Ruegeria marina sp. nov., isolated from Marine Sediment

Ying-Yi Huo,[1] Xue-Wei Xu,[2,3] Xue Li,[1] Chen Liu,[1] Heng-Lin Cui,[4] Chun-Sheng Wang[2,3] and Min Wu[1]

Correspondence
Min Wu
wumin@zju.edu.cn

[1]College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China

[2]Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration, Hangzhou 310012, PR China

[3]Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China

[4]School of Food & Biological Engineering, Jiangsu University, Zhenjiang 212013, PR China

A Gram-negative, neutrophilic and rod-shaped bacterium, strain ZH17^T, was isolated from a marine sediment of the East China Sea and subjected to a polyphasic taxonomic characterization. The isolate grew in the presence of 0–7.5 % (w/v) NaCl and at pH 6.5–9.0; optimum growth was observed with 0.5–3.0 % (w/v) NaCl and at pH 7.5. Chemotaxonomic analysis showed ubiquinone-10 as predominant respiratory quinone and $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{16:0}$, $C_{12:0}$ 3-OH and $C_{16:0}$ 2-OH as major fatty acids. The genomic DNA G+C content was 63.5 mol%. Comparative 16S rRNA gene sequence analysis revealed that the isolate belongs to the genus *Ruegeria*. Strain ZH17^T exhibited the closest phylogenetic affinity to the type strain of *Ruegeria pomeroyi*, with 97.2 % sequence similarity, and less than 97 % sequence similarity with respect to other described species of the genus *Ruegeria*. The DNA–DNA reassociation value between strain ZH17^T and *R. pomeroyi* DSM 15171^T was 50.7 %. On the basis of phenotypic and genotypic data, strain ZH17^T represents a novel species of the genus *Ruegeria*, for which the name *Ruegeria marina* sp. nov. (type strain ZH17^T =CGMCC 1.9108^T =JCM 16262^T) is proposed.

The genus *Ruegeria* was first proposed by Uchino *et al.* (1998) with reclassification of *Agrobacterium atlanticum* as the type species *Ruegeria atlantica*. At the same time, *Agrobacterium gelatinovorum and Roseobacter algicola* were reclassified into the genus as *Ruegeria gelatinovorans* and *Ruegeria algicola*, and were subsequently reclassified as *Thalassobius gelatinovorus* (Arahal *et al.*, 2005) and *Marinovum algicola* (Martens *et al.*, 2006). Yi *et al.* (2007) transferred *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi* to the genus *Ruegeria* as *Ruegeria lacuscaerulensis* and *Ruegeria pomeroyi*. Three further species of the genus *Ruegeria*, *Ruegeria mobilis* (Muramatsu *et al.*, 2007), *Ruegeria pelagia* (Lee *et al.*, 2007) and *Ruegeria scottomollicae* (Vandecandelaere *et al.*, 2008), were later described. Recently, *R. pelagia* was considered to be a later synonym of *R. mobilis* (Lai *et al.*, 2010). At the time of writing, the genus *Ruegeria* comprised five recognized species, *R. atlantica*, *R. lacuscaerulensis*, *R. mobilis*, *R. pomeroyi* and *R. scottomollicae*, all isolated from marine environments except for *R. lacuscaerulensis* (from a geothermal lake; Petursdottir & Kristjansson, 1997). Here we present the results of a polyphasic study describing a novel *Ruegeria* strain isolated from marine sediment of the East China Sea.

