INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Feng *et al.*, *Int J Syst Evol Microbiol* 2018;68:260–265
DOI 10.1099/ijsem.0.002494

MICROBIOLOGY SOCIETY

Aestuariicoccus marinus gen. nov., sp. nov., isolated from sea-tidal flat sediment

Tingye Feng,[1] Sang Eun Jeong,[1] Kyung Hyun Kim,[1] Hye Yoon Park[1,2] and Che Ok Jeon[1,*]

Abstract

A Gram-stain-negative, strictly aerobic and halotolerant bacterial strain, designated strain NAP41^T, was isolated from a sea tidal flat in the Yellow Sea of South Korea. Cells were non-motile cocci showing oxidase- and catalase-positive activities. Growth of strain NAP41^T was observed at 15–40 °C (optimum, 37 °C), at pH 6.5–9.0 (optimum, pH 7.0–7.5) and in the presence of 0.5–12 % (w/v) NaCl (optimum, 2 %). Strain NAP41^T contained summed feature 8 (comprising $C_{18:\omega7c}$/$_{C18:1\omega6c}$) and $C_{18:0}$ as the major fatty acids and ubiquinone-10 as the sole isoprenoid quinone. Phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, an unidentified aminolipid and three unidentified lipids were detected as the polar lipids. The G+C content of the genomic DNA was 56.0 mol%. Strain NAP41^T was most closely related to *Primorskyibacter insulae* SSK3-2^T, *Thalassococcus lentus* YCS-24^T and *Roseivivax lentus* DSM 29430^T with 96.67, 96.39 and 96.39 % 16S rRNA gene sequence similarities, respectively, and formed a phylogenetic lineage distinct from closely related taxa within the family *Rhodobacteraceae* with low bootstrap values. On the basis of phenotypic, chemotaxonomic and molecular properties, strain NAP41^T represents a novel species of a novel genus of the family *Rhodobacteraceae*, for which the name *Aestuariicoccus marinus* gen. nov., sp. nov. is proposed. The type strain of the type species is NAP41^T (KACC 18431^T=JCM 30739^T).

Although the family *Rhodobacteraceae* was recently proposed as a member of the class *Alphaproteobacteria*, it constitutes a very wide phylogenetic group including more than 100 genera, and numerous additional new members and 16S rRNA phylotypes have been continually described [1]. Members of the *Rhodobacteraceae* are fundamentally aquatic bacteria that were mostly isolated from marine environments and they are known as important players responsible for sulfur and carbon cycling in ocean environments [1–3]. Sea-tidal flats experiencing regular exposure to air and flooding by low and high tides of seawater are important in the fishery industry because they contain diverse valuable marine animals. In addition, it is known that sea-tidal flats are characterized by high nutrient and carbon cycling rates, probably being performed by microorganisms. Therefore, we have made efforts to isolate and characterize bacteria from sea-tidal flats [4–6] and in this study we isolated a bacterial strain that was presumably a member of a novel genus of the family *Rhodobacteraceae*. Here we describe its taxonomic characteristics using a polyphasic approach.

Strain NAP41^T was isolated from sea-tidal flat sediment, using a previously described procedure with some modifications [7]. Briefly, a tidal flat sediment sample that was obtained from the Taean coastal area (36° 48′ 50.82″ N, 126° 11′ 09.56″ E) of South Korea was serially diluted with artificial seawater (20 g NaCl, 2.9 g $MgSO_4$, 4.53 g $MgCl_2.6H_2O$, 0.64 g KCl, 1.75 g $CaCl_2.2H_2O$ per litre), spread onto marine agar 2216 (MA; BD) and incubated at 25 °C for 3 days under aerobic conditions. The 16S rRNA genes of colonies grown on MA were PCR-amplified using the universal primers F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) and double-digested with *Hae*III and *Hha*I, and then representative PCR amplicons showing distinct fragment patterns were partially sequenced using the primer F1, as described previously [8]. The resulting 16S rRNA gene sequences were compared with those of all validated type strains using the Nucleotide Similarity Search program in the EzBioCloud server (http://www.ezbiocloud.net/identify/) [9] and eventually a putative novel strain belonging to the family *Rhodobacteraceae*, designated strain NAP41^T, was selected for further phenotypic and phylogenetic analysis. Strain NAP41^T was routinely cultured aerobically on MA at 37 °C for 3 days. Strain NAP41^T was preserved at −80 °C in marine broth (MB;

