

Pseudoruegeria aestuarii sp. nov., of the family Rhodobacteraceae, isolated from a tidal flat

In-Tae Cha,¹† Inhye Park,²† Hae-Won Lee,³ Hyunjin Lee,² Jung-Min Park,⁴ Seong Woon Roh,⁵ Hak-Jong Choi,⁶ Young-Do Nam,⁷ Yoo Kyung Lee⁸ and Myung-Ji Seo^{1,2}

Correspondence
Myung-Ji Seo
mjseo@inu.ac.kr

¹Division of Bioengineering, Incheon National University, 22012 Incheon, Republic of Korea

²Department of Life Sciences, Graduate School of Incheon National University, 22012 Incheon, Republic of Korea

³Hygienic Safety and Analysis Center, World Institute of Kimchi, 61755 Gwangju, Republic of Korea

⁴Korean Culture Center of Microorganisms, 03641 Seoul, Republic of Korea

⁵Biological Disaster Analysis Group, Korea Basic Science Institute, 34133 Daejeon, Republic of Korea

⁶Microbiology and Functionality Research Group, World Institute of Kimchi, 61755 Gwangju, Republic of Korea

⁷Research Group of Gut Microbiome, Korea Food Research Institute, 13539 Sungnam, Republic of Korea

⁸Korea Polar Research Institute, KIOST, 21990 Incheon, Republic of Korea

A Gram-stain-negative, non-motile, aerobic and rod-shaped bacterium, designated strain MME-001^T, was isolated from the tidal flat of Muui-do in the Republic of Korea. Phylogenetic trees based on the 16S rRNA gene sequence showed that strain MME-001^T belonged to the genus *Pseudoruegeria* in the family Rhodobacteraceae and that it shared the highest 16S rRNA gene sequence similarity with *Pseudoruegeria sabulilitoris* GJMS-35^T (98.0% similarity of the 16S rRNA gene). Growth of strain MME-001^T occurred in the presence of 1.0–7.0% (w/v) NaCl at 15–40 °C and pH 7.0–9.0, with optimal growth in the presence of 2.0–3.0% (w/v) NaCl at 25–30 °C and pH 7.0. Ubiquinone-10 was the major respiratory quinone. Major polar lipids were phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unidentified lipid. The major cellular fatty acids were C_{18:1}ω7c and C_{16:0}. The genomic DNA G+C content was 62 mol%. DNA-DNA hybridization values between strain MME-001^T and *P. sabulilitoris* KCTC 42111^T, '*Pseudoruegeria limi*' KCTC 32460, *Pseudoruegeria lutimaris* KCTC 22690^T, *Pseudoruegeria aquimaris* KCTC 12737^T and *Pseudoruegeria halotis* KACC 17214^T was 36±5, 57±7, 34±4, 18±5 and 21±3%, respectively. Based on the phenotypic and phylogenetic taxonomical properties, this strain MME-001^T represents a novel species of the genus *Pseudoruegeria*, for which the name *Pseudoruegeria aestuarii* sp. nov. is proposed. The type strain is MME-001^T (=KCCM 43133^T=JCM 30751^T).

The genus *Pseudoruegeria*, a member of the class Alphaproteobacteria, was first proposed by Yoon *et al.* (2007). At the

time of writing, the genus comprised five species, *Pseudoruegeria aquimaris* (Yoon *et al.*, 2007), *P. lutimaris* (Jung

†These authors contributed equally to this work.

Abbreviations: DDH, DNA-DNA hybridization; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol. The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain MME-001^T is KP410678.

