Journal of Microbiology (2015) Vol. 53, No. 8, pp. 495–502
DOI 10.1007/s12275-015-5075-7
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Aliisedimentitalea scapharcae gen. nov., sp. nov., isolated from ark shell *Scapharca broughtonii*[§]

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(Received Feb 10, 2015 / Revised Jun 12, 2015 / Accepted Jun 19, 2015)

A Gram-negative, aerobic, non-spore-forming, motile and ovoid or rod-shaped bacterial strain, designated MA2-16^T, was isolated from ark shell (*Scapharca broughtonii*) collected from the South Sea, South Korea. Strain MA2-16^T was found to grow optimally at 30°C, at pH 7.0–8.0 and in the presence of 2.0% (w/v) NaCl. Neighbour-joining, maximum-likelihood and maximum-parsimony phylogenetic trees based on 16S rRNA gene sequences revealed that strain MA2-16^T clustered with the type strain of *Sedimentitalea nanhaiensis*. The novel strain exhibited a 16S rRNA gene sequence similarity value of 97.1% to the type strain of *S. nanhaiensis*. In the neighbour-joining phylogenetic tree based on *gyrB* sequences, strain MA2-16^T formed an evolutionary lineage independent of those of other taxa. Strain MA2-16^T contained Q-10 as the predominant ubiquinone and $C_{18:1}$ *ω7c* and 11-methyl $C_{18:1}$ *ω7c* as the major fatty acids. The major polar lipids of strain MA2-16^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and an unidentified lipid. The DNA G+C content of strain MA2-16^T was 57.7 mol% and its DNA-DNA relatedness values with the type strains of *S. nanhaiensis* and some phylogenetically related species of the genera *Leisingera* and *Phaeobacter* were 13–24%. On the basis of the data presented, strain MA2-16^T is considered to represent a novel genus and novel species within the family *Rhodobacteraceae*, for which the name *Aliisedimentitalea scapharcae* gen. nov., sp. nov. is proposed. The type strain is MA2-16^T (=KCTC 42119^T =CECT 8598^T).

Keywords: ark shell, *Scapharca broughtonii*, polyphasic taxonomy, novel genus and species, *Aliisedimentitalea scapharcae*

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

Introduction

During a screening of novel marine bacteria from ark shell (*Scapharca broughtonii*) collected from the South Sea, South Korea, many novel bacterial taxa have been isolated and characterized taxonomically. One of the bacterial isolates obtained, designated MA2-16^T, which belongs to the class *Alphaproteobacteria*, was selected for further taxonomic study. Comparative 16S rRNA gene sequence analysis indicated that the novel strain is most phylogenetically closely affiliated to representative of the genera *Leisingera* and *Phaeobacter*. The genera *Leisingera* and *Phaeobacter* were proposed by Schaefer *et al*. (2002) and Martens *et al*. (2006), respectively. However, members of the genera *Leisingera* and *Phaeobacter* have been found not to form monophyletic clusters encompassed by corresponding genera, thereby it have been recognized that they may have to be re-evaluated taxonomically (Gaboyer *et al*., 2013; Hameed *et al*., 2014; Liu *et al*., 2014; Park *et al*., 2014). Recently, *Phaeobacter daeponensis* (Yoon *et al*., 2007a), *Phaeobacter caeruleus* (Vandecandelaere *et al*., 2009) and *Phaeobacter aquaemixtae* (Park *et al*., 2014) were reclassified as members of the genus *Leisingera*, *Phaeobacter arcticus* (Zhang *et al*., 2008) and *Phaeobacter leonis* (Gaboyer *et al*., 2013) were reclassified as members of a novel genus *Pseudophaeobacter*, and *Leisingera nanhaiensis* (Sun *et al*., 2010) was reclassified as a member of a novel genus *Sedimentitalea* (Breider *et al*., 2014; Oren and Garrity, 2014). The aim of the present work was to determine the exact taxonomic position of strain MA2-16^T by using a polyphasic characterization that included the determination of chemotaxonomic and other phenotypic properties, detailed phylogenetic investigations based on 16S rRNA gene sequences and DNA-DNA hybridization.

Materials and Methods

Bacterial strains and culture conditions

Ark shell (*Scapharca broughtonii*) was collected from Gang-Jin bay on the South Sea, South Korea, and its whole body was used as a source for the isolation of bacterial strains. Strain MA2-16^T was isolated by the dilution plating technique on marine agar 2216 (MA; Becton, Dickinson and Company) at 25°C and cultivated routinely on MA at 30°C. For short-term preservation, strain MA2-16^T was maintained on MA at 4°C. For long-term preservation, cells of strain MA2-16^T was harvested from agar plates and transferred to cryo-tube containing 20% (w/v) glycerol solution. The tube is stored at -80°C. Strain MA2-16^T has been deposited in the Korean Collection for Type Cultures (KCTC) and the Spanish Type

Culture Collection (CECT; Spain) as KCTC 42119^T and CECT 8598^T, respectively.

