Ruegeria pelagia sp. nov., isolated from the Sargasso Sea, Atlantic Ocean

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Gram-negative, facultatively aerobic, chemoheterotrophic, short rod-shaped marine bacterial strains HTCC2662^T and HTCC2663, isolated from the Sargasso Sea by using a dilution-to-extinction culturing method, were investigated to determine their taxonomic position. Characterization of the two strains by phenotypic and phylogenetic analyses revealed that they belonged to the same species. The DNA G+C content of strain HTCC2662^T was 58.4 mol% and the predominant cellular fatty acids were $C_{18:1}\omega7c$ (52.5 %), $C_{16:0}$ 2-OH (13.5 %) and $C_{18:1}$ 11-methyl $\omega7c$ (12.2 %). Phylogenetic analysis of the 16S rRNA gene sequences showed that the strains represented a distinct line of descent within the genus *Ruegeria*, with highest sequence similarities to *Ruegeria atlantica* DSM 5823^T (97.2 %), *Ruegeria lacuscaerulensis* DSM 11314^T (96.5 %) and *Ruegeria pomeroyi* DSM 15171^T (95.6 %). Several phenotypic characteristics, including facultatively requiring NaCl and oxygen for growth, together with the cellular fatty acid composition, differentiated strain HTCC2662^T from other members of the genus *Ruegeria*. Based on phenotypic, chemotaxonomic and phylogenetic traits, it is suggested that strains HTCC2662^T and HTCC2663 represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria pelagia* sp. nov. is proposed. The type strain is HTCC2662^T (=KCCM 42378^T=NBRC 102038^T).

The genus *Ruegeria* in the order *Rhodobacterales* was erected by Uchino *et al.* (1998) and contains Gram-negative, aerobic, oxidase- and catalase-positive, non-phototrophic bacteria that require NaCl or sea salts for growth. At the time of writing, the genus comprises three recognized species, *Ruegeria atlantica*, *Ruegeria lacuscaerulensis* and *Ruegeria pomeroyi*. These three species have been isolated from saline environments. *R. lacuscaerulensis* and *R. pomeroyi* were originally classified as *Silicibacter lacuscaerulensis* (Petursdottir & Kristjansson, 1997) and *Silicibacter pomeroyi* (González *et al.*, 2003), respectively, but *Silicibacter* was recently combined with the genus *Ruegeria* by Yi *et al.* (2007) based on 16S rRNA gene sequence phylogeny and chemotaxonomy. In the present study, we describe the isolation and identification of a novel species of the genus *Ruegeria*.

Initial liquid cultures of two strains, designated HTCC2662^T and HTCC2663, were obtained by using the high-throughput culturing approaches of Cho & Giovannoni (2003) and Connon & Giovannoni (2002) according to a dilution-to-extinction methodology. The strains were subsequently purified as single colonies on marine agar 2216 (MA; Difco) after incubation for 4 days at 25 °C.

DNA extraction, PCR and sequencing of the 16S rRNA gene were performed as described by Cho & Giovannoni (2003). The resultant 16S rRNA gene sequences for strains HTCC2662^T (1425 bp) and HTCC2663 (1425 bp) were aligned by using the ARB software package (Ludwig *et al.*, 2004) and 1184 unambiguously aligned nucleotide positions were used for phylogenetic analyses in PAUP* 4.0 beta 10 (Swofford, 2002). Phylogenetic trees were generated according to the neighbour-joining (Saitou & Nei, 1987) – with Kimura two-parameter model correction (Kimura, 1980) – maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms. Bootstrap analyses were performed for the neighbour-joining and maximum-parsimony trees based on 1000 resamplings. The 16S rRNA gene sequences of strains HTCC2662^T and HTCC2663 were identical, suggesting that these two strains represented the same species. Preliminary comparisons with 16S rRNA gene sequences deposited in the GenBank database indicated that the two new strains were closely related to members of the genus *Ruegeria*. Based on multiple alignment in the ARB

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains HTCC2662^T and HTCC2663 are DQ916141 and EF134718, respectively.

