod shaped bacterium. Strain G-M8^T was indicated to be motile by means of peritrichous flagella (Supplementary Fig. 1), whereas the type strains of *R. atlantica* and *R. lacuscaerulensis* are non-motile as determined by the absence of flagella (Uchino et al. 1998; Petursdottir and Kristjansson 1997). Strain G-M8^T grew optimally at 30–37 °C, pH 7.0–7.5 and was found to be a moderate halophile as it grew optimally in the presence of 2 % (w/v) NaCl; the strain grew in the presence of 0.5–6.0 % NaCl. Mg^{2+} ions were required for growth, but K^+ ions were not required for growth. Strain G-M8^T showed catalase and oxidase activities but no urease activity. Strain G-M8^T reduced nitrate to nitrite. Strain G-M8^T was susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, neomycin (weak), oleandomycin, penicillin G, polymyxin B and streptomycin but not to gentamicin, kanamycin, lincomycin, novobiocin and tetracycline. Morphological, cultural, physiological and biochemical characteristics of strain G-M8^T are given in the species description (see below) and in Table 1.

Phylogenetic analysis

The almost-complete 16S rRNA gene sequence of strain G-M8^T comprised 1384 nucleotides. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain G-M8^T fell within the clade comprising *Ruegeria* species, joining the cluster comprising the type strains of *R. atlantica* and *R. lacuscaerulensis* by a bootstrap resampling value of 85.8 % (Fig. 1). Strain G-M8^T exhibited 16S rRNA gene sequence similarity values of 97.5 and 97.5 % to *R. atlantica* IAM 14463^T and *R. lacuscaerulensis* ITI-1157^T, respectively and 95.4–96.7 % to the type strains of the other validly named *Ruegeria* species. The *gyrB* sequence of strain G-M8^T determined in this study comprised 1,180 nucleotides. In the neighbour-joining phylogenetic tree based on *gyrB* sequences, strain G-M8^T clustered with the type strain of *R. lacuscaerulensis*, supported by a bootstrap resampling value of 99.7 %, with which it exhibited the highest sequence similarity (88.5 %) (Fig. 2). Strain G-M8^T exhibited 78.8–83.8 % *gyrB* sequence similarity values to the type strains of the other validly named *Ruegeria* species.

DNA–DNA relatedness

Strain G-M8^T exhibited mean DNA–DNA relatedness values of 18 ± 5.3 and 10 ± 3.6 % to *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T, respectively.

Chemotaxonomic characteristics

The predominant isoprenoid quinone detected in strain G-M8^T was ubiquinone-10 (Q-10), which is compatible with those of members of the genus *Ruegeria* (Yi et al. 2007; Muramatsu et al. 2007; Oh et al. 2011). In Table 2, the fatty acid profile of strain G-M8^T is compared with those of the type strains of two phylogenetically related *Ruegeria* species, *R. atlantica* and *R. lacuscaerulensis*, which were analysed under identical conditions and methods. The predominant fatty acid found in strain G-M8^T was $C_{18:1}$ $\omega 7c$ in line with *Ruegeria* species and the vast majority members of the *Alphaproteobacteria* (Yi et al. 2007; Muramatsu et al. 2007; Oh et al. 2011). The fatty acid profile of strain G-M8^T was similar to those of *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T, although there were differences in the proportions of some fatty acids (Table 2). In particular, strain G-M8^T was distinguished from *R. lacuscaerulensis* KCTC 2953^T in that some fatty acids which are present as significant amounts in strain G-M8^T are minor components or not detected in *R. lacuscaerulensis* KCTC 2953^T. The major polar lipids detected in strain G-M8^T were phosphatidylcholine, phosphatidylglycerol, one unidentified aminolipid and two unidentified lipids (Supplementary Fig. 2). The polar lipid profile of strain G-M8^T was compared with that of *R. atlantica* KCTC 12424^T grown and analysed under identical conditions by Kim et al. (2012). The profile of strain G-M8^T was similar with that of *R. atlantica* KCTC 12424^T in that phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and

Table 1 Differential phenotypic characteristics of *Ruegeria arenilitoris* G-M8^T and the type strains of *Ruegeria atlantica* and *Ruegeria lacuscaerulensis*

