Ruegeria faecimaris sp. nov., isolated from a tidal flat sediment

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A Gram-negative-staining, non-motile and rod-shaped bacterial strain, HD-28^T, was isolated from a tidal flat of the Yellow Sea, Korea. Strain HD-28^T grew optimally at pH 7.0–8.0 and 30 °C in the presence of 2–3 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain HD-28^T was most closely related to species of the genus *Ruegeria* and exhibited 95.5–96.9 % 16S rRNA gene sequence similarity to the type strains of *Ruegeria* species. A neighbour-joining phylogenetic tree based on *gyrB* gene sequences also showed that strain HD-28^T fell within the cluster comprising recognized species of the genus *Ruegeria*, showing 77.5–83.9 % sequence similarity. Strain HD-28^T contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c as the major fatty acid. The major polar lipids detected in strain HD-28^T were phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and two unidentified lipids. The DNA G + C content was 57.9 mol%. Differential phenotypic properties, together with phylogenetic distinctiveness, demonstrated that strain HD-28^T could be distinguished from recognized species of the genus *Ruegeria*. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain HD-28^T is considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria faecimaris* sp. nov. is proposed. The type strain is HD-28^T (=KCTC 23044^T=CCUG 58878^T).

The genus *Ruegeria* was created by Uchino *et al.* (1998) through the reclassification of *Agrobacterium atlanticum*, *Agrobacterium gelatinovorans* and *Roseobacter algicola* as *Ruegeria atlantica*, *Ruegeria gelatinovorans* and *Ruegeria algicola*, respectively. Later, *Ruegeria gelatinovorans* and *Ruegeria algicola* were reclassified as *Thalassobius gelatinovorans* (Arahal *et al.*, 2005, 2006) and as a member of a novel genus as *Marinovum algicola* (Martens *et al.*, 2006), respectively. More recently, two species of the genus *Silicibacter*, *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi*, were reclassified as members of the genus *Ruegeria* (Yi *et al.*, 2007). In addition, three further species of the genus *Ruegeria* have also been described recently: *Ruegeria mobilis* (Muramatsu *et al.*, 2007), *Ruegeria pelagia* (Lee *et al.*, 2007) and *Ruegeria scottomollicae* (Vandecastelaere *et al.*, 2008). However, it has recently been suggested that *Ruegeria pelagia* is a later heterotypic synonym of *Ruegeria mobilis* (Lai *et al.*, 2010). In this study, a novel bacterial strain, designated HD-28^T, which was isolated from a tidal flat off Hwang-do in the Yellow Sea, Korea, is described. Comparative 16S rRNA gene sequence analysis indicated that strain HD-28^T was

phylogenetically closely related to the genus *Ruegeria*. The aim of the present study was to determine the exact taxonomic position of strain HD-28^T by using a polyphasic approach that included determination of phenotypic properties and detailed phylogenetic investigations based on 16S rRNA and DNA gyrase B subunit (*gyrB*) gene sequences.

Strain HD-28^T was isolated by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). The type strains of six species of the genus *Ruegeria* were used as reference strains for phenotypic characterization and fatty acid analysis. *Ruegeria atlantica* KCTC 12424^T and *Ruegeria lacuscaerulensis* KCTC 2953^T were obtained from the Korean Collection for Type Cultures (Taejeon, Republic of Korea). *Ruegeria mobilis* CIP 109181^T was obtained from the Collection de l'Institut Pasteur (Paris, France). *Ruegeria pomeroyi* DSM 15171^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). *Ruegeria scottomollicae* CCUG 55858^T was obtained from the Culture Collection of the University of Göteborg (Göteborg, Sweden).

The morphological, physiological and biochemical characteristics of strain HD-28^T were investigated using routine cultivation at 30 °C on MA. Cell morphology was examined by using light (E600; Nikon) and transmission

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HD-28^T is GU057915. The accession numbers for the *gyrB* gene sequences of strain HD-28^T and *Ruegeria scottomollicae* CCUG 55858^T are GU057916 and GU057917, respectively.

Table 1. Differential phenotypic characteristics of strain HD-28^T and the type strains of other species of the genus *Ruegeria*

Strains: 1, HD-28^T; 2, *R. atlantica* KCTC 12424^T (data from Rüger & Höfle, 1992 and this study); 3, *R. lacuscaerulensis* KCTC 2953^T (Petersdottir & Kristjansson, 1997 and this study); 4, *R. mobilis* CIP 109181^T (Muramatsu *et al.*, 2007 and this study); 5, *R. pomeroyi* DSM 15171^T (González *et al.*, 2003 and this study); 6, *R. scottmollicae* CCUG 55858^T (Vandecastelaere *et al.*, 2008 and this study). +, Positive; –, negative; w, weakly positive. All strains are positive for activity of oxidase and catalase, utilization of D-glucose, acetate, L-malate and pyruvate (data from this study), acid production from D-ribose (data from this study), and activity of alkaline phosphatase (data from this study). All strains are weakly positive for activity of esterase (C4) (data from this study). All strains are negative for Gram staining, utilization of sucrose, trehalose, maltose, benzoate, salicin, formate and L-glutamate (data from this study), acid production from melezitose, lactose and raffinose (data from this study), and activity of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase (data from this study).

