Ruegeria mobilis sp. nov., a member of the *Alphaproteobacteria* isolated in Japan and Palau

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The taxonomic positions of two bacterial strains, MBIC01146^T and MBIC01099, isolated from marine environments of Japan and Palau, respectively, were determined by using a polyphasic approach. The bacteria were aerobic, motile, Gram-negative rods and formed star-shaped aggregations. The G + C content of the genomic DNA ranged from 58.5 to 58.7 mol%. The major respiratory quinone was ubiquinone-10 and the predominant cellular fatty acids were C_{16:0}, C_{18:1}ω6c and C_{18:1}ω7c. 16S rRNA gene sequence analyses based on both neighbour-joining and maximum-parsimony methods revealed that strains MBIC01146^T and MBIC01099 were positioned within the cluster comprising *Ruegeria atlantica* and *Silicibacter latuscaerulensis* within subgroup α-3 of the *Proteobacteria*. The phenotypic and chemotaxonomic characteristics of the novel strains were similar to those of *Ruegeria atlantica*; however, DNA–DNA hybridization tests showed that the isolates represented an independent species. The isolates could be differentiated from *Ruegeria atlantica* based on several characteristics. Therefore, strains MBIC01146^T and MBIC01099 are considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria mobilis* sp. nov. is proposed. The type strain is MBIC01146^T (=NBRC 101030^T =CIP 109181^T). An emended description of *Ruegeria atlantica* Uchino *et al.* 1999 is also given.

Strain MBIC01146^T was isolated from a slime attached to a glass slide that was floated on the sea surface for 1 day at Ishigaki island, Japan, in 1990. The slime was diluted with sterilized seawater and then cultured on a B2 agar plate (Izumida *et al.*, 1995). Strain MBIC01099 was isolated from a coelenterate (*Hexacorallia* sp.) recovered from Honeybee Channel, Palau, in 1991. The coelenterate was homogenized and diluted with sterilized seawater and then enriched in B6 medium [0.25 g Polypepton, 0.25 g Polypepton S, 0.25 g Polypepton Y (all Nihon Pharmaceuticals), 0.5 g brain heart infusion (Difco), 1.0 g yeast extract, 0.25 g malt extract, 0.25 g soluble starch, 0.5 g potato glucose broth (Difco), 26.0 g sea salt (Tropic Marin) and 0.5 g HEPES buffer, per litre distilled water, pH 7.7] supplemented with 10 mM eosin Y (Wako Pure Chemical Industries). The enriched sample was incubated at 25 °C for 1 day on a B6 agar

medium. Based on initial 16S rRNA gene sequence analysis, the isolates were tentatively identified as representing marine *Agrobacterium* species belonging to subgroup α-3 of the *Proteobacteria*, given that their phylogenetically closest relative was *Agrobacterium atlanticum*. *A. atlanticum* was reclassified within the genus *Ruegeria* as three species, *Ruegeria atlantica* (type species), *Ruegeria gelatinovorans* and *Ruegeria algicola* (Uchino *et al.*, 1998). Recently, the latter two species were reclassified as *Thalassobius gelatinovorans* and *Marinovum algicola*, respectively (Arahal *et al.*, 2005; Martens *et al.*, 2006). As a result, the genus *Ruegeria* now comprises a single recognized species. In addition, several new genera have been proposed within subgroup α-3 of the *Proteobacteria* (e.g. Labrenz *et al.*, 1999; Schaefer *et al.*, 2002). Here we characterized two strains, MBIC01146^T and MBIC01099, based on genomic, chemotaxonomic and phenotypic data.

Cellular morphology was observed under light and transmission electron microscopy. For the latter, fresh bacterial cultures were negatively stained with 1 % phosphotungstic acid (pH 7.0) and observed under a Hitachi H-7600 transmission electron microscope at an accelerating voltage of 80 kV. Cells of strains MBIC01146^T and MBIC01099 formed star-shaped aggregates, similar to those of *R. atlantica* NBRC 15792^T (Rüger & Höfle,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Ruegeria mobilis* MBIC01146^T and MBIC01099, *Ruegeria atlantica* NBRC 15792^T, *Thalassobius gelatinovorans* NBRC 15761^T and *Marinovum algicola* NBRC 16653^T are AB255401, AB255400, AB255399, AB289591 and AB289592, respectively.