Sedimentitalea nanhaiensis LMG 24841^T and *Leisingera aquimarina* LMG 24366^T and *Leisingera methylohalidivorans* DSM 14336^T, *Phaeobacter gallaeciensis* DSM 26640^T and *Phaeobacter inhibens* DSM 16374^T, which were used as reference strains for phenotypic characterization, fatty acid and polar lipid analyses, DNA-DNA hybridization and/or sequencing of DNA gyrase B subunit gene (*gyrB*), were obtained from the Laboratorium voor Microbiologie Universiteit Gent (LMG; Belgium) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Germany), respectively. Cell biomass of strain MA2-16^T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 2 days at 30°C in marine broth 2216 (MB), and cell biomass of *S. nanhaiensis* LMG 24841^T, *L. methylohalidivorans* DSM 14336^T, *L. aquimarina* LMG 24366^T, *P. gallaeciensis* DSM 26640^T, and *P. inhibens* DSM 16374^T for DNA extraction and for polar lipid analysis was obtained from cultures grown under the same culture conditions. For fatty acid methyl ester analysis, the cell mass of strain MA2-16^T, *S. nanhaiensis* LMG 24841^T, *L. methylohalidivorans* DSM 14336^T, *L. aquimarina* LMG 24366^T, *P. gallaeciensis* DSM 26640^T, and *P. inhibens* DSM 16374^T was harvested from MA after incubation for 3 days at 30°C.

Phenotypic characterization

The cell morphology, Gram reaction, pH range for growth and anaerobic growth were determined as described by Park *et al.* (2014). Growth at 4, 10, 20, 25, 30, 31, 32, 33, 34, 35, 37, and 40°C was measured on MA to determine the optimal temperature and temperature range for growth. Growth at various concentrations of NaCl (0, 0.5 and 1.0–10.0%, at increments of 1.0%) was investigated by supplementing with appropriate concentrations of NaCl in MB prepared according to the formula of the Becton, Dickinson and Company medium without NaCl. The requirement for Mg^{2+} ions was investigated by using MB, prepared according to the formula of the Becton, Dickinson and Company medium, that comprised of all the constituents except $MgCl_2$ and $MgSO_4$. Catalase and oxidase activities were determined as described by Lányí (1987). Hydrolysis of aesculin, casein, starch, hypoxanthine, Tween 80, L-tyrosine and xanthine was tested on MA using the substrate concentrations described by Barrow and Feltham (1993). Nitrate reduction was investigated as described previously (Lányí, 1987) with the modification that artificial seawater was used for the preparation of medium. Hydrolysis of gelatin and urea was investigated by using nutrient gelatin and urea agar base media (Becton, Dickinson and Company), respectively, with the modification that artificial seawater was used for the preparation of media. The artificial seawater contained (L^{-1} distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2 \cdot 6H_2O$, 5.94 g $MgSO_4 \cdot 7H_2O$, and 1.3 g $CaCl_2 \cdot 2H_2O$ (Bruns *et al.*, 2001). H_2S production was tested as described previously (Bruns *et al.*, 2001). Utilization of various substrates for growth was tested according to Baumann and Baumann (1981), using supplementation with 1% (v/v) vitamin solution (Staley, 1968) and 2% (v/v) Hutner's mineral salts (Cohen-Bazire *et al.*, 1957). Susceptibility to antibiotics was investigated on MA plates using antibiotic discs (Advantec) containing the following (μg per disc unless otherwise stated): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 IU), polymyxin B (100 IU), streptomycin (50), and tetracycline (30). Enzyme activities were determined, after incubation for 8 h at 30°C, by using the API ZYM system (bioMérieux); the strip was inoculated with cells suspended in artificial seawater from which $CaCl_2$ was excluded to avoid the formation of precipitates.

Molecular systematics

Chromosomal DNA was extracted and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR as described previously (Yoon *et al.*, 1998) using two universal primers (5′-GAGTT TGATCCTGGCTCAG-3′ and 5′-ACGGTTACCTTGTTA CGACTT-3′). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The amplification of the DNA gyrase B subunit gene (*gyrB*) was performed by using two primers, UP-1 and UP-2r, according to the method described previously (Yamamoto and Harayama, 1995) and the PCR products were purified with the QIAquick PCR purification kit (Qiagen). The amplified *gyrB* was cloned into pGEM T-easy vector (Promega) according to the manufacturer's instructions. Sequence of the *gyrB* was determined for both strands by extension from vector-specific priming sites (T7 and SP-6 primers from pGEM T-easy vector). Phylogenetic analysis of the *gyrB* sequence was performed as described by Yoon *et al.* (2007b). The sequence similarity values have been calculated using our own program from the alignments obtained from Clustal W program.

DNA-DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes for cross-hybridization in microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA-DNA relatedness values.

Chemosystematic characterization

Isoprenoid quinones were extracted according to the method of Komagata and Suzuki (1987) and analyzed using reversed-phase HPLC equipped with a YMC ODS-A (250 × 4.6 mm) column. The isoprenoid quinones were eluted by a mixture of methanol/isopropanol (2:1, v/v), using a flow rate of 1 ml/min at room temperature and detected by UV absorbance at 275 nm. Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI (Sherlock Microbial Identification System, version 6.2B). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified by using the TSBA6 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984), and separated by two-dimensional TLC using

chloroform/methanol/water (65:25:3.8, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (40: 7.5:6:1.8, by vol.) for the second dimension as described by Embley and Wait (1994). Individual polar lipids were identified by spraying with the ethanolic molybdophosphoric acid, molybdenum blue, ninhydrin and α-naphthol reagents (Minnikin *et al*., 1984; Komagata and Suzuki, 1987) and the Dragendorff's reagent (Sigma). The DNA G+C content of strain MA2-16^T was determined by the method of Tamaoka and Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC equipped with a YMC ODS-A (250 × 4.6 mm) column. The nucleotides were eluted by a mixture of 0.55 M $NH_4H_2PO_4$ (pH 4.0) and acetonitrile (40:1, v/v), using a flow rate of 1 ml/min at room temperature and detected by UV absorbance at 270 nm.