Characteristic	1	2	3
Optimum growth temperature (°C)	30–37	20–30	45
Motility	+	−	−
Anaerobic growth	+	−	−
Growth at			
4 °C	+	−	−
40 °C	+	−	+
50 °C	−	−	+
Hydrolysis of			
Asculin	−	+	−
Xanthine	−	+	+
Utilization of*			
D-Xylose	+	+	−
Acid production from*			
L-Arabinose	+	+	−
D-Cellobiose	w	+	−
D-Fructose	−	−	w
D-Galactose	+	+	−
Maltose	−	+	−
D-Ribose	−	+	+
D-Trehalose	−	+	−
D-Xylose	−	+	−
Enzyme activity (by API ZYM)*			
Esterase lipase (C 8)	−	−	w
β-Galactosidase	−	−	+
DNA G+C content (mol%)	64.6	55	66.2

Strains: 1, *R. arenilitoris* G-M8^T; 2, *R. atlantica* KCTC 12424^T, data from Rüger and Höfle (1992) and Oh et al. (2011); 3, *R. lacuscaerulensis* KCTC 2953^T, data from Petursdottir and Kristjansson (1997) and Oh et al. (2011). All strains are positive for activity of oxidase and catalase; nitrate reduction; utilization of* D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, acetate, citrate, L-malate, pyruvate and succinate; and activity of* alkaline phosphatase. All strains are weakly positive for activity of* esterase (C 4) and leucine arylamidase. All strains are negative for Gram staining; hydrolysis of casein, starch and Tween 80; utilization of* L-arabinose, sucrose, D-trehalose, maltose, benzoate, salicin, formate and L-glutamate; acid production from* D-glucose, lactose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, sucrose, *myo*-inositol, D-mannitol and D-sorbitol; and activity of* lipase (C 14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, *β*-glucuronidase, α-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase and α-fucosidase. *Data of reference strains taken from Oh et al. (2011). *Symbol*: +, positive reaction; –, negative reaction

w, weakly positive reaction

an unidentified lipid are major polar lipids, but was distinguishable from that of *R. atlantica* KCTC 12424^T in that a minor amount of diphosphatidylglycerol and one additional unidentified lipid are absent. The G+C content of strain G-M8^T was 64.6 mol%, a value in the range reported for *Ruegeria* species (Huo et al. 2011; Oh et al. 2011).

Conclusion

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 G-M8^T is a member of the genus *Ruegeria*. Phenotypic characteristics of