A marine sediment sample was collected from Zhenhai in the Zhejiang Province of China in October, 2008. Approximately 100 mg of the sample was suspended in 3 ml sterile seawater and vortexed for 15 min. The dispersed sediment suspension was plated on modified ZoBell medium (ZoBell, 1941) agar plates, using a tenfold dilution series method. The modified ZoBell medium contained (l^{-1} distilled water): NaCl 19.45 g, $MgCl_2$ 8.8 g, Na_2SO_4 3.24 g, $CaCl_2$ 1.8 g, KCl 0.55 g, $NaHCO_3$ 0.16 g, ferric citrate 0.1 g, KBr 0.08 g, $CsCl_2$ 34 mg, H_3BO_3 22 mg, Na_2SiO_3 4.0 mg, NaF 2.4 mg, NH_4NO_3 1.6 mg, Na_3PO_4 8.0 mg, peptone (BD) 0.5 g, yeast extract (BD) 0.1 g; pH 7.4. After 3 days of incubation aerobically at 37 °C, a cream coloured colony, named ZH17^T, was picked. The strain was purified by repeated restreaking; purity was confirmed by the uniformity of colony morphology.

Growth at various NaCl concentrations (0, 0.5, 1.0, 3.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0, w/v) was investigated in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ZH17^T is FJ872535.

Two supplementary tables and one supplementary figure are available with the online version of this paper.

marine broth 2216 (MB). The pH range for growth was determined at pH 5.0–10.0 (at intervals of 0.5 pH unit) in MB using the following buffers: MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.0) at a concentration of 40 mmol l^{-1}. The temperature range for growth was determined by incubating at 4, 10, 15, 20, 25, 30, 35, 37, 40, 42, 45 and 48 °C. Cell morphology and motility were examined by optical microscopy (BX40, Olympus) and transmission electron microscopy (JEM-1230, JEOL).

Single carbon source assimilation tests were performed using modified basal medium and determined as González *et al.* (2003) described. The modified basal medium contained (l^{-1} distilled water): NH_4Cl 1.0 g, K_2HPO_4 0.044 g, $FeSO_4.7H_2O$ 0.028 g, yeast extract (BD) 0.1 g, artificial seawater 500 ml, Tris/HCl (1 M, pH 7.5) 50 ml. Artificial seawater contained (l^{-1} distilled water): NaCl 40.0 g, $MgSO_4.7H_2O$ 24.6 g, KCl 1.5 g, $CaCl_2$ 2.9 g. The filter-sterilized sugar (0.2 %), alcohol (0.2 %), organic acid (0.1 %) or amino acid (0.1 %) being tested was added into liquid medium. Biochemical and nutritional tests were performed on marine agar 2216 (MA). API ZYM, API 20 NE and API 20 E (bioMérieux) tests were used to determine physiological and biochemical characteristics. API ZYM strips were read after 12 h, API 20 E and API 20 NE strips after 72 h. Susceptibility to antibiotics was detected on agar plates using antibiotic discs with the following concentrations (μg unless otherwise stated): amoxicillin (10), ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), cefotaxime (30), cefoxitin (30), chloramphenicol (30), erythromycin (15), kanamycin (30), nitrofurantoin (300), novobiocin (30), nystatin (100), penicillin (10), polymyxin B (300 IU), rifampicin (5), streptomycin (10), tetracycline (30) and tobramycin (10).

Fatty acid methyl esters obtained from cells grown in MA (BD) for 3 days at 35 °C were analysed by using GC/MS (Kuykendall *et al.*, 1988). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Phospholipids and glycolipids were separated on silica gel plates (10 × 10 cm) by thin layer chromatography and were analysed according to Kates (1986) and Vaskovsky & Kostetsky (1968). Genomic DNA was obtained using the method described by Marmur & Doty (1962). The purified DNA was hydrolysed with P1 nuclease and the nucleotides dephosphorylated with calf intestine alkaline phosphatase; the G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989). DNA–DNA hybridizations were performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983), using a Beckman DU 800 spectrophotometer.

The 16S rRNA gene of strain ZH17^T was amplified and PCR products were cloned into the pMD 19-T vector (TaKaRa) for sequencing (Xu *et al.*, 2007). An almost complete 16S rRNA gene sequence (1386 nt) was obtained and compared with closely related sequences of reference organisms from the FASTA and EzTaxon service (Chun *et al.*, 2007). Sequence data were aligned by using CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA4 program package (Tamura *et al.*, 2007) and by the maximum-likelihood method (Felsenstein, 1981) with the PHYLIP 3.6 program. Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the neighbour-joining method.