A maximum-parsimony tree showing the phylogenetic positions of strains MBIC01146^T and MBIC01099 based on 16S rRNA gene sequences is available as supplementary material with the online version of this paper.

Fig. 1. Transmission electron micrographs of negatively stained cells of strain MBIC01146^T. (a) Star-shaped aggregate formation; (b) cell showing several polar flagella. Bars, 1 µm.

1992), when they were statically cultivated to late exponential phase for 20 h in one-fifth-strength LBM medium (Suzuki *et al.*, 2001) (Fig. 1a). Bruhn *et al.* (2005) reported that *Roseobacter* sp. 27-4 formed star-shaped aggregates with pigment production. The two new isolates formed star-shaped aggregates without colour changes. Cells were motile and possessed several polar flagella (Fig. 1b). In contrast, we confirmed that cells of *R. atlantica* NBRC 15792^T were non-motile, as previously reported (Rüger & Höfle, 1992; Uchino *et al.*, 1998).

Genomic DNA extraction and 16S rRNA gene sequencing were performed as described by Nakagawa *et al.* (2002). Sequence data obtained were aligned with those of representative members of subgroup α -3 of the *Proteobacteria* by using CLUSTAL X (Thompson *et al.*, 1997) and then modified manually by referring to the 16S rRNA gene secondary structure of *Escherichia coli* (Gutell *et al.*, 1994) and using the Se-AI v2.0 alignment editor (A. Rambaut; available at <http://>

evolve.zoo.ox.ac.uk/). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) with calculated K_{nuc} values (Kimura, 1980), as well as the maximum-parsimony method with PAUP 4.0b10 (Swofford, 2002). Topology of the trees was evaluated by the bootstrap analysis method of Felsenstein (1985) with 1000 replicates. Almost complete sequences of the 16S rRNA genes from positions 28–1494 according to the *E. coli* numbering system (Brosius *et al.*, 1978) were determined for strains MBIC01146^T and MBIC01099 as well as for *R. atlantica* NBRC 15792^T. Analysis of the 16S rRNA gene sequences of the unclassified strains resulted in their clustering with *R. atlantica*, *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi* within subgroup α -3 of the *Proteobacteria* (Fig. 2 and Supplementary Fig. S1 in IJSEM Online). Levels of similarity in the 16S rRNA gene sequences between strain MBIC01146^T and *R. atlantica* NBRC 15792^T, *S. lacuscaerulensis* ITI-1157^T and *S. pomeroyi* DSS-3^T were 97.1, 96.6 and 96.3 %, respectively.

DNA–DNA hybridization experiments were carried out in microplate wells by using photobiotin-labelled probes as described by Ezaki *et al.* (1989). The hybridization temperature was 48 °C in 2 × SSC containing 50 % formamide.

Fig. 2. Neighbour-joining tree showing the phylogenetic positions of strains MBIC01146^T (=NBRC 101030^T) and MBIC01099 (=NBRC 101029) based on 16S rRNA gene sequences. *Escherichia coli* K-12 (GenBank accession no. E05133) was used as an outgroup (not shown). Bootstrap values of 700 or more in 1000 replicates are shown. Bar, 0.02 K_{nuc} . A maximum-parsimony tree is available as Supplementary Fig. S1 in IJSEM Online.

The results indicated that strains MBIC01146^T and MBIC01099 represented a single species, given that their DNA–DNA relatedness values were above 75 % (Wayne *et al.*, 1987). Strain MBIC01146^T showed 4–5 % DNA–DNA relatedness with *R. atlantica* NBRC 15792^T. Hybridizations between the new isolates and *S. lacuscaerulensis* ITI-1157^T were not performed because 16S rRNA gene sequence similarities between these taxa were below 96.6 %. It has been reported that if 16S rRNA sequence similarities between two organisms are lower than 97 %, they must be classified as separate species (Wayne *et al.*, 1987). G+C contents of the DNA samples were subsequently determined according to Mesbah *et al.* (1989). The values for strains MBIC01146^T and MBIC01099 were 58.5 and 58.7 mol%, respectively, similar to the value of 59.4 mol% obtained in this study for *R. atlantica* NBRC 15792^T. Reported DNA G+C content values for the genus *Silicibacter* are 64.1–66.2 mol% (Petursdottir & Kristjansson, 1997; González *et al.*, 2003; Whitman, 2006).

