Ruegeria pelagia is a later heterotypic synonym of *Ruegeria mobilis*

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The 16S rRNA genes of *Ruegeria pelagia* NBRC 102038^T and *Ruegeria mobilis* NBRC 101030^T were resequenced and the results confirmed that they differ by only one base in their almost full-length sequences (1425 nt). The *gyrB* gene sequence similarity between the two strains was also high (97.7 %). The outcome of API 20NE, API ZYM and antibiotic susceptibility tests showed that the two strains show only one difference, in β -galactosidase activity, in API tests and five differences in susceptibility among 30 tested antibiotics. In addition, similar BOX-PCR fingerprints were obtained and the DNA–DNA relatedness between the two strains was 91 ± 4 %. On the basis of these results, it is concluded that *Ruegeria pelagia* Lee *et al.* 2007 is a later heterotypic synonym of *Ruegeria mobilis* Muramatsu *et al.* 2007.

Ruegeria mobilis Muramatsu *et al.* 2007 and *Ruegeria pelagia* Lee *et al.* 2007 were reported separately as novel species in June and August 2007. They were originally isolated from marine environments of Japan and Palau and from the Sargasso Sea, respectively. Although the two type strains differ by only one base in their almost full-length 16S rRNA gene sequences, a comparative study of *R. pelagia* and *R. mobilis* has not been reported at the time of writing. The present study aims to determine the exact taxonomic positions of *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T by using a polyphasic taxonomic approach.

R. pelagia NBRC 102038^T and *R. mobilis* NBRC 101030^T were obtained from the NITE Biological Resource Center (NBRC), Chiba, Japan. Genomic DNA was prepared according to the method of Ausubel *et al.* (1995) and the 16S rRNA gene was amplified by PCR using previously described primers (Liu & Shao, 2005). Sequences of related taxa were obtained from the GenBank database (<http://www.ncbi.nlm.nih.gov/>). Phylogenetic analysis was

performed using MEGA version 4 (Tamura *et al.*, 2007) after multiple sequence alignment by using DNAMAN (version 5.1; Lynnon Biosoft). Distances (distance options according to Kimura's two-parameter model) and clustering with the neighbour-joining method of Saitou & Nei (1987) and minimum-evolution method of Rzhetsky & Nei (1992, 1993) were determined by using bootstrap values based on 1000 replications.

Resequencing of the 16S rRNA genes showed that the two strains differ in only one nucleotide position, as indicated by the previously reported sequences. The phylogenetic tree obtained in this study (not shown) was similar to that of Vandecandelaere *et al.* (2008). In the tree, the two strains formed a clade within the genus *Ruegeria*. DNA–DNA hybridization between genomic DNA from *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T was performed as described previously (Liu & Shao, 2005) with duplicate reciprocal hybridizations. Genomic DNA from *Escherichia coli* DH5 α was used as an outgroup sample. Salmon-sperm DNA was used as a negative control. DNA–DNA hybridization between *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T was 91 ± 4 %, suggesting that the two strains belong to a single species (Wayne *et al.*, 1987).

To elucidate further the taxonomic relationship between *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T, biochemical tests were carried out with API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, except that the NaCl concentration in all tests was adjusted to 3.0 %. Antibiotic susceptibility tests were performed by the disc diffusion method (Shieh *et al.*,

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Abbreviation: rep-PCR, repetitive element-based PCR.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this study are EU977138 (*R. pelagia* NBRC 102038^T 16S rRNA gene), EU977136 (*R. pelagia* NBRC 102038^T partial *gyrB* gene), EU977137 (*R. mobilis* NBRC 101030^T 16S rRNA gene) and EU977135 (*R. mobilis* NBRC 101030^T partial *gyrB* gene).

A transmission electron micrograph of cells of *R. pelagia* NBRC 102038^T, a dendrogram based on *gyrB* sequences and a comparison of rep-PCR patterns are available as supplementary material with the online version of this paper.