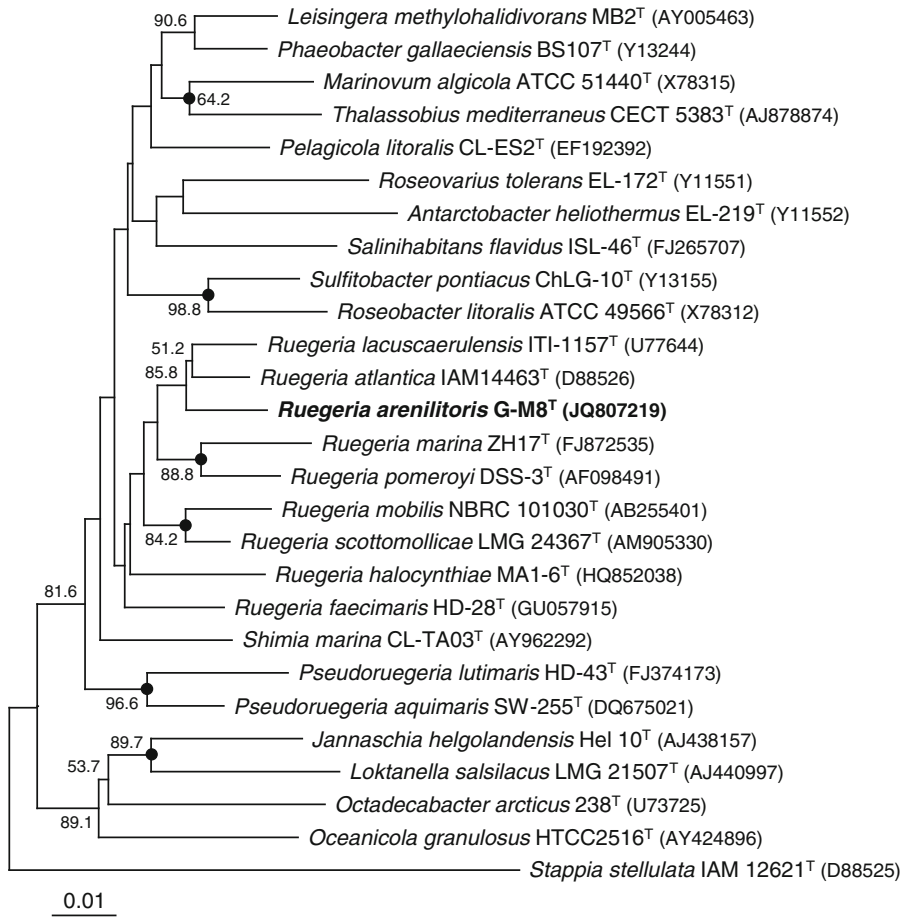

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Ruegeria arenilitoris* G-M8^T, *Ruegeria* species and representative of some other related taxa. Only bootstrap values (expressed as percentages of 1,000 replications) of >50 % are shown at branching points. *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 (GenBank accession no. D88525) was used as an outgroup. *Bar* 0.01 substitutions per nucleotide position

strain G-M8^T were compared with those of *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T, i.e. related species that showed 16S rRNA gene sequence similarity of >97 % (Table 1). Strain G-M8^T was clearly distinguishable from *R. atlantica* KCTC 12424^T and *R. lacuscaerulensis* KCTC 2953^T by differences in several phenotypic characteristics, including motility, anaerobic growth, growth at 4 °C, xanthine hydrolysis and acid production from some substrates, most of which were determined under the same conditions and methods (Table 1). These differences, together with the phylogenetic distinctiveness and genetic distinctiveness of strain G-M8^T, are sufficient to show that this strain is separate from recognized species of the genus *Ruegeria* (Wayne et al. 1987; Stackebrandt and Goebel 1994). Therefore, on the basis of the phenotypic, chemotaxonomic, phylogenetic and genetic data, strain G-M8^T is considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria arenilitoris* sp. nov. is proposed.

Description of *Ruegeria arenilitoris* sp. nov

Ruegeria arenilitoris (a.re.ni.li.to'ris. L. n. *arena* sand; L. n. *litus -oris* the seashore, coast; N.L. gen. n. *arenilitoris* of sand of seashore, from which the type strain was isolated).