For analysis of whole-cell fatty acid composition, all strains were grown on Bacto marine agar 2216 (Difco) at 25 °C for 48 h. Fatty acid methyl esters were prepared and analysed according to the standard protocol described in the Microbial Identification System (Microbial ID). Strains MBIC01146^T and MBIC01099 showed similar fatty acid profiles, in which C_{16:0}, C_{18:1}ω6c and C_{18:1}ω7c were the dominant components (more than 5 % of the total) (Table 1). The major fatty acids in *R. atlantica* NBRC 15792^T were C_{16:0}, C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The dominant fatty acids in *S. lacuscaerulensis* and *S. pomeroyi* were reported to be C_{18:0}, C_{12:0} 3-OH and C_{18:1}ω7c, and C_{16:0}, C_{12:0} 3-OH and C_{18:1}ω7c, respectively (González *et al.*, 2003). *R. atlantica*, *S. lacuscaerulensis*, *S. pomeroyi* and the new isolates had similar fatty acid contents in which the dominant component was C_{18:1}ω7c. Q-10 was found to be the major respiratory quinone in strains MBIC01146^T and MBIC01099 as well as in *R. atlantica*, when extracted according to Nakagawa & Yamasato (1993) and analysed by using an LC-MS 8000α spectrometer (Shimadzu).

Production of indole, H₂S, oxidase and nitrate reductase was examined as described by Smibert & Krieg (1981). Utilization of urea, sodium nitrate, ammonium sulfate, sodium glutamate, peptone (Difco) and Casamino acids as nitrogen sources was examined by using basal medium (0.1 % glucose, 0.02 % sodium acetate, 70 % artificial seawater, pH 7.2) supplemented with each element. Decomposition of urea was examined as described by Rustigian & Stuart (1941). To analyse the growth response at different temperatures (increments of 5 °C) and pH values (pH 3–11, increments of 1 pH unit), we used one-fifth-strength LBM medium for cultivation with shaking. NaCl range for growth was determined by using one-fifth-strength LB medium supplemented with 0, 1, 3, 5, 7 or 10 % NaCl. Carbohydrate assimilation was determined by using the API 50CH system (bioMérieux). Several colonies on agar plates were suspended in artificial seawater supplemented with

Table 1. Cellular fatty acid compositions (%) of strains MBIC01146^T and MBIC01099 and members of the genera *Ruegeria* and *Silicibacter*

Strains: 1, MBIC01146^T; 2, MBIC01099; 3, *R. atlantica* NBRC 15792^T; 4, *S. lacuscaerulensis* ITI-1157^T (data from González *et al.*, 2003); 5, *S. pomeroyi* DSS-3^T (González *et al.*, 2003). –, Not detected/not reported.

Fatty acid	1	2	3	4	5
Straight-chain					
C _{10:0}	–	–	2.1	2.5	3.1
C _{12:0}	–	0.2	2.6	2.1	1.1
C _{16:0}	10.3	10.8	6.9	1.6	7.8
C _{17:0}	0.2	0.3	–	0.3	–
C _{18:0}	1.0	1.1	1.0	2.6	1.6
Unsaturated					
C _{18:1} ω6c	6.4	6.5	–	–	–
C _{18:1} ω7c	74.9	74.0	71.2	81.7	76.3
11-Methyl C _{18:1} *	–	–	–	2.2	1.9
11-Methyl C _{18:1} ω7c	1.9	1.2	7.1	–	–
C _{20:1} ω7c	–	–	0.2	–	–
Hydroxy					
C _{10:0} 3-OH	2.0	2.2	0.4	1.6	0.6
C _{12:0} 3-OH	0.3	0.5	4.7	4.0	4.9
C _{16:0} 2-OH	0.4	0.5	3.4	–	2.8
Summed feature 3†	0.5	0.5	0.3	0.3	–
Unknown	2.2	2.2	0.1	–	–

*The site of the double bond was not assigned by González *et al.* (2003).