Two supplementary figures and a supplementary table are available with the online Supplementary Material.

et al., 2010), *P. haliotis* (Hyun *et al.*, 2013), *P. sabulilitoris* (Park *et al.*, 2014), and '*P. limi*' (Lee *et al.*, 2014). The major features of the genus *Pseudoruegeria* are that members are Gram-stain-negative, aerobic and rod-shaped, and have ubiquinone-10 as the predominant respiratory quinone. The major fatty acid is C_{18:1} ω 7c and the common major polar lipid components are identified phosphatidylglycerol (PG), an unidentified aminolipid and an unidentified lipid (Yoon *et al.*, 2007; Jung *et al.*, 2010). The west coast regions of Korea, have widely developed tidal flats, from which many bacterial strains have been isolated. One of these isolates, designated strain MME-001^T, belonging to the genus *Pseudoruegeria* is described in this study. We determined the taxonomic position of strain MME-001^T by a polyphasic characterization that included determination of chemotaxonomic and other phenotypic properties, detailed phylogenetic analysis based on 16S rRNA gene sequences, and DNA–DNA hybridization.

Strain MME-001^T was isolated from the tidal flat of Muuido in Incheon, Republic of Korea. Tidal flat samples were collected (37° 24' 16" N 126° 24' 50" E) in September 2014 and serially diluted into natural seawater agarose medium. To manufacture this medium, 0.5 g yeast extract and 1.5 % (w/v) agarose were added to 1 l natural seawater. The solution was autoclaved and supplemented with 1 ml trace element solution SL-6 (DSM medium no. 27) and 1 ml vitamin solution (Wolin, 1963) which was filtered using a 0.2 μ m syringe filter. The plates were then incubated at 30 °C for 2 weeks. At the end of the incubation period, colonies were streaked on the same fresh medium at least four times to obtain pure colonies. The pure colonies were then transferred onto marine agar 2216 (MA). The pH was adjusted to 7.2–7.4 with 1 M HCl or NaOH. One of the isolates was designated MME-001^T and routinely cultivated on MA at 30 °C. For analysis of the polar lipids, fatty acids, respiratory quinone and genomic DNA G+C content, and DNA–DNA hybridization experiments, the reference strains, *Pseudoruegeria sabulilitoris* KCTC 42111^T, '*P. limi*' KCTC 32460, *P. lutimaris* KCTC 22690^T and *P. aquimaris* KCTC 12737^T, were purchased from the Korean Collection for Type Cultures (Jeongup, Korea), and *P. haliotis* KACC 17214^T was bought from the Korean Agriculture Collection (Jeonju, Korea).

Genomic DNA of isolate MME-001^T was extracted using a HiYield Genomic DNA Mini Kit (Real Biotech Corporation). The 16S rRNA gene was amplified using PCR (Weisburg *et al.*, 1991) with the universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Lane, 1991) and the products were sequenced by CosmogeneTech. The nearest 16S rRNA gene full sequences were obtained using the universal bacterial primers, 27F, 337F (5'-GACTCC TACGGGAGGCWGCAG-3'), 518R (5'-ATTACCGCGGCTGCTGG-3'), 785F (5'-GGATTAGATACCCTGGTA-3'), and 1492R (Weisburg *et al.*, 1991) and assembled as described previously by Roh *et al.* (2008) using the SeqMan software (DNASTar). The 16S rRNA gene sequence of strain

MME-001^T was aligned with sequences of related species with validly published names using SILVA (<http://www.arb-silva.de/aligner>) (Pruesse *et al.*, 2012). Phylogenetic neighbors were identified using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>) and the EzTaxon-e server (<http://www.ezbiocloud.net/eztaxon/>) (Kim *et al.*, 2012). The evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981), and maximum-parsimony (Fitch, 1971) methods using MEGA6 (Tamura *et al.*, 2013), and the bootstrap values were calculated based on 1000 replicates. In this study, the 16S rRNA gene sequence of strain MME-001^T was determined to be 1376 bp in length, and was related to those of members of the genus *Pseudoruegeria*. The 16S rRNA gene sequence similarities between strain MME-001^T and *P. sabulilitoris* GJMS-35^T, '*P. limi*' D-17, *P. lutimaris* HD-43^T, *P. aquimaris* SW-255^T, and *P. haliotis* WM67^T were 98.0, 97.4, 97.3, 96.9 and 96.7 %, respectively, while all others showed less than 96.4 % similarity. Upon phylogenetic analysis, strain MME-001^T was found to belong to the genus *Pseudoruegeria* (Fig. 1).