Cells of strain ZH17^T were Gram-negative, rod-shaped and approximately 0.5–1.0 μm wide and 2.0–4.5 μm long (Supplementary Fig. S1, available in IJSEM Online). The detailed phenotypic characteristics of strain ZH17^T are given in the species description. A comparison of the phenotypic properties of strain ZH17^T and *R. pomeroyi* DSM 15171^T is shown in Table 1. Detailed results are given in the species description, Table 1 and Supplementary Table S1.

Comparisons of 16S rRNA gene sequences showed that strain ZH17^T should be positioned within the genus *Ruegeria*, related most closely to the type strain of *R. pomeroyi* with 97.2 % similarity; sequence similarities with respect to type strains of other recognized species of the genus *Ruegeria* were 94.9–95.7 %. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain ZH17^T had the closest phylogenetic affinity to the type strain of *R. pomeroyi* with high levels of bootstrap support (Fig. 1).

Table 1. Differentiating characteristics of strain ZH17^T and its closest phylogenetic relative, *Ruegeria pomeroyi* DSM 15171^T

Strains: 1, ZH17^T; 2, *Ruegeria pomeroyi* DSM 15171^T. Data were obtained from this study under identical growth conditions. +, Positive; −, negative; w, weakly positive.

Characteristics	1	2
Hydrolysis of:		
Casein	w	−
Tween 40	−	+
Tween 60	−	+
Tween 80	−	+
Utilization of:		
Gluconate	−	+
Malonate	+	−
Propionate	+	−
API 20NE:		
Lysine decarboxylase	−	+
Ornithine decarboxylase	−	+
API ZYM:		
Naphthol-AS-BI-phosphohydrolase	w	−
Valine arylamidase	+	−
DNA G+C content (mol%)	63.5	68.0*

*Data from González *et al.* (2003).

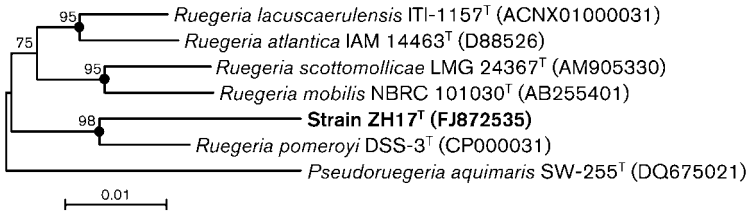

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolate and related taxa. Bootstrap values are based on 1000 replicates; only values >70 % are shown. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate nodes recovered with bootstrap values >80 % in both maximum-likelihood and maximum-parsimony trees.

The DNA–DNA relatedness value of 50.7 % between strain ZH17^T and *R. pomeroyi* DSM 15171^T was significantly below the value of 70 % considered to be the threshold for the delineation of species (Wayne *et al.*, 1987). Chemotaxonomic characteristics of strain ZH17^T were typical of the genus *Ruegeria* in having ubiquinone-10 as the predominant quinone, a polar lipid profile comprising phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, three unidentified phospholipids and three unidentified lipids, and $C_{18:1}\omega7c$ and 11-methyl $C_{18:1}\omega7c$ as predominant cellular fatty acids (Martens *et al.*, 2006; Yi *et al.*, 2007). The major fatty acids of strain ZH17^T were $C_{18:1}\omega7c$ (54.8 %), 11-methyl $C_{18:1}\omega7c$ (17.5 %), $C_{16:0}$ (8.5 %), $C_{12:0}$ 3-OH (5.3 %) and $C_{16:0}$ 2-OH (5.0 %). Nevertheless, the proportion of $C_{18:1}\omega7c$ in strain ZH17^T was higher than that in *R. pomeroyi* DSM 15171^T (36.1 %) and in *R. atlantica* KCTC 12017 (44.7 %), whereas the proportion of 11-methyl $C_{18:1}\omega7c$ in strain ZH17^T was lower than that found in *R. pomeroyi* DSM 15171^T (25.5 %) and *R. atlantica* KCTC 12017 (26.9 %) (Supplementary Table S2). In addition, strain ZH17^T could be differentiated from the recognized species of the genus *Ruegeria* on the basis of some phenotypic characteristics, including growth at different temperatures and NaCl concentrations, nitrate reduction, hydrolysis of substrates, utilization of substrates and susceptibility to antibiotics (Supplementary Table S1). Strain ZH17^T could also be distinguished from *Ruegeria pomeroyi* DSM 15171^T by several phenotypic characteristics, including hydrolysis of casein and Tweens 40, 60 and 80, utilization of gluconate, malonate and propionate, and activities of lysine and ornithine decarboxylases and valine arylamidase (Table 1).