Characteristic	1	2	3	4	5	6
Optimum growth temperature (°C)	30	20–30	45	25–30	30	20
Growth at						
4 °C	w	–	–	–	–	+
40 °C	–	–	+	–	+	w
Motility	–	–	–	+	+	+
Anaerobic growth	–	–	–	+	–	–*
Nitrate reduction	+	+	+	–	–	–
Hydrolysis of						
Aesculin	w	+	–	+	–	+
Xanthine	–	+	+	–*	+	–*
Tween 80	+	–	–	–	–	+
Utilization of*						
L-Arabinose	–	–	–	+	–	–
Cellobiose	+	+	+	+	–	+
D-Fructose	+	+	+	+	–	+
D-Galactose	+	+	+	+	–	+
D-Mannose	+	+	+	+	–	+
D-Xylose	–	+	–	+	+	+
Citrate	+	+	+	–	+	+
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	–	–	+	–	–	–
DNA G + C content (mol%)	57.9	55	66.2	58.5	67.9–68.1	61

*Data from reference type strains obtained in this study.

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). H_2S production was tested as described previously (Bruns *et al.*, 2001). Susceptibility to antibiotics was investigated by spreading bacterial suspension on MA and applying paper discs impregnated with the following antibiotics (concentration per disc); ampicillin (10 μ g), carbenicillin (100 μ g), cephalothin (30 μ g), chloramphenicol (100 μ g), gentamicin (30 μ g), kanamycin (30 μ g), lincomycin (15 μ g), neomycin (30 μ g), novobiocin (5 μ g), oleandomycin (15 μ g), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 μ g) and tetracycline (30 μ g). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Baumann & Baumann (1981), using supplementation with 2 % (v/v) Hutner's mineral salts solution (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). The carbon sources were added at a concentration of 0.2 % (w/v) after sterilization by filtration. The API ZYM system (bioMérieux) was used to determine enzyme activities.

Strain HD-28^T was cultivated at 30 °C in MB to obtain the cell biomass required for DNA extraction and for the analyses of isoprenoid quinones and polar lipids. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize the contamination of RNA. The 16S

rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analyses were performed as described by Yoon *et al.* (2003). PCR amplification of the *gyrB* gene was performed by using two primers, UP-1 and UP-2r, according to the method described previously (Yamamoto & Harayama, 1995). The PCR product was purified with a QIAquick PCR purification kit (Qiagen). The amplified *gyrB* gene was cloned into pGEM T-easy vector (Promega) according to the manufacturer's instructions. The sequence of the *gyrB* gene was determined for both strands by extension from vector-specific priming sites (T7 and SP-6 primers from pGEM T-easy). Phylogenetic analysis of *gyrB* gene sequences was performed as described by Yoon *et al.* (2007).

Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by means of two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The presence of phosphatidylcholine was tested by spraying Dragendorff's reagent (Sigma). For cellular fatty acid analysis, cell mass of strain HD-28^T, *R. atlantica* KCTC 12424^T, *R. lacuscaerulensis* KCTC 2953^T, *R. mobilis* CIP 109181^T, *R. pomeroyi* DSM 15171^T and *R. scottomollicae* CCUG 55858^T was harvested from MA plates after cultivation for 3 days at 30 °C. The fatty acids were extracted and fatty acid methyl

esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

The morphological, cultural, physiological and biochemical characteristics of strain HD-28^T are given in the species description (see below) and in Table 1. The almost-complete 16S rRNA gene sequence of strain HD-28^T comprised 1417 nt. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain HD-28^T joined the cluster comprising species of the genus *Ruegeria* with a bootstrap resampling value of 52.7 % (Fig. 1). The relationship between strain HD-28^T and the cluster comprising members of the genus *Ruegeria* was also maintained in trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain HD-28^T exhibited 96.9 % 16S rRNA gene sequence similarity to *R. scottomollicae* LMG 24367^T and 95.5–96.4 % similarity to the type strains of the other species of the genus *Ruegeria*. The *gyrB* gene sequence of strain HD-28^T determined in this study comprised 1177 nt. In the neighbour-joining phylogenetic tree based on *gyrB* gene sequences, strain HD-28^T fell within the cluster comprising species of the genus *Ruegeria* (Fig. 2). Strain HD-28^T exhibited 77.5–83.9 % *gyrB* gene sequence similarity to members of the genus *Ruegeria* and less than 80.6 % to other species used in this study.