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

0.05 % yeast extract (Lau *et al.*, 2005) and were added to test strips. Colour was compared with medium in which no cells were suspended. No specific signals or acid production were observed with the carbon sources used in the API 20E, API 20NE, API ZYM and Biolog systems. Strains MBIC01146^T, MBIC01099 and *R. atlantica* NBRC 15792^T were positive for utilization of glucose in the API 50CH system. In contrast, it was reported that utilization of glucose was negative for *S. lacuscaerulensis* ITI-1157^T (Petursdottir & Kristjansson, 1997). Other phenotypic characteristics of strains MBIC01146^T and MBIC01099 are summarized in the species description below and in Table 2.

In the phylogenetic analysis of the 16S rRNA gene sequences, strains MBIC01146^T and MBIC01099 clustered with species belonging to the genera *Ruegeria* and *Silicibacter*. 16S rRNA gene sequence analysis suggested that the genera *Ruegeria* and *Silicibacter* were closely related and should be integrated into one genus. However, their differential phenotypic properties did not support this unification. The genus *Ruegeria* Uchino *et al.* 1999 (Validation List No. 68) has priority over *Silicibacter* Petursdottir and Kristjansson 1999 (Validation

Table 2. Differential phenotypic characteristics between strains MBIC01146^T and MBIC01099 and closely related taxa

Taxa: 1, strain MBIC01146^T; 2, strain MBIC01099; 3, *R. atlantica* NBRC 15792^T; 4, *S. lacuscaerulensis* ITI-1157^T (data from Petursdottir & Kristjansson, 1997); 5, *S. pomeroyi* DSS-3^T (González *et al.*, 2003; Whitman, 2006). +, Positive; –, negative; w, weakly positive; (w), weakly positive after 2 weeks; ND, no data. Strains MBIC01146^T and MBIC01099 and *R. atlantica* NBRC 15792^T are all negative for utilization of i-erythritol, D-arabinose, L-xylose, methyl β -D-xyloside, sorbose, dulcitol, methyl γ -D-mannoside, methyl γ -D-glucoside, amygdalin, salicin, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, D-turanose, D-lyxose, D-tagatose, L-fucose and 5-ketoglucuronate (API 50CH).

Characteristic	1	2	3	4	5
Cell size (μ m) (diameter $\times$ length)	0.6 $\times$ 2.0	0.8 $\times$ 1.0	0.8 $\times$ 4.0	0.6–0.8 $\times$ 9–18	ND
Motility	+	+	–	–	+
Flagella	Multiple	Multiple	–	–	Single
Vacuole (inclusion body)	–	–	–	+	+
Growth conditions					
Temperature range ($^{\circ}$ C) [optimum]	5–35 [30]	5–35 [30]	5–30 [25]	22–50 [45]	10–40 [30]
pH range [optimum]	5–11 [7]	6–11 [7]	6–11 [7]	6.5–8.5 [7.5]	ND
NaCl range (%) [optimum]	0–10 [1]	0–10 [3]	3–10 [3]	1.5–7 [3.5]	1.5–5.8
Hydrolysis of gelatin	–	+	–	ND	+
Nitrate reduction to nitrite	–	w	+	+	–
Nitrite reduction to gas	–	–	+	ND	–
Utilization, as sole nitrogen source, of:					
Ammonium sulfate	+	w	–	ND	ND
Urea	+	+	–	ND	ND
Assimilation of (API 50CH test):					
L-Arabinose	+	+	–	ND	ND
Adonitol	+	+	–	ND	ND
Glucose	+	+	+	–	+
Fructose	+	+	–	ND	–
Rhamnose	–	+	–	ND	–
Mannitol	+	+	(w)	ND	–
Sorbitol	w	w	–	ND	ND
Arbutin	–	–	+	ND	ND
Xylitol	+	+	–	ND	ND
D-Fucose	+	+	–	ND	ND
D-Arabitol	+	+	–	ND	ND
L-Arabitol	w	+	–	ND	ND
Gluconate	–	–	w	ND	ND
DNA G + C content (mol%)	58.5	58.7	59.4	66.2	64.1

List No. 71). We conclude that strains MBIC01146^T and MBIC01099 should be included in the genus *Ruegeria* based on similar morphological features, fatty acid compositions and DNA G + C contents. However, DNA–DNA hybridization experiments indicated that they constituted an independent species that was clearly differentiated from *R. atlantica* based on motility, ability to grow without NaCl and other characteristics summarized in Table 2. Thus, strains MBIC01146^T and MBIC01099 are considered to represent a novel species of the genus *Ruegeria*, for which the name *Ruegeria mobilis* sp. nov. is proposed. We emend below the species description of *Ruegeria atlantica*, based on detailed phenotypic characteristics obtained in the present study.