A transmission electron micrograph of cells of strain HTCC2662^T is available with the online version of this paper.

database, strain HTCC2662^T showed highest 16S rRNA gene sequence similarity to *R. atlantica* DSM 5823^T (97.2 %), followed by *R. lacuscaerulensis* DSM 11314^T (96.5 %) and *R. pomeroyi* DSM 15171^T (95.6 %). In all the phylogenetic trees generated in this study (Fig. 1), strains HTCC2662^T and HTCC2663 formed a monophyletic clade together with the three recognized species of the genus *Ruegeria*, with relatively high bootstrap support (97 % in the neighbour-joining tree and 71 % in the maximum-parsimony tree). The new strains formed an independent phylogenetic lineage within the genus *Ruegeria*, suggesting that they were not associated with any of the recognized species of the genus and thus represented a novel species in the genus *Ruegeria*.

Phenotypic characterizations of strains HTCC2662^T and HTCC2663 were carried out as described by Cho & Giovannoni (2003) and Smibert & Krieg (1994) with MA as the basal medium at 30 °C. Cell morphology was examined by energy-filtering transmission electron microscopy (LIBLA120; Carl Zeiss), and phase-contrast and epifluorescence microscopy (Nikon 80i). Anaerobic growth was tested on MA at 30 °C using both the MGC anaerobic system and the AnaeroPack Anaero (Mitsubishi Gas Chemical Company, Inc.). Biochemical tests, enzyme activities and oxidative carbon source utilization tests were carried out on API 20NE (bioMérieux), API ZYM (bioMérieux) and Biolog GN2 microplates, respectively, with artificial seawater (ASW; per litre: 25.0 g NaCl, 1.0 g $MgCl_2.6H_2O$, 5.0 g $MgSO_4.7H_2O$, 0.7 g KCl, 0.15 g $CaCl_2.2H_2O$, 0.5 g NH_4Cl, 0.1 g KBr, 0.27 g KH_2PO_4, 0.04 g $SrCl_2.6H_2O$, 0.025 g H_3BO_3) following the manufacturer's instructions. Susceptibility to ten different antimicrobial agents (listed in the species description below) was tested by the diffusion plate method. The DNA G+C content and cellular fatty acid composition were analysed for strain HTCC2662^T. The DNA G+C content was analysed by using HPLC with a Discovery C18 column (5 μm, 15 cm × 4.6 mm; Supelco) (Mesbah *et al.*, 1989). Cellular fatty acid methyl esters were prepared from a culture grown on MA at 30 °C for 3 days, and analysed according to the instructions of the Microbial Identification System (MIDI) and performed at the Korean Culture Center of Micro-organisms (KCCM).

The results of morphological, physiological and biochemical tests are given in the species description and in Table 1. Strains HTCC2662^T and HTCC2663 showed identical phenotypic and physiological traits. The two strains were therefore regarded as representing the same species based on 16S rRNA gene sequence similarities (100 %) and phenotypic characterizations. The two strains could clearly be differentiated from recognized *Ruegeria* species, mainly by NaCl requirement for growth, oxygen requirement for growth and acid production from glucose (Table 1). The DNA G+C content of strain HTCC2662^T was 58.4 mol%, which is within the range of values (55–68 mol%) reported for the genus *Ruegeria*. The major cellular fatty acids of strain HTCC2662^T were generally consistent with those of the three recognized *Ruegeria* species (Table 1); however, strain HTCC2662^T could be differentiated based on the proportions of several fatty acids, including $C_{18:1}\omega7c$, $C_{16:0}$ 2-OH, $C_{18:1}$ 11-methyl $\omega7c$, $C_{16:0}$ and $C_{12:0}$ 3-OH.