2003). All antibiotic sensitivity tests were performed with Oxoid discs. *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T had almost identical reactions in these tests (Table 1). Lee *et al.* (2007) reported that *R. pelagia* NBRC

Table 1. Characteristics of *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T

In this study, both strains were positive for catalase and oxidase, sensitive to (µg per disc unless indicated) ampicillin (10), carbenicillin (100), cefobid (30), chloromycetin (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), minomycin (30), norfloxacin (10), ofloxacin (5), piperacillin (100), polymyxin B (30 U), rifampicin (5), rocephin (30), tetracycline (30) and vibramycin (30) and resistant to cefalexin (30), cefazolin (30), cephradine (30), clindamycin (2), cotrimoxazole (25), lincomycin (2), metronidazole (5), oxacillin (1) and penicillin G (10). In API 20NE tests, both strains were positive for D-glucose fermentation, β-glucosidase activity and utilization of D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malic acid, N-acetylglucosamine and phenylacetic acid and negative for nitrate reduction, indole production, activities of arginine dihydrolase and urease, gelatin hydrolysis and utilization of adipic acid, capric acid, potassium gluconate and trisodium citrate. In API ZYM tests, both strains were positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine aminopeptidase, lipase (C14), N-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, valine aminopeptidase and α-glucosidase, weakly positive for cystine aminopeptidase and negative for trypsin, α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. +, Positive; w, weakly positive; –, negative. Data were obtained in this study unless indicated.

Characteristic	<i>R. mobilis</i> NBRC 101030 ^T	<i>R. pelagia</i> NBRC 102038 ^T
Cell size (µm)		
Length	1.0–2.0 ^a	0.8–2.1 ^b
Width	0.6–0.8 ^a	0.7–1.2 ^b
pH for growth		
Range	5–11 ^a	4–12 ^b
Optimum	7 ^a	5–6 ^b
Temperature for growth (°C)		
Range	5–35 ^a	16–42 ^b
Optimum	20–25 ^a	30–37 ^b
NaCl concentration for growth (% w/v)		
Range	0–10 ^a	0–10 ^b
Optimum	1–3 ^a	3.5 ^b
Antibiotic susceptibility		
Neomycin (10 µg)	w	–
Vancomycin (30 µg)	w	–
Furazolidone (15 µg)	+	–
Kanamycin (30 µg)	+	w
Streptomycin (10 µg)	+	w
DNA G + C content (mol%)	58.5 ^a	58.4 ^b
β-Galactosidase (API 20NE)	+	w

*Data from: a, Muramatsu *et al.* (2007); b, Lee *et al.* (2007).

102038^T was not motile, in contrast to *R. mobilis* NBRC 101030^T (Muramatsu *et al.*, 2007). In this report, however, we found that *R. pelagia* NBRC 102038^T had a polar flagellum (Supplementary Fig. S1, available in IJSEM Online). Electron microscopy was performed according to the method of Lai *et al.* (2009). Motility was tested by penetrating the strains into soft agar according to Dong & Cai (2001), and the result showed that both *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T were motile.

Further comparison of *R. pelagia* NBRC 102038^T with *R. mobilis* NBRC 101030^T was based on sequencing of the housekeeping gene *gyrB* and repetitive element-based PCR (rep-PCR) fingerprinting. The *gyrB* genes of the two type strains were sequenced (about 1072 bp) (Yamamoto *et al.*, 2000), and the similarity between strain *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T was 97.7%; the deduced GyrB amino acid sequence identity between the two strains was 99.2%. As shown in Supplementary Fig. S2, a phylogenetic tree based on *gyrB* gene sequences demonstrated that *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T formed a separate branch within the genus *Ruegeria*, as observed with 16S rRNA gene sequence analysis. In addition, the primer BOX-A1R (5'-CTACGGCAAGGCG-ACGCTGACG-3') was used for rep-PCR fingerprint analysis (Versalovic *et al.*, 1991). The PCR was carried out according to Versalovic *et al.* (1991) and the PCR products were separated by agarose (2%) gel electrophoresis. *R. pelagia* NBRC 102038^T and *R. mobilis* NBRC 101030^T had similar rep-PCR fingerprints (Supplementary Fig. S3). This supports the high relatedness at the DNA level found by DNA–DNA hybridization.

On the basis of data described above, we conclude that the strains described as *Ruegeria pelagia* match the genomic characteristics of *Ruegeria mobilis*, and that *Ruegeria pelagia* Lee *et al.* 2007 must therefore be seen as a later heterotypic synonym of *Ruegeria mobilis* Muramatsu *et al.* 2007.

Acknowledgements

This work was supported financially by the Natural Science Foundation of Fujian Province of China (no. 2009J01177) and the National Infrastructure of Natural Resources for Science and Technology Program of China (no. 2005DKA21209).

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