Author affiliations: [1]Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea; [2]National Institute of Biological Resources, Incheon 22689, Republic of Korea.
***Correspondence:** Che Ok Jeon, cojeon@cau.ac.kr
Keywords: *Aestuariicoccus marinus*; tidal flat; *Rhodobacteraceae*; new taxa.
Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; RDP, Ribosomal Database Project.
The GenBank accession number for the 16S rRNA gene sequence of strain NAP41^T is MF113251.
One supplementary table and three supplementary figures are available with the online version of this article.

BD) containing 15 % (v/v) glycerol. *Primorskyibacter insulae* KCTC 42602^T, *Thalassococcus lentus* KCTC 32084^T, *Roseivivax lentus* KCTC 22708^T and *Maliponia aquimaris* KCTC 42721^T were used as reference strains for the comparisons of phenotypic properties and fatty acid analysis.

To obtain a longer RNA sequence, the 16S rRNA gene of strain NAP41^T was cloned into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer's instructions and sequenced using the M13 reverse and T7 primers of the TOPO cloning kit at Macrogen (Korea). The 16S rRNA gene sequences of strain NAP41^T and closely related type strains were aligned using the Fast Secondary-Structure Aware Infernal Aligner available in the Ribosomal Database Project (RDP) [10]. Phylogenetic trees based on the neighbour-joining (NJ) and maximum-parsimony (MP) algorithms were reconstructed using the PHYLIP software (version 3.695) [11] and their tree topologies were evaluated through a bootstrap analysis based on a 1000-resampled dataset. A phylogenetic tree with bootstrap values based on the maximum-likelihood (ML) algorithm was also reconstructed using RAxML-HPC BlackBox (version 8.2.4) of the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org) [12]. An additional taxonomic analysis was performed using the RDP Naïve Bayesian rRNA Classifier tool (http://rdp.cme.msu.edu/classifier/) [13].

Comparative analysis based on the 16S rRNA gene sequences revealed that strain NAP41^T was most closely related to *P. insulae* SSK 3-2^T, *T. lentus* YCS-24^T, *R. lentus* DSM 29430^T and *M. aquimaris* MM-10^T with 96.67, 96.39, 96.39 and 96.14 % 16S rRNA gene sequence similarities, respectively. The phylogenetic analysis using the NJ algorithm revealed that strain NAP41^T formed a phyletic lineage distinct from other genera including *Roseivivax*, *Yangia* and *Citreimonas* within the family *Rhodobacteraceae* (Fig. 1). The phylogenetic trees based on the MP and ML algorithms also showed that strain NAP41^T formed a distinct phyletic lineage within the family *Rhodobacteraceae* (Fig. S1, available in the online version of this article). The taxonomic analysis using the RDP Naïve Bayesian rRNA Classifier tool showed that strain NAP41^T was classified as an unclassified_*Rhodobacteraceae* even at low confidence threshold (50 %), suggesting that strain NAP41^T cannot be affiliated with currently validated genus members, and probably represents a new genus of the family *Rhodobacteraceae*. In conclusion, the phylogenetic analyses clearly suggest that strain NAP41^T represents a novel genus of the family *Rhodobacteraceae*.