The morphological, physiological and biochemical characteristics of strain MME-001^T were examined by routine cultivation on MA at 30 °C. Cell morphology and the presence of flagella were determined by phase contrast microscopy (Primo Star; Carl Zeiss) and transmission electron microscopy (JEM-1010; JEOL), respectively. The motility was determined using semi-solid MA (containing 0.5 % agarose) after incubation at 30 °C for 7 days (Tittsler & Sandholzer, 1936). The Gram reaction was investigated using a Gram-stain kit (Bioworld) according to the manufacturer's instructions. Catalase and oxidase activities were performed as described by Smibert & Krieg (1994). Growth under anaerobic conditions was determined by incubating samples on MA in a GasPak EZ anaerobe gas generating pouch system with indicator (BD) for 2 weeks at 30 °C. Colonies of strain MME-001^T were circular, smooth, convex, glistening, pale yellow and 1.0 mm after incubation on MA at 30 °C for 3 days. The isolate was Gram-stain-negative, aerobic and rod-shaped. The cells were 0.5–0.6 μ m wide by 2.4–2.5 μ m long (Fig. S1, available in the online Supplementary Material). The motility of cells was not observed in semi-solid MA and flagella were not discernible upon transmission electron microscopy.

To obtain the optimum growth temperature, strain MME-001^T was cultivated at 4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C on MA for 2 weeks. The salinity range for growth of strain MME-001^T was determined with 0–10.0 % (w/v) NaCl using modified artificial seawater medium as described by Cha *et al.* (2013). The pH range for growth was determined in marine broth 2216 (MB) that was adjusted to pH 5.0–11.0 (in increments of 1.0 pH unit). Hydrolysis of starch and casein was determined according to the methods of Benson (2002). Hydrolysis of Tweens 20, 40, and 80 was determined according to the method