Description of *Ruegeria mobilis* sp. nov.

Ruegeria mobilis (mo'bi.lis. L. adj. *mobilis* mobile).

Cells are Gram-negative, aerobic, motile rods that are 1.0–2.0 μ m long and 0.6–0.8 μ m wide. Growth is detected at 5–35 $^{\circ}$ C with an optimum at 25–30 $^{\circ}$ C. pH range for growth is 5–11 with an optimum at pH 7. Growth occurs at 0–10 % NaCl with an optimum at 1–3 %. Oxidase- and catalase-positive. Alginate acid, tyrosine and aesculin are degraded, but agar, casein, cellulose, chitin, inulin, carboxymethylcellulose, Tween 80, DNA and yeast cells are not. H₂S and indole are not produced. Starch is not hydrolysed. Nitrate is not reduced to gas. Ammonium sulfate and urea are used as sole nitrogen sources for growth. Positive for utilization of

L-arabinose, adonitol, fructose, mannitol, xylitol, D-fucose and D-arabitol, weakly positive for sorbitol and L-arabitol and negative for arbutin and gluconate as sole carbon sources with the API 50CH system. Major fatty acids are $C_{16:0}$, $C_{18:1\omega6c}$ and $C_{18:1\omega7c}$. $C_{18:0}$, 11-methyl $C_{18:1\omega7c}$ and $C_{10:0}$ 3-OH are present as minor components. Major ubiquinone is Q-10. DNA G + C content of the type strain is 58.5 mol%.

The type strain, MBIC01146^T (=NBRC 101030^T=CIP 109181^T), was isolated from a marine slime in Japan. Strain MBIC01099 (=NBRC 101029) is also included in the species.

Emended description of *Ruegeria atlantica* (Rüger and Höfle 1992) Uchino *et al.* 1999

The emended description here is based on data from Rüger & Höfle (1992), Uchino *et al.* (1998), Martens *et al.* (2006) and this study. Cells are Gram-negative, aerobic, non-motile rods that are 1.5–2.0 µm long and 0.6–0.8 µm wide. Growth is detected at 5–30 °C with an optimum at 25 °C. pH range for growth is 6–11 with an optimum at pH 7. Growth occurs at 3–10 % NaCl with an optimum at 3 %. Oxidase- and catalase-positive. Alginate, casein, tyrosine and aesculin are degraded, but agar, casein, cellulose, chitin, inulin, carboxymethylcellulose, Tween 80, DNA and yeast cells are not. H₂S and indole are not produced. Starch is not hydrolysed. Nitrate is reduced to nitrite and gas. Ammonium sulfate and urea are not used as sole nitrogen sources for growth. Negative for utilization of L-arabinose, adonitol, fructose, rhamnose, sorbitol, xylitol, D-fucose, D-arabitol and L-arabitol, positive for arbutin and weakly positive for mannitol and gluconate as sole carbon sources with the API 50CH system. Major fatty acids are $C_{16:0}$, $C_{18:1\omega7c}$ and 11-methyl $C_{18:1\omega7c}$. $C_{10:0}$, $C_{12:0}$, $C_{18:0}$, $C_{12:0}$ 3-OH and $C_{16:0}$ 2-OH are present as minor components. Major ubiquinone is Q-10. DNA G + C content of the type strain is 55–59.4 mol%.

The type strain is NBRC 15792^T (=ATCC 700000^T=CIP 105975^T=DSM 5823^T=IAM 14463^T).

Acknowledgements

We thank Dr K. Isono (NBRC) for valuable comments on an earlier version of the manuscript.

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