The predominant isoprenoid quinone detected in strain HD-28^T was ubiquinone-10 (Q-10), in line with other

Fig. 2. Neighbour-joining phylogenetic tree based on *gyrB* gene sequences showing the positions of strain HD-28^T and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. *Oceanospirillum linum* ATCC 11336^T (GenBank accession no. AB014935) was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

members of the genus *Ruegeria* (Yi *et al.*, 2007; Muramatsu *et al.*, 2007). The fatty acid profile of strain HD-28^T is shown in Table 2, together with those of the type strains of members of the genus *Ruegeria* also analysed in this study. The major fatty acid found in strain HD-28^T was C_{18:1}ω7c (Table 2). The fatty acid profile of strain HD-28^T was similar to those of the type strains of species of the genus *Ruegeria*, even though there were differences in the proportions of some fatty acids (Table 2). The major polar lipids detected in strain HD-28^T were phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and two unidentified lipids; a minor amount of diphosphatidylglycerol was also detected (Fig. 3). The DNA G + C content for strain HD-28^T was 57.9 mol%. The results obtained from the chemotaxonomic analyses supported the results from the phylogenetic analysis based on 16S rRNA gene sequences, suggesting that strain HD-28^T belonged to the genus *Ruegeria*.

Strain HD-28^T was clearly distinguishable from recognized species of the genus *Ruegeria* by differences in several phenotypic characteristics, including hydrolysis and utilization of, and acid production from certain substrates, most of which were determined using the same methods in this study (Table 1). Phylogenetic distinctiveness, together with differential phenotypic properties, was sufficient to

Fig. 3. TLC chromatogram of polar lipids of strain HD-28^T. PC, phosphatidylcholine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; AL, unidentified aminolipid; L, unidentified lipid.

allocate strain HD-28^T as a species distinct from recognized species of the genus *Ruegeria* (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain HD-28^T is considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria faecimaris* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of strain HD-28^T and the type strains of species of the genus *Ruegeria*

Strains: 1, HD-28^T; 2, *R. atlantica* KCTC 12424^T; 3, *R. lacuscaerulensis* KCTC 2953^T; 4, *R. mobilis* CIP 109181^T; 5, *R. pomeroyi* DSM 15171^T; 6, *R. scottomollicae* CCUG 55858^T. All data are from this study. Fatty acids that represented <0.5% in all strains were omitted. –, Not detected.

Fatty acid	1	2	3	4	5	6
Straight-chain fatty acid						
C _{10:0}	3.1	2.6	6.3	–	2.8	–
C _{12:0}	3.4	3.1	4.5	–	1.2	0.1
C _{16:0}	4.4	3.6	1.8	5.3	8.2	2.5
C _{18:0}	0.7	0.6	2.0	1.5	2.0	0.6
Unsaturated fatty acid						
C _{17:1} ω7c	–	–	–	0.5	0.4	1.0
C _{18:1} ω7c	73.6	63.5	69.8	74.1	66.7	79.1
C _{20:1} ω7c	–	0.3	0.8	0.3	0.4	0.3
Hydroxy fatty acid						
C _{10:0} 3-OH	0.9	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	3.9	6.9	–	5.9	5.2	4.3
C _{18:1} 2-OH	1.1	1.2	–	1.1	0.2	3.0
11 methyl C _{18:1} ω7c	2.1	11.3	1.8	4.2	5.4	2.5
Cyclo C _{19:0} ω8c	–	–	0.6	–	–	–
Unknown fatty acid*						
ECL 11.799	0.3	0.2	1.2	3.0	0.1	2.5

*ECL, Equivalent chain-length.

Description of *Ruegeria faecimaris* sp. nov.

Ruegeria faecimaris (fae.ci.ma'ris. L. n. *faex faecis* sediment; L. gen. n. *maris* of the sea; N.L. gen. n. *faecimaris* of the sediment of the sea).

Cells stain Gram-negative, are non-motile and rod-shaped. Cells are 0.3–0.6 × 1.0–8.0 μm; a few cells longer than 10.0 μm may occur. Colonies on MA are circular, glistening, convex, smooth, greyish yellow in colour and 0.5–2.0 mm in diameter after incubation for 5 days at 30 °C. Growth occurs at 4 and 37 °C, but not at 40 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of 7.0% (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 8.0% (w/v) NaCl. Hypoxanthine, L-tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed and aesculin is weakly hydrolysed, but casein, gelatin, starch, urea and xanthine are not hydrolysed. Cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, acetate, citrate, L-malate, pyruvate and succinate are utilized as carbon and energy sources, but L-arabinose, maltose, sucrose, trehalose, D-xylose, benzoate, formate, salicin, L-glutamate are not utilized. Acid is produced from D-galactose, L-rhamnose, D-ribose and sucrose, but not from L-arabinose, cellobiose, D-fructose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, raffinose, D-sorbitol, trehalose or D-xylose. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, penicillin

G, polymyxin B and streptomycin, but not to lincomycin, novobiocin, oleandomycin or tetracycline. In assays with the API ZYM system, alkaline phosphatase and acid phosphatase are present and esterase (C4) is weakly present, but esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. The predominant ubiquinone is Q-10. The major fatty acid (>10 % of total fatty acids) is C_{18:1} ω 7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and two unidentified lipids. The DNA G+C content of the type strain is 57.9 mol% (determined by HPLC).

The type strain, HD-28^T (=KCTC 23044^T=CCUG 58878^T), was isolated from a tidal flat of Hwang-do, Korea.

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