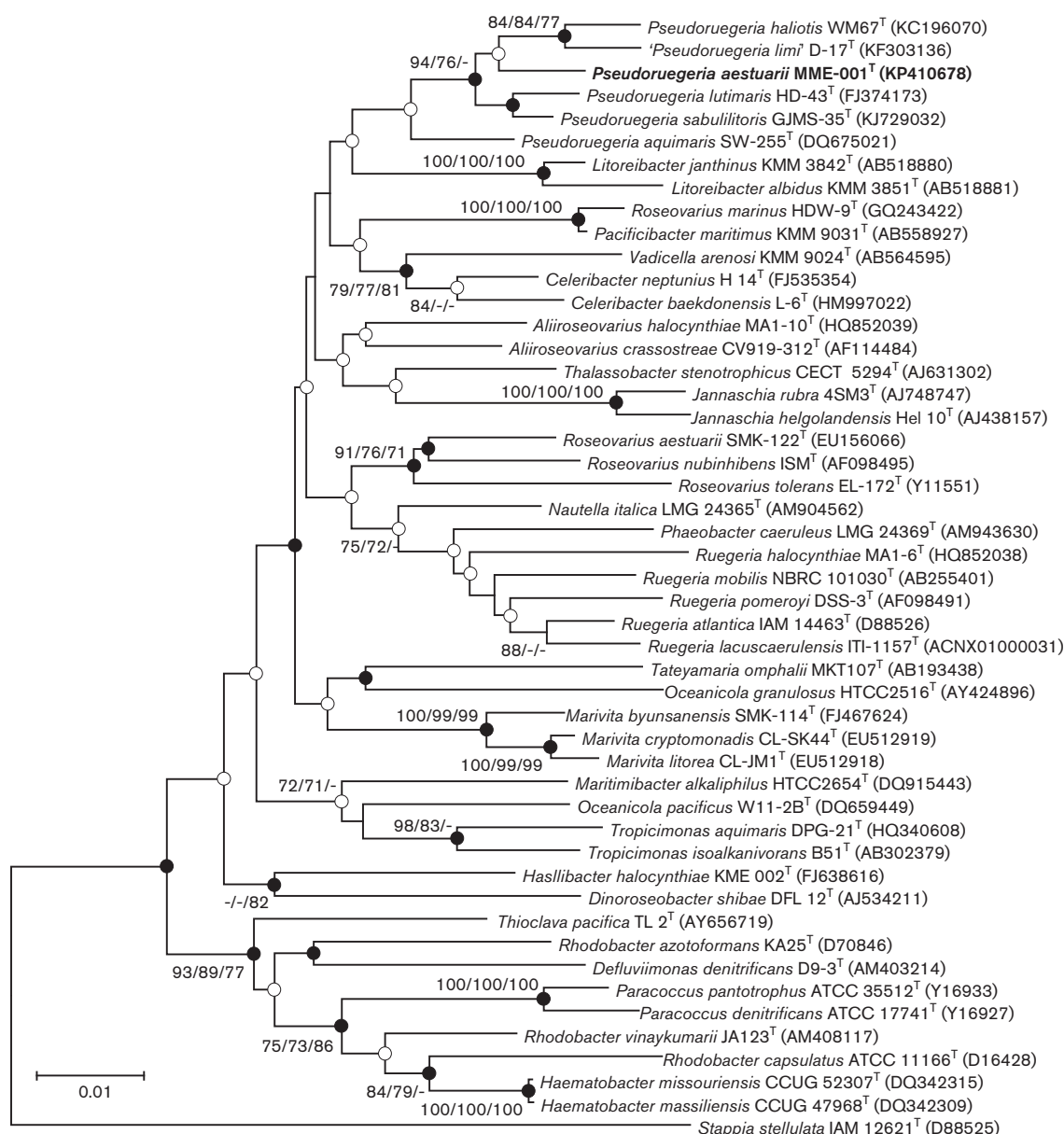


Fig. 1. Neighbour-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences showing the position and relationship between strain MME-001^T and other species of the genus *Pseudoruegeria* and other related taxa. Numbers at nodes indicate bootstrap values (>70%) calculated based on the NJ/maximum-likelihood (ML)/maximum-parsimony (MP) algorithms. Closed circles indicate that the corresponding nodes are also recovered by the ML and MP methods. Open circles indicate that the corresponding nodes are also recovered by the ML or MP methods. *Stappia stellulata* IAM 12621^T served as an outgroup. Bar, 0.01 substitutions per nucleotide position.

described by González *et al.* (1978). Hydrolysis of gelatin and L-tyrosine was evaluated as described by Smibert & Krieg (1994). Other enzyme activities were measured using the API 20NE and API ZYM systems (bioMérieux).

Growth of strain MME-001^T occurred at 15–40 °C in the presence of 1.0–7.0% (w/v) NaCl at pH 7.0–9.0. The optimal growth ranges of this strain were at 25–30 °C in the presence of 2.0–3.0% (w/v) NaCl at pH 7.0. Catalase and

oxidase activities were positive. Strain MME-001^T hydrolyzed gelatin, but not starch, casein, Tweens 20, 40 and 80, or L-tyrosine. Additionally, this strain could not reduce nitrate to nitrite. The API 20NE test revealed that the strain was positive for β -glucosidase, protease and β -galactosidase. In API ZYM, the enzyme activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -

Table 1. Differential phenotypic characteristics of strain MME-001^T and related members of the genus *Pseudoruegeria*