On the basis of the phylogenetic, genotypic, chemotaxonomic and phenotypic data, we propose to classify strain ZH17^T as the type strain of a new species within the genus *Ruegeria*, *Ruegeria marina* sp. nov.

Description of *Ruegeria marina* sp. nov.

Ruegeria marina (ma.ri′na. L. fem. adj. *marina* marine, of the sea, where the type strain was isolated).

Cells are Gram-negative, rod-shaped and non-motile. Cells are 0.5–1.0 μm wide and 2.0–4.5 μm long. Colonies on MA are 1.5–2 mm in diameter, rough, slightly elevated and cream-coloured with regular edges after 3 days at 35 °C. Growth occurs at NaCl concentrations of 0–7.5 % (w/v), with optimum growth at 0.5–3.0 %. The pH and temperature ranges for growth are pH 6.5–9.0 and 10–42 °C (optimum growth at pH 7.5 and 35–37 °C). Oxidase- and catalase-positive. Casein, gelatin, tyrosine and Tween 20 are hydrolysed. Aesculin, starch, Tween 40, Tween 60, Tween 80 and urea are not hydrolysed. Indole production and activities of arginine dihydrolase, *o*-nitrophenyl-β-D-galactopyranosidase, lysine and ornithine decarboxylases and tryptophan deaminase are negative. Citrate utilization is positive. Nitrate is not reduced to nitrite. The following substrates are utilized for growth: acetate, L-alanine, L-arginine, citrate, L-cysteine, ethanol, glucose, L-glutamate, L-glutamine, glycerol, L-histidine, L-isoleucine, lactate, L-lysine, malate, malonate, L-ornithine, propionate, pyruvate, succinate, L-serine and D-xylose. The following compounds are not utilized as sole carbon and energy sources: L-arabinose, cellobiose, formate, D-fructose, fumarate, D-galactose, gluconate, glycine, inositol, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-salicin, D-sorbitol, starch, sucrose and trehalose. In the API ZYM system, acid and alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activities are present, whereas α-chymotrypsin, cystine arylamidase, α- and β-galactosidases, *N*-acetyl-β-glucosaminidase, α- and β-glucosidases, β-fucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin activities are absent. Susceptible to amoxicillin (10 μg), ampicillin (10 μg), carbenicillin (100 μg), cefotaxime (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), nitrofurantoin (300 μg), novobiocin (30 μg), penicillin G (10 IU), polymyxin B (300 IU), rifampicin (5 μg), streptomycin (10 μg), tetracycline (10 μg) and tobramycin (10 μg), but not susceptible to bacitracin (0.04 IU) and nystatin (100 μg). The predominant quinone is ubiquinone-10. The major polar lipids of strain ZH17^T include phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, three unidentified phospholipids and three unidentified lipids. The major fatty acids (>5 %) include $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{16:0}$, $C_{12:0}$ 3-OH and $C_{16:0}$ 2-OH. The DNA G+C content of the type strain is 63.5 mol%.

The type strain, ZH17^T (=CGMCC 1.9108^T =JCM 16262^T), was isolated from marine sediment of the East China Sea.

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