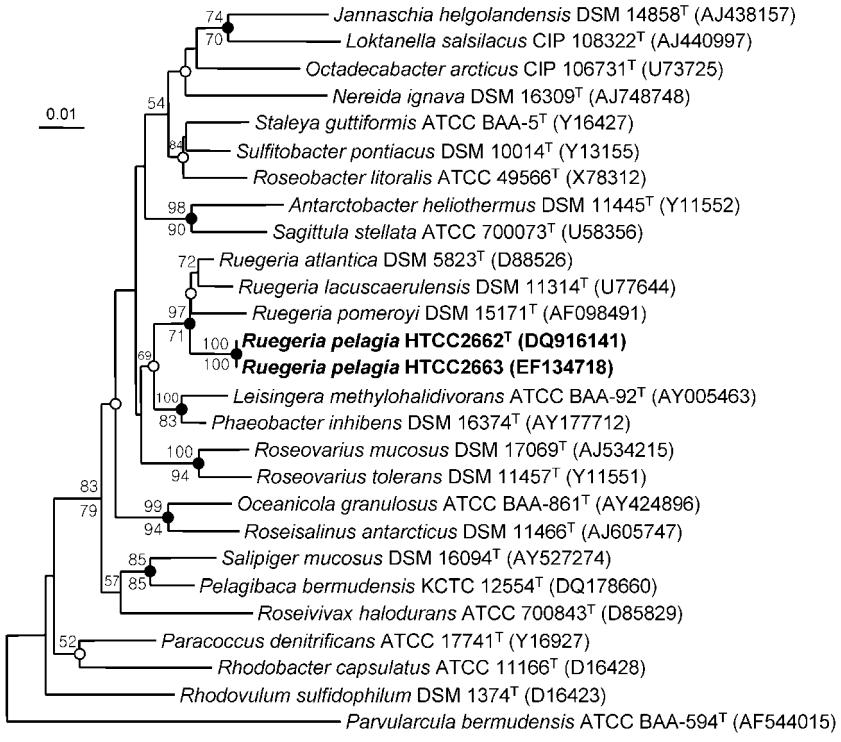

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains HTCC2662^T and HTCC2663 and members of the order *Rhodobacterales*. Bootstrap percentages (above 50 %) from both neighbour-joining (above nodes) and maximum-parsimony (below nodes) methods are shown. Filled and open circles at each node indicate nodes recovered reproducibly by all treeing methods or by two treeing methods. Bar, 0.01 substitutions per nucleotide position.

Table 1. Characteristics that differentiate strain HTCC2662^T from members of the genus *Ruegeria*

Strains: 1, HTCC2662^T; 2, *R. atlantica* DSM 5823^T; 3, *R. lacuscaerulensis* DSM 11314^T; 4, *R. pomeroyi* DSM 15171^T. +, Positive; −, negative; w, weak reaction. Data are taken from this study and from Rüger & Höfle (1992), Uchino *et al.* (1998), Petursdottir & Kristjansson (1997), González *et al.* (2003) and Yi *et al.* (2007).

Characteristic	1	2	3	4
Cell shape	Short rod	Short rod	Long rod	Short rod
Motility	−	−	−	+
Colony colour	Dark brown	Beige	Tan	Cream
Growth at 10 °C	−	+	+	−
Growth at 40 °C	+	−	+	+
Salt requirement	−	+	+	+
Anaerobic growth	+	−	−	−
Nitrate reduction	−	+	+	−
Acid production from glucose	+	−	−	−
Aesculin hydrolysis	+	+	+	−
Gelatinase	−	−	−	+
β-Galactosidase	+	+	+	−
Esterase (C4)	+	−	−	−
Esterase lipase (C8)	+	−	−	w
Valine arylamidase	+	w	−	−
Acid phosphatase	+	−	+	−
Naphthol-AS-BI-phosphohydrolase	+	−	+	−
α-Glucosidase	+	w	+	−
N-Acetyl-β-glucosaminidase	+	−	−	−
Glucose utilization	+	+	−	+
Acetic acid utilization	+	+	−	+
DNA G+C content (mol%)	58.4	55	66	68
Major fatty acid composition (%):				
$C_{18:1}\omega7c$	52.5	44.7	72.9	76.3
$C_{16:0}$ 2-OH	13.5	8.9	0.4	2.8
$C_{18:1}$ 11-methyl $\omega7c$	12.2	26.9	7.3	1.9
$C_{16:0}$	2.0	3.4	2.1	7.8
$C_{12:0}$ 3-OH	1.0	5.9	4.1	4.9

On the basis of the combined phenotypic, chemotaxonomic and phylogenetic evidence presented, strains HTCC2662^T and HTCC2663 are considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria pelagia* sp. nov. is proposed.