Nucleotide sequence accession number

The 16S rRNA gene sequence of strain MA2-16^T has been deposited in the GenBank database under accession number KJ889016. The *gyrB* sequences of strain MA2-16^T, *Sedimentitalea nanhaiensis* LMG 24841^T and *Leisingera aquima-*

Table 1. Differential characteristics of strain MA2-16^T and the type strains of *Sedimentitalea nanhaiensis*, two *Leisingera* species and two *Phaeobacter* species

Strains: 1, MA2-16^T; 2, *S. nanhaiensis* LMG 24841^T; 3, *L. methylohalidivorans* DSM 14336^T; 4, *L. aquimarina* LMG 24366^T; 5, *P. gallaeciensis* DSM 26640^T; 6, *P. inhibens* DSM 16374^T. Data of reference strains obtained from this study unless indicated otherwise. +, positive reaction; –, negative reaction; w, weakly positive reaction. All strains are positive for the followings: motility*; catalase and oxidase activities*; susceptibility to carbenicillin, chloramphenicol, kanamycin (weak for *L. aquimarina* LMG 24366^T, *P. gallaeciensis* DSM 26640^T and *P. inhibens* DSM 16374^T), neomycin, polymyxin B and streptomycin; and activity of esterase (C4), esterase lipase (C8) (weak for *L. methylohalidivorans* DSM 14336^T and *L. aquimarina* LMG 24366^T), leucine arylamidase and acid phosphatase (weak for *L. aquimarina* LMG 24366^T). All strains are negative for the followings: Gram staining; utilization of L-arabinose, maltose, sucrose, D-trehalose, benzoate, formate and L-glutamate; susceptibility to lincomycin and oleandomycin; and activity of lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

Characteristic	1	2	3	4	5	6
Growth at*						
4°C	+	+	+	+	–	+
37°C	–	+	–	+	+	–
Maximum NaCl concentration (%)*	5.0	6.0	6.0	7.0	11.7	8.7
Nitrate reduction*	+	–	–	–	–	–
Hydrolysis of*						
Gelatin	–	–	–	w	–	–
Starch	–	–	+	–	–	–
Tween 80	+	–	–	–	–	–
Utilization of						
D-Cellobiose	–	–	–	–	+	+
D-Fructose	–	–	–	–	+	+
D-Galactose	–	–	–	–	+	+
D-Glucose	–	–	–	–	+	+
D-Mannose	–	–	–	–	+	+
D-Xylose	–	–	–	–	+	+
Acetate	+	+	–	+	+	+
Citrate	–	–	–	+	+	+
L-Malate	–	+	–	+	+	+
Pyruvate	–	+	–	+	+	+
Succinate	–	+	w	–	+	+
Salicin	–	–	–	–	w	+
Susceptibility to						
Ampicillin	+	–	–	+	–	+
Cephalothin	+	+	–	+	+	+
Gentamicin	+	–	+	+	+	+
Novobiocin	+	+	–	–	–	–
Penicillin G	+	+	–	+	+	+
Tetracycline	+	+	+	–	+	+
Enzyme activity (API ZYM)						
Alkaline phosphatase	+	+	–	w	+	+
Valine arylamidase	–	+	–	–	w	+
Naphthol-AS-BI-phosphohydrolase	–	w	–	–	–	–
α-Galactosidase	–	–	–	–	w	+
α-Glucosidase	–	–	–	–	+	w
DNA G+C content (mol%)*	57.7	60.5	60.5	61.4	58	55.7

* Data of reference strains taken from Ruiz-Ponte *et al.* (1998), Schaefer *et al.* (2002), Martens *et al.* (2006), Vandecandelaere *et al.* (2008), and Sun *et al.* (2010).

rina LMG 24366^{T} have been deposited in the GenBank database under accession numbers KT033420, KT033421, and KT033422, respectively.

Results and Discussion

Morphological, cultural, physiological, and biochemical characteristics

Strain MA2-16^{T} was aerobic, Gram-staining-negative, non-spore-forming and ovoid or rod-shaped (approximately 0.2–0.8 μm in diameter and 0.6->10.0 μm in length); a few cells greater than 10 μm in length are also observed. It was motile by means of a single polar flagellum (Supplementary data Fig. S1). Colonies were circular, slightly convex, smooth, glistening and light greyish yellow in colour on MA. Strain MA2-16^{T} grew at 4 and 32°C with an optimum temperature of 30°C. It grew optimally at pH 7.0–8.0 and in the presence of 2.0% (w/v) NaCl. Strain MA2-16^{T} showed catalase and oxidase activities and reduced nitrate to nitrite. Morphological, cultural, physiological and biochemical characteristics of strain MA2-16^{T} are given in the species description (see below) or in Table 1.