Growth of strain NAP41^T was tested on MA, R2A agar (BD), laboratory prepared Luria–Bertani (LB) agar, nutrient agar (NA; BD) and tryptic soy agar (TSA; BD) containing 2 % (w/v) NaCl at 37 °C for 3 days. Growth of strain NAP41^T at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) and pH values (4.5–11.0 at 0.5 pH unit intervals) was evaluated in marine broth (MB) for 3 days. MB of below pH 7.5 and pH 8.0–11.0 were prepared using Na_2HPO_4/NaH_2PO_4 and Tris-HCl buffers, respectively [14]. After autoclaving (121 °C, 15 min), the pH values were adjusted again if necessary. Growth of strain NAP41^T at different NaCl concentrations (0, 0.5, 1–15 % at 1 % intervals) was tested in MB prepared in the laboratory according to the BD formula. Anaerobic growth was assessed on MA and MA supplemented with various electron acceptors [sodium nitrate (10 mM), sodium nitrite (2 mM), disodium fumarate (10 mM) and dimethyl sulfoxide (10 mM)] under anaerobic conditions (with 4–10 % CO_2) using the GasPak Plus system (BBL) at 37 °C for 20 days. The following physiological and biochemical tests were conducted using cells grown on MA for 3 days at 37 °C. Gram staining was investigated using the bioMérieux Gram stain kit according to the manufacturer's instructions. Oxidase activity was evaluated by the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), and catalase activity was tested by the production of oxygen bubbles in 3 % (v/v) aqueous H_2O_2 [15]. Cell morphology was investigated using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) with cells grown on MA at 37 °C. The following properties of strain NAP41^T and four reference strains were investigated under the same conditions in parallel. Hydrolysis of casein, starch, aesculin, tyrosine, Tween 20 and Tween 80 was tested on MA according to the methods described previously [15, 16]. Nitrate reduction was assessed in MB according to the method described previously [16]. Additional enzymatic activities, biochemical features and oxidation of various carbon sources were evaluated using API ZYM, API 20NE (bioMérieux) and GN2 MicroPlate (Biolog) testing systems, respectively. The manufacturer's protocol was applied, except that cells resuspended in artificial seawater were used as the inocula and the test strains were incubated at their optimal growth temperatures.

Strain NAP41^T grew well on MA, but grew slowly on R2A agar, NA, LB agar and TSA containing 2 % NaCl. Cells of strain NAP41^T were Gram-stain-negative, non-motile cocci without flagella and were 0.9–1.3 µm in diameter; extracellular vesicles were observed on cell surfaces (Fig. S2). Anaerobic growth of strain NAP41^T was not observed under any tested electron acceptor conditions. In the Biolog GN2 MicroPlate, strain NAP41^T oxidized α-cyclodextrin, succinic acid monomethyl ester, β-hydroxybutyric acid, α-ketoglutaric acid, succinamic acid, L-alanine, dextrin, *cis*-aconitic acid, sucrose, D-arabitol, D-mannitol, succinic acid, gentiobiose, D-psicose, D-galactonic acid lactone, L-asparagine, L-serine, cellobiose, D-fructose, D-mannose, D-sorbitol, inosine, glycerol, turanose, D,L-lactic acid, L-ornithine and thymidine, but strain NAP41^T did not oxidize other carbon compounds in the Biolog GN2 MicroPlate (Table S1). The phenotypic characteristics of strain NAP41^T are presented in Table 1 and in the genus description. Some of the characteristics were in agreement with those considered to be characteristic of the family *Rhodobacteraceae*, whereas many other properties including morphology, optimal growth temperature and tyrosine hydrolysis allowed the