Taxa: 1, MME-001^T (data from this study); 2, *P. sabulilitoris* KCTC 42111^T (Park *et al.*, 2014); 3, '*P. limi*' KCTC 32460 (Lee *et al.*, 2014); 4, *P. lutimaris* KCTC 22690^T (Jung *et al.*, 2010); 5, *P. aquimaris* KCTC 12737^T (Yoon *et al.*, 2007); 6, *P. haliotis* KACC 17214^T (Hyun *et al.*, 2013). All taxa are positive for catalase and oxidase, and enzyme activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, and naphthol-AS-BI-phosphohydrolase. All taxa are negative for hydrolysis of L-tyrosine, indole production and enzyme activities of lipase (C14), β -glucuronidase, arginine dihydrolase, cystine arylamidase, trypsin, α -chymotrypsin, α -mannosidase, and α -fucosidase. +, Positive; –, negative.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|------------------------------------|---------|---------|---------|---------|---------|---------|
| Optimal growth range of: | | | | | | |
| Temperature (°C) | 25–30 | 28–30 | 25 | 25–30 | 37 | 30 |
| NaCl concentration (% w/v) | 2.0–3.0 | 2.0 | 2.0–3.0 | 2.0–3.0 | 2.0 | 1.0 |
| pH | 7.0 | 7.0–8.0 | 7.0–7.5 | 7.5–8.5 | 7.0–8.0 | 7.0–8.0 |
| Hydrolysis of:* | | | | | | |
| Casein | – | – | – | – | – | + |
| Starch | – | – | – | + | – | – |
| Tween 20 | – | – | – | + | – | – |
| Tween 40 | – | – | – | + | – | + |
| Tween 80 | – | – | – | – | – | + |
| Gelatin | + | – | – | – | – | + |
| Reduction of nitrate to nitrite* | – | + | + | + | – | – |
| Glucose fermentation* | – | + | – | – | – | – |
| Urease activity* | – | – | – | – | + | + |
| Enzyme activity* | | | | | | |
| Leucine arylamidase | + | + | + | + | + | – |
| Valine arylamidase | – | – | – | – | + | – |
| α -Galactosidase | – | – | + | – | + | – |
| β -Galactosidase | + | + | + | – | + | – |
| α -Glucosidase | + | + | + | – | + | + |
| β -Glucosidase | + | – | + | – | + | – |
| N-Acetyl- β -glucosaminidase | – | – | + | – | + | – |
| DNA G+C content (mol%) | 62 | 64.1 | 63.6 | 73.5 | 67 | 66.5 |

*Data from this study.

glucosidase and β -glucosidase were positive. Other characteristics are provided in Table 1.

To observe utilization of carbohydrates, 1 % (w/v) acetate, L-arabinose, benzoate, cellobiose, citrate, formate, D-fructose, D-galactose, D-glucose, L-glutamate, glycerol, inositol, lactose, malate, maltose, D-mannitol, D-mannose, melibiose, L-ornithine, pyruvate, raffinose, L-rhamnose, D-ribose, D-sorbitol, succinate, sucrose, trehalose and D-xylose were added to modified MB from which the peptone was removed and yeast extract was reduced to 0.01 % (w/v) (González *et al.*, 1997). Acid production was measured as described by Leifson (1963) with the following carbohydrates (1 %, w/v): L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, melibiose, L-rhamnose, D-ribose, trehalose and D-xylose. The test results of carbohydrate utilization and acid production are given in the species description and Table S1.

Susceptibility to antibiotics was determined by spreading the bacterial suspension on MA plates and using discs

containing the following antibiotics ($\mu\text{g ml}^{-1}$ unless otherwise stated): ampicillin (10), carbenicillin (100), cephalothin (30), ciprofloxacin (10), erythromycin (25), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), norfloxacin (20), novobiocin (10¹), penicillin G (20 UI), polymyxin B (100 UI), streptomycin (50) and tetracycline (30). Strain MME-001^T was sensitive to carbenicillin, erythromycin, gentamicin, kanamycin, neomycin, novobiocin, penicillin G, polymyxin B and streptomycin, but resistant to ampicillin, cephalothin, ciprofloxacin, lincomycin, norfloxacin and tetracycline.

Strain MME-001^T for analysis of quinone and polar lipids was cultivated on MA at 30 °C for 3 days. The isoprenoid quinones were extracted from freeze-dried cells using chloroform/methanol (2:1, v/v) (Collins & Jones, 1981) and identified using an HPLC system (YL9100; Younglin). The polar lipids were extracted according to the protocols described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata &

Suzuki, 1987): sulfuric acid-ethanol (1:2, v/v) for total lipids, Zinzadze reagent (5% of ethanolic molybdophosphoric acid) for total phospholipids, ninhydrin for amino-containing lipids, Dragendorff reagent for phosphatidylcholine, and α -naphthol reagent for glycolipids. The cellular fatty acid composition was analysed as described by Miller (1982) using an Agilent 6890 gas chromatography system and a crosslinked methyl siloxane column (HP-1; A30 m \times 0.320 mm \times 0.25 μ m). The profile analysis was determined using the Sherlock MIS Software version 6.2, based on the TSBA6 database (Sasser, 1990). The identities of fatty acid methyl esters detected by MIDI were further confirmed by GC-MS using an Agilent GC Series 6890 GC-MS. The genomic DNA G+C content was determined by the method described by Mesbah & Whitman (1989) using reversed-phase HPLC.