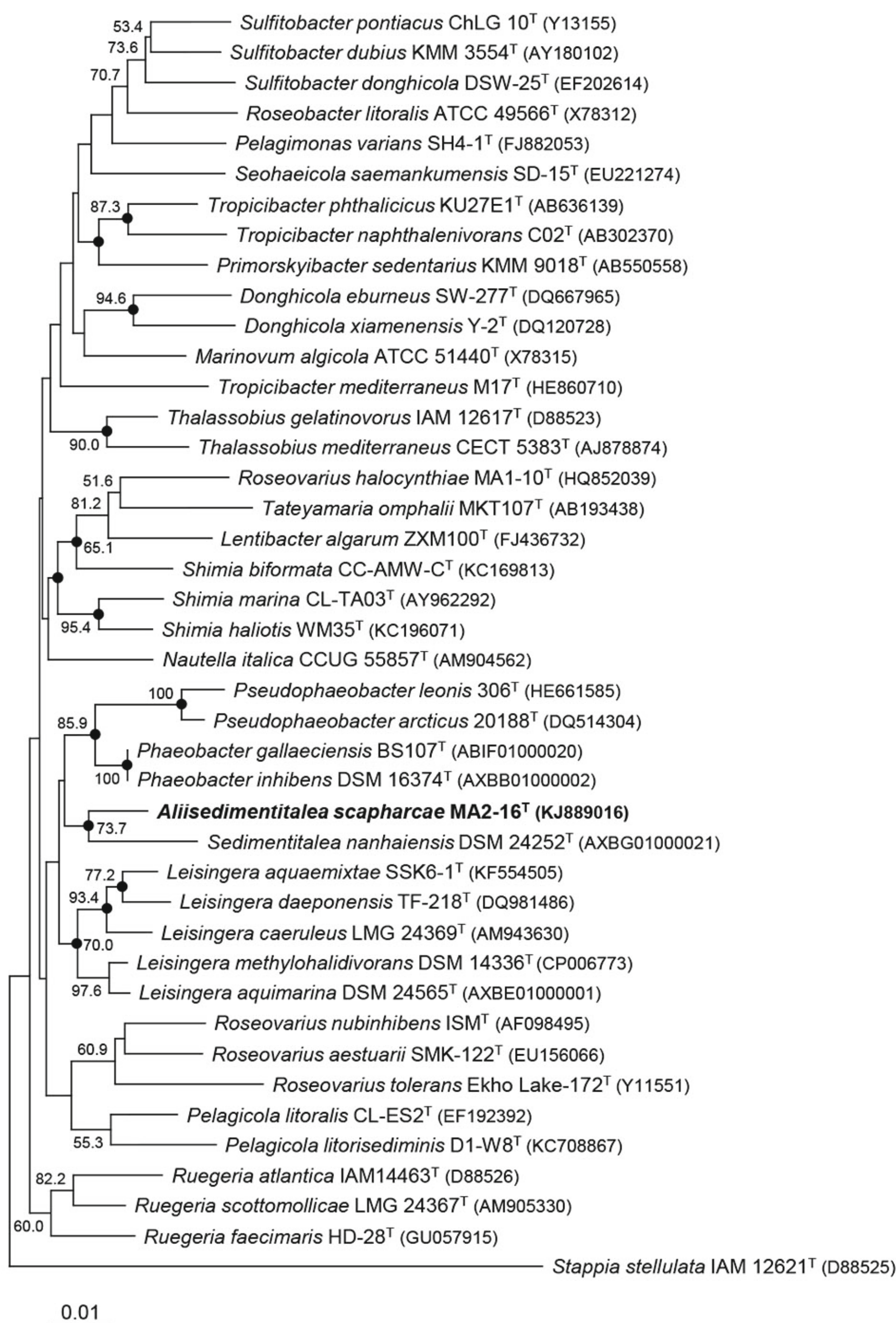

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain MA2-16^{T} and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1,000 replications) of > 50% are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum parsimony algorithms. *Stappia stellulata* IAM 12621^{T} (GenBank accession no., D88525) was used as an outgroup. Scale bar, 0.01 substitutions per nucleotide position.

Phylogenetic analysis

The almost complete 16S rRNA gene sequence of strain MA2-16^T determined in this study comprised 1386 nucleotides, approximately 95% of the *Escherichia coli* 16S rRNA sequence. In the neighbour-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences, strain MA2-16^T clustered with the type strain of *Sedimentitalea nanhaiensis* with a bootstrap resampling value of 73.7% (Fig. 1). The relationship between strain MA2-16^T and the type strain of *S. nanhaiensis* was also maintained in the trees constructed using the maximum-likelihood (ML) and maximum-parsimony (MP) algorithms (Fig. 1). Strain MA2-16^T exhibited 16S rRNA gene sequence similarity value of 97.1% to *S. nanhaiensis* DSM 24252^T. It exhibited 16S rRNA gene sequence similarity values of 97.8, 97.8, 97.6 and 97.3% to the type strains of *P. gallaeciensis*, *P. inhibens*, *L. methylohalidivorans*, and *L. aquimarina*, respectively, and of less than 97.0% to the type strains of the other recognized species.