Description of *Ruegeria pelagia* sp. nov.

Ruegeria pelagia (pe.la′gi.a. L. fem. adj. *pelagia* of the sea).

Cells are Gram-negative and non-motile short rods that are 0.8–2.1 μm in length and 0.7-1.2 μm in width (see Supplementary Fig. S1 available in IJSEM Online). Colonies on MA are circular, smooth, convex, butyrous or (sometimes) viscous with entire margins and dark brown-coloured. Dark-brown precipitates are observed around the colonies. Growth occurs at 16–42 °C (optimally at 30–37 °C), pH 4–12 (optimally at pH 5–6) and 0–10 % NaCl (optimally at 3.5 %). NaCl is not essential for growth. Chemoheterotrophic and facultatively aerobic. No bacteriochlorophyll *a* is present. Does not produce poly-β-hydroxyalkanoate granules. Oxidase- and catalase-positive. In API 20NE tests, positive for β-galactosidase, aesculin hydrolysis and acid production from glucose, but negative for nitrate reduction, indole production, urease, arginine dihydrolase and gelatin liquefaction. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and *N*-acetyl-β-glucosaminidase activity, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galatosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activity. In tests with Biolog GN2 microplates, the following carbon substrates produce positive results: α-cyclodextrin, dextrin, glycogen, Tweens 40 and 80, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, D-fructose, D-galactose, α-D-glucose, *myo*-inositol, α-D-lactose, maltose, D-mannitol, D-mannose, D-psicose, D-sorbitol, sucrose, trehalose, turanose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid, D-galactonic acid lactone, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid,

propionic acid, D-saccharic acid, succinic acid, bromo-succinic acid, succinamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL-α-glycerol phosphate. The following carbon substrates produce negative results in Biolog GN2 plates: *N*-acetyl-D-galactosamine, i-erythritol, L-fucose, gentiobiose, lactulose, D-melibiose, methyl β-D-glucoside, D-raffinose, L-rhamnose, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, *p*-hydroxyphenlyacetic acid, itaconic acid, malonic acid, quinic acid, sebacic acid, glucuronamide, phenylethylamine, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Susceptible to chloramphenicol (25 μg), erythromycin (15 μg), rifampicin (50 μg), streptomycin (10 μg) and tetracycline (30 μg), but resistant to ampicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), penicillin G (10 μg) and vancomycin (30 μg). The cellular fatty acid profile comprises $C_{18:1}\omega7c$ (52.5 %), $C_{16:0}$ 2-OH (13.5 %), $C_{18:1}$ 11-methyl $\omega7c$ (12.2 %), $C_{18:1}$ 2-OH (6.8 %), $C_{10:0}$ 3-OH (2.9 %), $C_{16:0}$ (2.0 %), $C_{12:0}$ 3-OH (1.0 %), $C_{18:0}$ (0.9 %), $C_{18:0}$ 2-OH (0.5 %), $C_{16:1}$ 2-OH (0.4 %), $C_{17:0}$ 2-OH (0.3 %), $C_{12:0}$ (0.3 %) and $C_{20:1}\omega7c$ (0.3 %). The DNA G+C content is 58.4 mol% (by HPLC).

The type strain, HTCC2662^T (=KCCM 42378^T=NBRC 102038^T), was isolated from Bermuda Atlantic Time Series Station in the western Sargasso Sea, Atlantic Ocean.

Acknowledgements

This study was supported by the 21C Frontier programme of Microbial Genomics and Applications (grant MG05-0102-1-0) from the Ministry of Science and Technology, Republic of Korea. Strains HTCC2662^T and HTCC2663 were isolated in S. J. G.'s laboratory by J.-C. C., with grant support from the Gordon and Betty Moore Foundation.

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