The predominant respiratory quinone of strain MME-001^T was ubiquinone-10 (at a peak area ratio of approximately 95%), which is the same as other members of the genus *Pseudoruegeria* as described by Yoon *et al.* (2007). The composition of polar lipids was phosphatidylcholine (PC), phosphatidylethanolamine (PE), PG, diphosphatidylglycerol (DPG), and four unidentified lipids (Fig. S2). The polar lipid profile of strain MME-001^T was similar to the type strains of *P. sabulilitoris* KCTC 42111^T, showing PC, PE and PG as major polar lipids. The common major polar lipids of the genus *Pseudoruegeria* were PC, PE, PG, an unidentified aminolipid, and an identified lipid (Jung *et al.*, 2010); however, the unidentified aminolipid was not detected from strain MME-001^T in the present study whereas it was detected from the reference strains, *P. sabulilitoris* KCTC 42111^T, *P. aquimaris* KCTC 12737^T and *P. lutimaris* KCTC 22690^T. Although DPG has been a well-known common 'minor' lipid in species of the genus *Pseudoruegeria* (Yoon *et al.*, 2007; Jung *et al.*, 2010; Park *et al.*, 2014), it was only detected from strain MME-001^T but not from *P. sabulilitoris* KCTC 42111^T, *P. aquimaris* KCTC 12737^T or *P. lutimaris* KCTC 22690^T in this study.

The fatty acid profile of strain MME-001^T is shown in Table 2 with strains of other species of the genus *Pseudoruegeria* analysed in this study. The major cellular fatty acids (>5%) of strain MME-001^T were C_{18:1} ω 7c (85.4%) and C_{16:0} (6.6%), which are similar to other reference species of the genus *Pseudoruegeria*. Furthermore, the major fatty acid found in members of the family *Rhodobacteraceae* was reported to be C_{18:1} ω 7c and the detected fatty acid, summed feature 8, which includes C_{18:1} ω 7c (Pujalte *et al.*, 2014). The DNA G+C content of strain MME-001^T was 62 mol%, which is lower than the values of other members of the genus *Pseudoruegeria* (65–74 mol%) (Yoon *et al.*, 2007; Jung *et al.*, 2010; Hyun *et al.*, 2013; Lee *et al.*, 2014; Park *et al.*, 2014).

DNA–DNA hybridization (DDH) was performed fluorometrically by the membrane filter method using a DIG High Prime DNA Labelling and Detection Starter Kit II (Roche Applied Science). The DNA of strain MME-001^T was labelled and the other reference strains were

immobilized, with the experiment repeated three times. The DNA–DNA relatedness between strain MME-001^T and *P. sabulilitoris* KCTC 42111^T, '*P. limi*' KCTC 32460, *P. lutimaris* KCTC 22690^T, *P. aquimaris* KCTC 12737^T, and *P. haliotis* KACC 17214^T was 36 \pm 5, 57 \pm 7, 34 \pm 4, 18 \pm 5 and 21 \pm 3%, respectively. Reciprocal experiments were also performed with the labelled DNA of *P. sabulilitoris* KCTC 42111^T, resulting in a DNA–DNA relatedness value of 33 \pm 8% with strain MME-001^T. Current prokaryotic systematics define DDH values of <70% as indicative of a distinct species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Thus the DDH values indicated that strain MME-001^T was a distinct member of the genus *Pseudoruegeria*.