In the NJ phylogenetic tree based on *gyrB* sequences, strain MA2-16^T formed a distinct evolutionary lineage independent of those of other taxa of the family *Rhodobacteraceae* (Fig. 2). Strain MA2-16^T exhibited the highest *gyrB* sequence similarity value (82.7%) to the type strain of *L. methylohalidivorans* followed by sequence similarities more than 80% to the type strains of *Ruegeria lacuscaerulensis* (81.9%), *L. aquimarina* (81.6%), *Ruegeria pomeroyi* (81.0%), *Ruegeria arenilitoris* (80.7%), *Ruegeria scottomollicae* (80.6%), *Sulfitobacter dubius* (80.3%), *Roseovarius nubinhibens* (80.3%), *Ruegeria atlantica* (80.3%), *Sulfitobacter pontiacus* (80.2%), and *Ruegeria faecimaris* (80.1%). It exhibited *gyrB* sequence similarity values of 79.9, 79.7, and 78.5% to the type strains of *P. inhibens*, *P. gallaeciensis*, and *S. nanhaiensis*, respectively.

DNA-DNA relatedness

Strain MA2-16^T exhibited mean DNA-DNA relatedness values of 22, 13, 17, 24, and 19% to *S. nanhaiensis* LMG 24841^T, *P. gallaeciensis* DSM 26640^T, *P. inhibens* DSM 16374^T, *L. methylohalidivorans* DSM 14336^T, and *L. aquimarina* LMG 24366^T, respectively.

Chemosystematic characteristics

The predominant isoprenoid quinone detected in strain MA2-16^T was ubiquinone-10 (Q-10) which is typical of the vast majority of the class *Alphaproteobacteria*. In Table 2, the fatty acid profile of strain MA2-16^T is compared with those of the type strains of *S. nanhaiensis*, *L. methylohalidivorans*, *L. aquimarina*, *P. gallaeciensis*, and *P. inhibens*, which were grown and analysed under identical conditions in this study. The major fatty acids (> 10% of the total fatty acids) found in strain MA2-16^T were $C_{18:1}$ $\omega 7c$ (63.6%) and 11-methyl $C_{18:1}$ $\omega 7c$ (18.7%) (Table 2). The fatty acid profile of strain MA2-16^T was similar to those of the type strains of *S. nanhaiensis*, *L. methylohalidivorans*, *L. aquimarina*, *P. gallaeciensis*, and *P. inhibens* in that the predominant fatty acid is $C_{18:1}$ $\omega 7c$, although there were differences in the proportions of some fatty acids (Table 2). The major polar lipids detected in strain MA2-16^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid, and one unidentified lipid; a minor amount of one additional unidentified lipid was also present (Supplementary data Fig. S2). The polar lipid profile of strain MA2-16^T was similar to those of the type strains of *S. nanhaiensis*, *L. methylohalidivorans*, and *P. gallaeciensis* in that phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid and one unidentified lipid are major polar lipids, but distinguishable from the type strains of *S. nanhaiensis* and *L. methylohalidivorans* by the absence of a minor amount of diphosphatidylglycerol (Supplementary data Fig. S2). The DNA G+C content of strain MA2-16^T was 57.7 mol%, a value lower than that of the type strain of *S. nanhaiensis* (Table 1).

Table 2. Cellular fatty acid compositions (%) of strain MA2-16^T and the type strains of *Sedimentitalea nanhaiensis*, two *Leisingera* species and two *Phaeobacter* species

Strains: 1, MA2-16^T; 2, *S. nanhaiensis* LMG 24841^T; 3, *L. methylohalidivorans* DSM 14336^T; 4, *L. aquimarina* LMG 24366^T; 5, *P. gallaeciensis* DSM 26640^T; 6, *P. inhibens* DSM 16374^T. All data obtained from this study. Fatty acids that represented < 0.5% in all strains were omitted. TR, Trace (< 0.5%); –, Not detected.

Fatty acid	1	2	3	4	5	6
Straight-chain						
$C_{12:0}$	–	0.6	0.7	0.6	0.9	0.7
$C_{16:0}$	4.4	3.6	3.3	5.3	7.7	6.3
$C_{18:0}$	3.8	1.4	0.6	1.6	3.8	2.6
Branched						
iso-$C_{14:1}$ E*	–	–	–	4.8	–	–
Unsaturated						
$C_{18:1}$ $\omega 7c$	63.6	65.5	71.5	68.7	70.4	75.2
Hydroxy						
$C_{10:0}$ 3-OH	2.8	2.8	1.9	1.7	2.0	1.8
$C_{12:0}$ 3-OH	1.4	2.3	2.9	2.5	2.3	2.0
$C_{16:0}$ 2-OH	4.8	5.4	6.2	7.0	4.1	2.2
$C_{18:1}$ 2-OH	–	TR	0.6	TR	–	–
11-methyl $C_{18:1}$ $\omega 7c$	18.7	18.0	12.0	7.3	8.6	9.1

* Double bond position indicated by a capital letter is unknown.