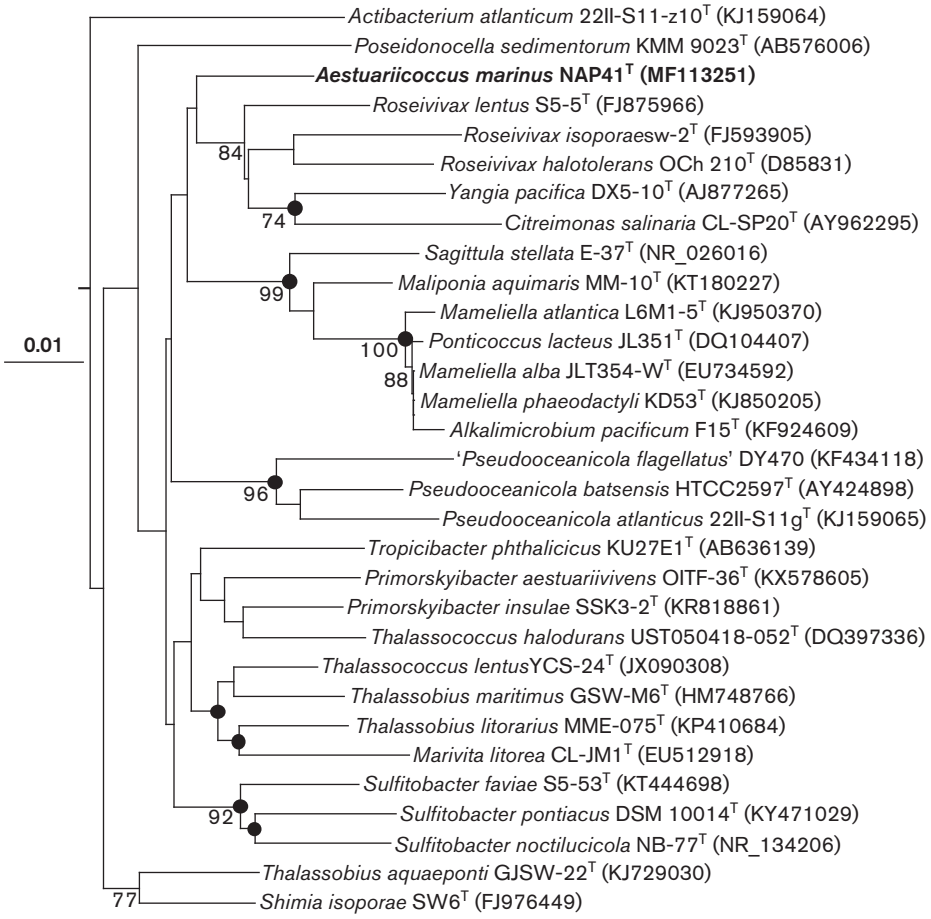

Fig. 1. Phylogenetic relationship between strain NAP41^T and closely related strains within the family *Rhodobacteraceae*, based on 16S rRNA gene sequences. The tree was reconstructed with the NJ algorithm, and filled circles (•) indicate the corresponding nodes that were also recovered in the trees generated with the ML and MP algorithms. Bootstrap values shown on nodes are percentages of 1000 replicates; only values over 70 % are shown. *Stappia stellulata* IAM 12621^T (D88525) was used as an outgroup (not shown). The scale bar equals 0.01 changes per nucleotide position.

differentiation of strain NAP41^T from the closely related genera (Tables 1 and S1).

The isoprenoid quinones of strain NAP41^T were extracted according to the method of Minnikin *et al.* [17] and analysed using a model LC-20A HPLC system (Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250×4.6 mm, Kromasil; Akzo Nobel) as described by Komagata and Suzuki [18]. The DNA G+C content of strain NAP41^T was determined by the fluorometric method [19] using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). Strain NAP41^T and four reference strains were cultivated in MB at their respective optimal temperatures and their cells were harvested at the same growth stage (exponential phase, OD_{600} 0.8) for the cellular fatty acid analysis. The cellular fatty acids of the microbial cells were saponified, methylated and extracted using the standard MIDI protocol. The fatty acid methyl esters were analysed by using a gas chromatograph (Hewlett Packard 6890) and identified by using the TSBA6 database of the Microbial Identification System (Sherlock ver. 6.0B) [20]. The polar lipids of strain NAP41^T were analysed by TLC using cells harvested during the exponential growth phase according to the procedure described by Minnikin *et al.* [21]. The following reagents were used to detect different polar lipids: 10 % ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer–Lester (for phospholipids) and α-naphthol/sulfuric acid (for glycolipids) reagents.