Although some characteristics, such as the relatively low G+C content, absence of unidentified amino lipids, and DDH values, distinguished strain MME-001^T from species of the genus *Pseudoruegeria*, phylogenetic, phenotypic, and chemotaxonomic properties of strain MME-001^T revealed that it belonged to the genus *Pseudoruegeria* within the family *Rhodobacteraceae*. Therefore, based on the data presented herein, strain MME-001^T is considered to represent a novel species of the genus *Pseudoruegeria*, for which the name *Pseudoruegeria aestuarii* sp. nov. is proposed.

Description of *Pseudoruegeria aestuarii* sp. nov.

Pseudoruegeria aestuarii (aes.tu.a'ri.i. L. gen. n. aestuarii of a tidal flat).

Cells are Gram-stain-negative, non-motile, aerobic, rod-shaped and 0.5–0.6 μ m in width by 2.4–2.5 μ m in length. Colonies on MA are circular, convex, smooth, glistening, pale yellow and 1.0 mm in diameter after incubation for 3 days at 30 °C. Growth occurs in presence of 1.0–7.0% (w/v) NaCl (optimum 2.0–3.0%) at 15 and 40 °C (optimum 25–30 °C) and pH 7.0–9.0 (optimum pH 7.0). Na⁺ is required for growth, nitrate is not reduced to nitrite and anaerobic growth does not occur on MA. Gelatin is hydrolysed, while starch, casein, Tweens 20, 40 and 80, and L-tyrosine are not. In the API 20NE kit, β -glucosidase, protease and β -galactosidase are positive, while indole production, glucose fermentation, and activities of arginine dihydrolase and urease are negative. In the API ZYM test, enzyme activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase and β -glucosidase are positive, but lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities are negative. Utilizes acetate, L-arabinose, cellobiose, citrate, formate, D-fructose, D-galactose, D-glucose, L-glutamate, glycerol, inositol, lactose, malate, maltose, D-mannitol, D-mannose, melibiose, pyruvate, L-rhamnose, D-ribose, D-sorbitol, succinate, sucrose, trehalose and D-xylose as carbon and energy sources, but benzoate, L-ornithine and raffinose are not utilized. Acid is produced from L-arabinose, cellobiose,

Table 2. Cellular fatty acid contents (%) of strain MME-001^T and strains of related species of the genus *Pseudoruegeria*

Strains: 1, MME-001^T; 2, *P. sabulilitoris* KCTC 42111^T; 3, '*P. limi*' KCTC 32460; 4, *P. lutimaris* KCTC 22690^T; 5, *P. aquimaris* KCTC 12737^T; 6, *P. haliotis* KACC 17214^T. All data are from this study. TR, Trace <1.0 %; —, not detected.

| Fatty acid (%) | 1 | 2 | 3 | 4 | 5 | 6 |
|---------------------------------|------|------|------|------|------|------|
| Straight-chain | | | | | | |
| C _{16:0} | 6.6 | 7.1 | 6.5 | 6.2 | TR | 7.7 |
| C _{17:0} | TR | TR | 4.0 | — | TR | 1.0 |
| C _{18:0} | 2.6 | 2.0 | 1.8 | 3.2 | 3.2 | 2.0 |
| Unsaturated | | | | | | |
| C _{18:1} ω7c | 85.4 | 86.4 | 78.7 | 82.7 | 80.7 | 81.4 |
| Hydroxy | | | | | | |
| C _{10:0} 3-OH | — | — | — | — | 3.8 | — |
| C _{12:0} 3-OH | 1.5 | 1.8 | 2.7 | 1.1 | — | TR |
| 11-Methyl C _{18:1} ω7c | 1.5 | TR | TR | 1.4 | 3.7 | — |
| Cyclo C _{19:0} ω8c | — | — | — | — | 3.5 | 2.4 |
| Summed feature* | | | | | | |
| 1 | — | — | 1.1 | TR | — | TR |
| 7 | TR | TR | 1.1 | TR | TR | TR |

*Summed features are groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 comprised iso-C_{15:1} H and/or C_{13:0} 3-OH; summed feature 7 comprised C_{19:1}ω7c and/or C_{19:1}ω6c.

D-fructose, D-galactose, D-glucose, D-mannose, melibiose, L-rhamnose, D-ribose, trehalose and D-xylose. The predominant respiratory quinone is Q-10. The polar lipids are PC, PE, PG, DPG and four unidentified lipids. The major fatty acids are C_{18:1}ω7c and C_{16:0}.