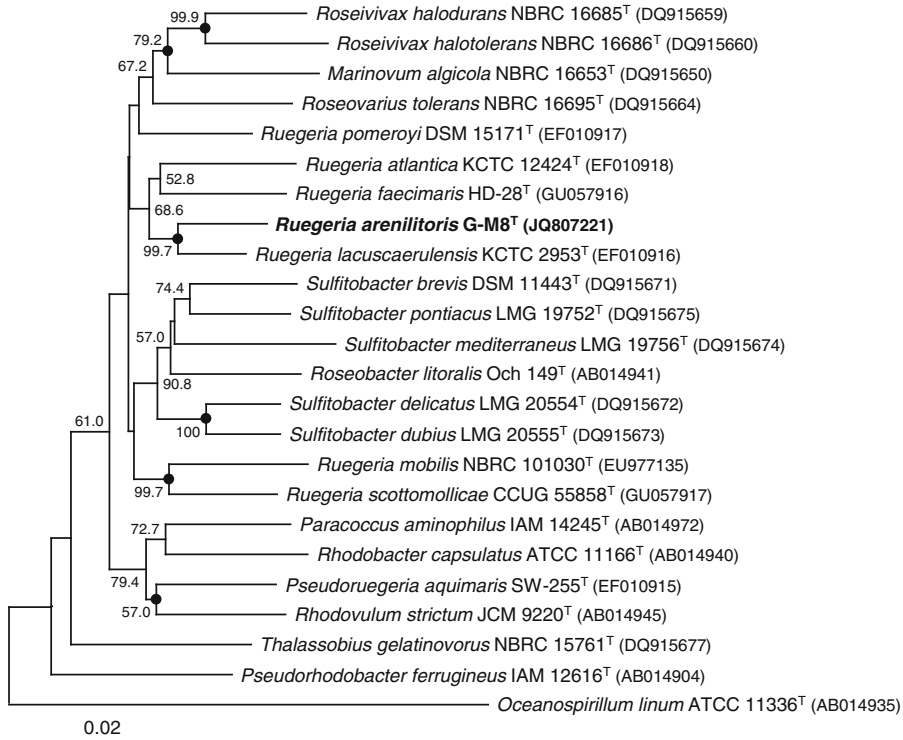

Fig. 2 Neighbour-joining phylogenetic tree based on *gyrB* sequences showing the positions of *Ruegeria arenilitoris* G-M8^T, some *Ruegeria* species and representative of some other related taxa. Only bootstrap values (expressed as percentages of 1,000 replications) of >50 % are shown at branching points. *Filled circles* indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum parsimony algorithms. *Oceanospirillum linum* ATCC 11336^T (GenBank accession number, AB014935) was used as an outgroup. *Scale bar* 0.02 substitutions per nucleotide position

Cells are Gram-negative, non-spore-forming and rods-shaped, approximately 0.2–0.6 μm in diameter 0.8–4.0 μm in length. Motile by means of peritrichous flagella. Colonies on marine agar are circular to slightly irregular, flat to raised, glistening, smooth, grayish yellow in colour and 2.0–3.0 mm after incubation for 3 days at 30 °C. Anaerobic growth occurs on marine agar. Optimal growth occurs at 30–37 °C; growth occurs at 4 and 45 °C, but not at 50 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2.0 % (w/v) NaCl; growth occurs in the presence of 0.5–6.0 % (w/v) NaCl. Mg^{2+} ions are required for growth but K^+ ions are not. Catalase- and oxidase-positive. Nitrate reduction is positive. Hypoxanthine and L-tyrosine are hydrolysed but aesculin, casein, gelatin, starch, Tweens 20, 40, 60 and 80, urea and xanthine are not. D-Cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-xylose, acetate, citrate, L-malate, pyruvate and succinate are utilized as carbon and energy sources but L-arabinose, maltose, sucrose, D-trehalose, benzoate, formate, salicin and L-glutamate are not. Acid is produced from L-arabinose, D-cellobiose (weak) and D-galactose but not from D-fructose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, D-trehalose and D-xylose. In assays with the API ZYM system, alkaline phosphatase activity is present and esterase (C 4) and leucine arylamidase activities are weakly present, but esterase lipase (C 8), lipase (C 14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are

Table 2 Cellular fatty acid compositions (%) of *Ruegeria arenilitoris* G-M8^T and the type strains of *Ruegeria atlantica* and *Ruegeria lacuscaerulensis*

Fatty acid	1	2	3
Straight-chain fatty acid			
$C_{10:0}$	2.9	2.6	6.3
$C_{12:0}$	3.4	3.1	4.5
$C_{16:0}$	3.7	3.6	1.8
$C_{18:0}$	0.6	0.6	2.0
Unsaturated fatty acid			
$C_{17:1}$ $\omega 7c$	0.6	–	–
$C_{18:1}$ $\omega 7c$	51.3	63.5	69.8
$C_{20:1}$ $\omega 7c$	–	tr	0.8
Hydroxy fatty acid			
$C_{10:0}$ 3-OH	tr	tr	3.3
$C_{12:0}$ 3-OH	5.6	5.2	7.5
$C_{16:0}$ 2-OH	7.4	6.9	–
$C_{18:1}$ 2-OH	1.1	1.2	–
11 methyl $C_{18:1}$ $\omega 7c$	17.6	11.3	1.8
Cyclo $C_{19:0}$ $\omega 8c$	4.7	–	0.6
Unknown fatty acid			
ECL 11.799	tr	tr	1.2

Strains: 1, *R. arenilitoris* G-M8^T; 2, *R. atlantica* KCTC 12424^T; 3, *R. lacuscaerulensis* KCTC 2953^T. Data of reference strains taken from Oh et al. (2011) obtained under the same conditions and methods. Fatty acids that represented <0.5 % in all strains were omitted. tr, traces (<0.5 %); –, not detected

ECL equivalent chain length

absent. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of the total fatty acids) are $C_{18:1}$ $\omega 7c$ and 11-methyl $C_{18:1}$ $\omega 7c$. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, one unidentified aminolipid and two unidentified lipids. The DNA G+C content of the type strain is 64.6 mol% (determined by HPLC).

The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequence and *gyrB* sequence of strain G-M8^T are JQ807219 and JQ807221, respectively. The type strain, G-M8^T (=KCTC 23960^T = CCUG 62412^T), was isolated from seashore sand around a seaweed farm at Geoje island, South Korea.

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