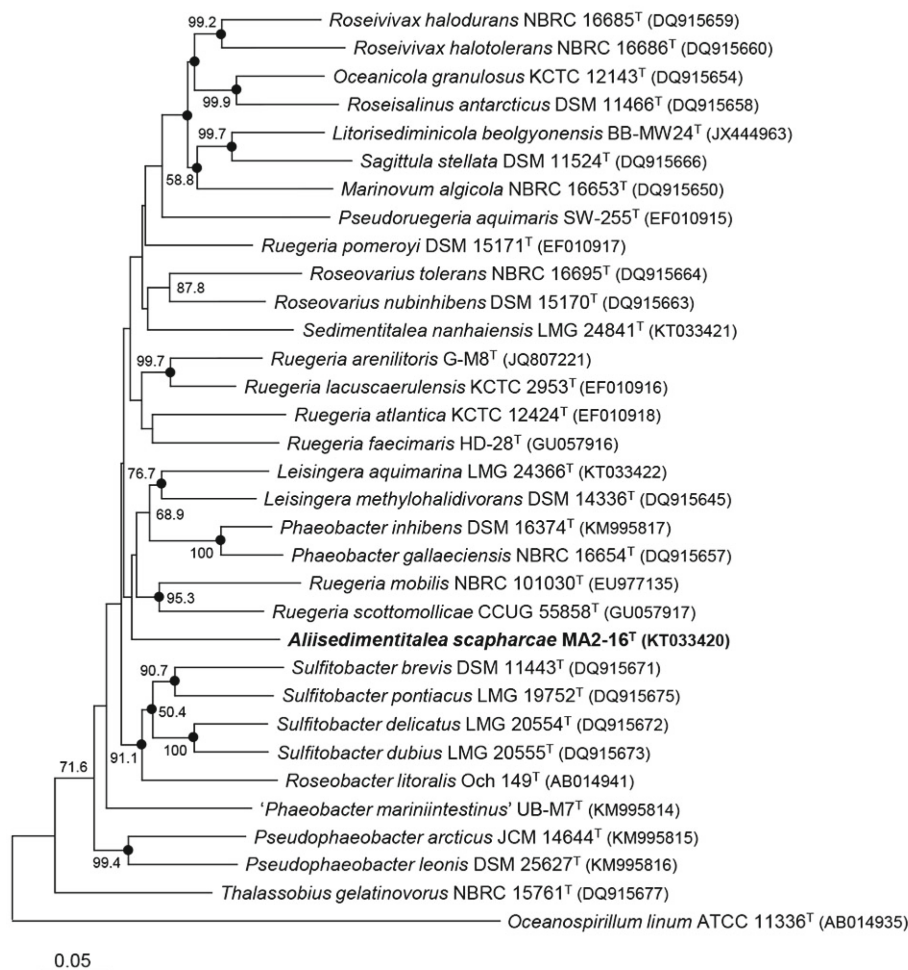

Fig. 2. Neighbour-joining phylogenetic tree based on *gyrB* sequences showing the positions of strain MA2-16^{T} and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1,000 replications) of > 50% are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum parsimony algorithms. *Oceanospirillum linum* ATCC 11336^{T} (GenBank accession no., AB014935) was used as an outgroup. Scale bar, 0.05 substitutions per nucleotide position.

Conclusion

In the NJ-, ML-, and MP-phylogenetic trees based on 16S rRNA gene sequences, strain MA2-16^{T} was found to form a cluster with the type strain of *S. nanhaiensis* (Fig. 1). Nevertheless, the novel strain did not exhibit the highest 16S rRNA gene sequence similarity to the type strain of *S. nanhaiensis*. The cluster comprising strain MA2-16^{T} and the type strain of *S. nanhaiensis* may not fall in the clusters comprising the type strains of *Leisingera* species and *Phaeobacter* species or may form an independent phylogenetic lineage, depending on which reference strains are included or excluded and depending on treeing methods used. Moreover, strain MA2-16^{T} was found to form a phylogenetic lineage independent of those of other taxa as well as the type strain of *S. nanhaiensis* in the phylogenetic tree based on *gyrB* sequences (Fig. 2). Strain MA2-16^{T} exhibited higher *gyrB* sequence similarities to the type strain of *L. methylohalidivorans* and some members of the genera *Ruegeria*, *Sulfitobacter*, and *Roseovarius* than to the type strain of *S. nanhaiensis*. It is likely not to be appropriate to assign strain MA2-16^{T} to any of the recognized genera of the family *Rhodobacteraceae* from the chemotaxonomic properties and the phylogenetic data based on 16S rRNA gene and *gyrB* sequences.

Strain MA2-16^{T} could be distinguished from the type strains of some phylogenetically related species by differences in some phenotypic characteristics, as shown in Table 1. These differences, in combination with the phylogenetic and genetic distinctiveness of strain MA2-16^{T}, suggest that the novel strain is separated from recognized species of the genera *Sedimentitalea*, *Leisingera* and *Phaeobacter* (Wayne *et al*., 1987; Stackebrandt and Goebel, 1994). Accordingly, on the basis of the phylogenetic and chemotaxonomic distinctiveness and other differential phenotypic properties, strain MA2-16^{T} is considered to represent a novel genus and species within the family *Rhodobacteraceae*, for which the name *Aliisedimentitalea scapharcae* gen. nov., sp. nov., is proposed.