The type strain, MME-001^T (=KCCM 43133^T=JCM 30751^T) was isolated from the tidal flat sediment of Muuido in Incheon, Republic of Korea. The genomic DNA G+C content of the type strain is 62 mol% (HPLC).

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2014R1A1A1002980 and 201405670001). This study was also supported by the Korea Polar Research Institute (KOPRI, PE16030). We thank the Nano-Bio Electron Microscopy Research Team members for their technical help with BIO-TEM installed at the Korea Basic Science Institute (KBSI).

References

- Benson, H. J. (2002). *Microbiological Application: a Laboratory Manual in General Microbiology*. New York: McGraw-Hill.
- Cha, I. T., Park, S. J., Kim, S. J., Kim, J. G., Jung, M. Y., Shin, K. S., Kwon, K. K., Yang, S. H., Seo, Y. S. & Rhee, S. K. (2013). *Marinoscillum luteum* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **63**, 3475–3480.
- Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20**, 406–416.

Gonzalez, C., Gutierrez, C. & Ramirez, C. (1978). *Halobacterium vallismortis* sp. nov. An amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* **24**, 710–715.

González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst Bacteriol* **47**, 369–376.

Hyun, D. W., Shin, N. R., Kim, M. S., Kim, P. S., Kim, J. Y., Whon, T. W. & Bae, J. W. (2013). *Pseudoruegeria haliotis* sp. nov., isolated from the gut of the abalone *Haliotis discus hannai*. *Int J Syst Evol Microbiol* **63**, 4626–4632.

Jung, Y. T., Kim, B. H., Oh, T. K. & Yoon, J. H. (2010). *Pseudoruegeria lutimaris* sp. nov., isolated from a tidal flat sediment, and emended description of the genus *Pseudoruegeria*. *Int J Syst Evol Microbiol* **60**, 1177–1181.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackbrandt & M. Goodfellow. Chichester: Wiley.

Lee, J. B., Kim, H., Park, D. S., Yang, J. H., Chun, Y. Y., Lee, K. H. & Bae, K. S. (2014). *Pseudoruegeria limi* sp. nov. isolated from mud flats in the Yellow Sea in Korea. *Antonie Van Leeuwenhoek* **105**, 987–994.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

- Mesbah, M. & Whitman, W. B. (1989).** Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.
- Miller, L. T. (1982).** Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Park, S., Jung, Y. T., Won, S. M. & Yoon, J. H. (2014).** *Pseudoruegeria sabulilitoris* sp. nov., isolated from seashore sand. *Int J Syst Evol Microbiol* **64**, 3276–3281.
- Pruesse, E., Peplies, J. & Glöckner, F. O. (2012).** SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829.
- Pujalte, M. J., Lucena, T., Ruvira, M. A., Arahal, D. R. & Macián, M. C. (2014).** Alphaproteobacteria and Betaproteobacteria. In *The Prokaryotes*, pp. 439–512. Edited by E. Rosenberg, E. F. DeLong, S. Lory, E. Stackbrandt & F. Thompson. Heidelberg, Germany: Springer.
- Roh, S. W., Sung, Y., Nam, Y. D., Chang, H. W., Kim, K. H., Yoon, J. H., Jeon, C. O., Oh, H. M. & Bae, J. W. (2008).** *Arthrobacter soli* sp. nov., a novel bacterium isolated from wastewater reservoir sediment. *J Microbiol* **46**, 40–44.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013).** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.
- Tittsler, R. P. & Sandholzer, L. A. (1936).** The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* **31**, 575–580.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987).** International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Wolin, E. A., Wolin, M. J. & Wolfe, R. S. (1963).** Formation of methane by bacterial extracts. *J Biol Chem* **238**, 2882–2886.
- Yoon, J. H., Lee, S. Y., Kang, S. J., Lee, C. H. & Oh, T. K. (2007).** *Pseudoruegeria aquimaris* gen. nov., sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* **57**, 542–547.