Description of *Aliisedimentitalea* gen. nov.

Aliisedimentitalea (A.li.i.se.di.men.ti.ta'le.a. L. pronoun *alius* other, another; N.L. fem. n. *Sedimentitalea* a bacterial generic name; N.L. fem. n. *Aliisedimentitalea* the other *Sedimentitalea*).

Cells are Gram-stain-negative, aerobic, motile, and ovoid or rod-shaped. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-10. The major fatty acids are $C_{18:1}$ *ω7c* and 11-methyl $C_{18:1}$ *ω7c*. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified amino-

lipid and an unidentified lipid. The DNA G+C content is 57.7 mol%. The type species is *Aliisedimentitalea scapharcae*. A member of the family *Rhodobacteraceae*, the class *Alphaproteobacteria*, according to 16S rRNA gene sequence analysis.

Description of *Aliisedimentitalea scapharcae* sp. nov.

Aliisedimentitalea scapharcae (sca.phar'cae. N.L. gen. n. *scapharcae* of *Scapharca*, named after the generic name of the ark shell *Scapharca broughtonii*, from which the type strain was isolated).

Cells are Gram-negative, non-spore-forming, and ovoid or rod-shaped, approximately 0.2–0.8 μm in diameter and 0.6->10.0 μm in length; a few cells greater than 10 μm in length are also observed. Motile by means of a single polar flagellum. Colonies on MA are circular, slightly convex, smooth, glistening, light greyish yellow in colour and 1.0–1.5 mm in diameter after incubation for 5 days at 30°C. Optimal growth occurs at 30°C; growth occurs at 4 and 32°C, but not at temperatures of more than 33°C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 6.0, but not at pH 5.5. Growth occurs in the presence of 1.0–5.0% (w/v) NaCl with an optimum of approximately 2.0% (w/v) NaCl. Mg^{2+} ions are required for growth. Anaerobic growth does not occur on MA and on MA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. Hypoxanthine, Tween 80 and L-tyrosine are hydrolysed and casein and xanthine are weakly hydrolysed, but aesculin, gelatin, starch, and urea are not. Aetate is utilized, but L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, D-trehalose, D-xylose, benzoate, citrate, formate, L-malate, pyruvate, succinate, salicin, and L-glutamate are not. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, and acid phosphatase activities are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are absent. The predominant ubiquinone is Q-10. The major fatty acids (> 10% of the total fatty acids) are $C_{18:1}$ $\omega 7c$ and 11-methyl $C_{18:1}$ $\omega 7c$. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and an unidentified lipid. The DNA G+C content of the type strain is 57.7 mol%.

The type strain, MA2-16^T (=KCTC 42119^T =CECT 8598^T), was isolated from ark shell (*Scapharca broughtonii*) collected from Gang-Jin bay on the South Sea, South Korea.

Acknowledgements

This work was supported by grant from the National Fisheries Research and Development Institute (NFRDI) and the Program for Collection, Management and Utilization of Biological Resources (grant NRF-2013M3A9A5075953) from the Ministry of Science, ICT & Future Planning (MSIP) of the Republic of Korea.

References

Barrow, G.I. and Feltham, R.K.A. 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.

Baumann, P. and Baumann, L. 1981. The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*, pp. 1302–1331. *In* Starr, M.P., Stolp, H., Trüper, H.G., Balows, A., and Schlegel, H.G. (eds.), The Prokaryotes. Springer-Verlag, Berlin, Germany.

Breider, S., Scheuner, C., Schumann, P., Fiebig, A., Petersen, J., Pradella, S., Klenk, H.P., Brinkhoff, T., and Göker, M. 2014. Genome-scale data suggest reclassifications in the *Leisingera-Phaeobacter* cluster including proposals for *Sedimentitalea* gen. nov. and *Pseudophaeobacter* gen. nov. *Front Microbiol*. **5**, 416.

Bruns, A., Rohde, M., and Berthe-Corti, L. 2001. *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int. J. Syst. Evol. Microbiol*. **51**, 1997–2006.

Cohen-Bazire, G., Sistrom, W.R., and Stanier, R.Y. 1957. Kinetic studies of pigment synthesis by nonsulfur purple bacteria. *J. Cell Comp. Physiol*. **49**, 25–68.

Embley, T.M. and Wait, R. 1994. Structural lipids of eubacteria, pp. 121–161. *In* Goodfellow, M. and O'Donnell, A.G. (eds.), Modern Microbial Methods. Chemical Methods in Prokaryotic Systematics. John Wiley & Sons, Chichester, England.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol*. **39**, 224–229.

Gaboyer, F., Tindall, B.J., Ciobanu, M.C., Duthoit, F., Romancer, M.L., and Alain, K. 2013. *Phaeobacter leonis* sp. nov., a novel alphaproteobacterium from Mediterranean Sea sediments. *Int. J. Syst. Evol. Microbiol*. **63**, 3301–3306.

Hameed, A., Shahina, M., Lim, S.Y., Nakayan, P., Liu, Y.C., Lai, W.A., and Hsu, Y.H. 2014. *Youngimonas vesicularis* gen. nov., sp. nov., of the family *Rhodobacteraceae*, isolated from surface seawater, reclassification of *Donghicola xiamenensis* Tan *et al*. 2009 as *Pseudodonghicola xiamenensis* gen. nov., comb. nov. and emended description of the genus *Donghicola* Yoon *et al*. 2007. *Int. J. Syst. Evol. Microbiol*. **64**, 2729–2737.

Komagata, K. and Suzuki, K.I. 1987. Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol*. **19**, 161–207.

Lanyi, B. 1987. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol*. **19**, 1–67.

Liu, C., Zhang, X.Y., Su, H.N., Zhou, M.Y., Chen, B., Li, H., Chen, X.L., Zhao, D.L., Zhou, B.C., Shi, M., *et al.* 2014. *Puniceibacterium antarcticum* gen. nov., sp. nov., isolated from seawater. *Int. J. Syst. Evol. Microbiol*. **64**, 1566–1572.

Martens, T., Heidorn, T., Pukall, R., Simon, M., Tindall, B.J., and Brinkhoff, T. 2006. Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte *et al*. 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay *et al*. 1995) Uchino *et al*. 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter, Ruegeria* and *Leisingera. Int. J. Syst. Evol. Microbiol*. **56**, 1293–1304.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Oren, A. and Garrity, G.M. 2014. List of new names and new combinations previously effectively, but not validly, published, Validation List no. 160. *Int. J. Syst. Evol. Microbiol*. **64**, 3603–

3606.

Park, S., Park, D.S., Bae, K.S., and Yoon, J.H. 2014. *Phaeobacter aquaemixtae* sp. nov., isolated from the junction between the ocean and a freshwater spring. *Int. J. Syst. Evol. Microbiol.* **64**, 1378–1383.

Ruiz-Ponte, C., Cilia, V., Lambert, C., and Nicolas, J.L. 1998. *Roseobacter gallaeciensis* sp. nov., a new marine bacterium isolated from rearings and collectors of the scallop *Pecten maximus*. *Int. J. Syst. Bacteriol.* **48**, 537–542.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Inc., Newark, DE, USA.

Schaefer, J.K., Goodwin, K.D., McDonald, I.R., Murrell, J.C., and Oremland, R.S. 2002. *Leisingera methylohalidivorans* gen. nov., sp. nov., a marine methylotroph that grows on methyl bromide. *Int. J. Syst. Evol. Microbiol.* **52**, 851–859.

Stackebrandt, E. and 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.

Staley, J.T. 1968. *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J. Bacteriol.* **95**, 1921–1942.

Sun, F., Wang, B., Liu, X., Lai, Q., Du, Y., Li, G., Luo, J., and Shao, Z. 2010. *Leisingera nanhaiensis* sp. nov., isolated from marine sediment. *Int. J. Syst. Evol. Microbiol.* **60**, 275–280.

Tamaoka, J. and Komagata, K. 1984. Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**, 125–128.

Vandecandelaere, I., Segaert, E., Mollica, A., Faimali, M., and Vandamme, P. 2008. *Leisingera aquimarina* sp. nov., isolated from a marine electroactive biofilm, and emended descriptions of *Leisingera methylohalidivorans* Schaefer *et al.* 2002, *Phaeobacter daeponensis* Yoon *et al.* 2007 and *Phaeobacter inhibens* Martens *et al.* 2006. *Int. J. Syst. Evol. Microbiol.* **58**, 2788–2793.

Vandecandelaere, I., Segaert, E., Mollica, A., Faimali, M., and Vandamme, P. 2009. *Phaeobacter caeruleus* sp. nov., a blue-coloured, colony-forming bacterium isolated from a marine electroactive biofilm. *Int. J. Syst. Evol. Microbiol.* **59**, 1209–1214.

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., Stackebrandt, E., *et al.* 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.

Yamamoto, S. and Harayama, S. 1995. PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl. Environ. Microbiol.* **61**, 1104–1109.

Yoon, J.H., Kang, S.J., Lee, S.Y., and Oh, T.K. 2007a. *Phaeobacter daeponensis* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int. J. Syst. Evol. Microbiol.* **57**, 856–861.

Yoon, J.H., Km, H., Kim, S.B., Kim, H.J., Kim, W.Y., Lee, S.T., Goodfellow, M., and Park, Y.H. 1996. Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int. J. Syst. Bacteriol.* **46**, 502–505.

Yoon, J.H., Kim, I.G., Shin, D.Y., Kang, K.H., and Park, Y.H. 2003. *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int. J. Syst. Evol. Microbiol.* **53**, 53–57.

Yoon, J.H., Lee, S.Y., Kang, S.J., Lee, C.H., and Oh, T.K. 2007b. *Pseudoruegeria aquimaris* gen. nov., sp. nov., isolated from seawater of the East Sea in Korea. *Int. J. Syst. Evol. Microbiol.* **57**, 542–547.

Yoon, J.H., Lee, S.T., and Park, Y.H. 1998. Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rRNA gene sequences. *Int. J. Syst. Bacteriol.* **48**, 187–194.

Zhang, D.C., Li, H.R., Xin, Y.H., Liu, H.C., Chi, Z.M., Zhou, P.J., and Yu, Y. 2008. *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. *Int. J. Syst. Evol. Microbiol.* **58**, 1384–1387.