The only respiratory quinone detected from strain NAP41^T was ubiquinone-10, which was consistent with other closely related genus members [22–25]. The genomic DNA G+C content of strain NAP41^T was approximately 56.0 mol%. The major cellular fatty acids (>8 % of the total fatty acids) of strain NAP41^T were summed feature 8 (comprising $C_{18:1}\omega7c$/$C_{18:1}\omega6c$) and $C_{18:0}$. The overall fatty acid profile of strain NAP41^T was similar to those of the reference

Table 1. Comparison of phenotype characteristics of strain NAP41^T and closely related taxa of the family *Rhodobacteraceae*

Taxa: 1, strain NAP41^T (this study); 2, *P. insulae* KCTC 42602^T [22]; 3, *T. lentus* KCTC 32084^T [23]; 4, *R. lentus* KCTC 22708^T [24], 5; *M. aquimaris* KCTC 42721^T [25]. All strains are positive for the following characteristics: catalase activity, activity* of esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase. All strains are negative for the following characteristics: motility; hydrolysis* of casein, Tween 20, starch; activity* of lipase (C14), crystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase; assimilation* of capric acid, trisodium citrate, phenylacetic acid. Symbols: +, positive; –, negative.

Characteristic	1	2	3	4	5
Morphology	Coccus	Rod	Rod/ovoid	Rod	Coccus/ovoid
Oxidase	+	+	+	+	–
Nitrate reduction	+	+	–	+	+
Optimal temperature (°C)	37	30	25–28	30	30
Growth range of NaCl (%, w/v)	0.5–12.0	0.5–7.0	0.5–5.0	2.0–13.0	0–9.0
Hydrolysis* of:					
Tween 80	+	–	+	+	–
Tyrosine	+	–	–	–	–
Aesculin	+	+	–	+	+
Enzyme activity (API ZYM)* of:					
Alkaline phosphatase	+	+	–	–	+
Acid phosphatase	+	+	+	+	–
α-Glucosidase	+	–	–	+	–
β-Glucosidase	–	+	–	–	–
Assimilation (API 20NE)* of:					
D-Glucose, L-arabinose, D-mannose	+	+	–	–	–
D-Mannitol	+	–	–	–	+
N-Acetyl-glucosamine, potassium gluconate	–	–	–	–	+
Maltose	+	–	–	+	+
Adipic acid	–	+	–	–	+
Malic acid	+	+	–	–	+
Major polar lipids†	PC, PG, PE, AL, L	PC, PG, AL, L	PG, PC, AL, L	PG, PE, AL, PL, L, DPG	PC, PG, PE, DPG, PME, PL, L
DNA G+C content (mol%)	56.0	60.6	58.0	68.2	66.0

*Data were obtained from this study.

†PC, phosphatidylcholine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanol-amine; PME, phosphatidyl-*N*-methylethanol-amine; AL, unidentified aminolipid; PL, unidentified phospholipid; L, unidentified lipid.

taxa of the family *Rhodobacteraceae*. However, there were some differences in the respective proportions of some fatty acid components (Table 2). For example, anteiso-$C_{14:0}$ and anteiso-$C_{15:0}$ were detected from strain NAP41^T, while they were not detected or just detected as a trace from other reference strains. Phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, an unidentified aminolipid and three unidentified lipids were detected from strain NAP41^T as the major polar lipids (Fig. S3). The profiles of polar lipids also differentiated strain NAP41^T from closely related strains (Table 1). For example, phosphatidylethanolamine was detected from strain NAP41^T, while it was not detected from *P. insulae* and *T. lentus* [22, 23]. In addition, diphosphatidylglycerol was identified from *R. lentus* and *M. aquimaris*, but it was not detected from strain NAP41^T [24, 25]. In conclusion, phylogenetic, physiological and chemotaxonomic features clearly support that strain NAP41^T represents a novel species of a novel genus of the family *Rhodobacteraceae*, for which the name *Aestuariicoccus marinus* gen. nov., sp. nov. is proposed.

DESCRIPTION OF THE GENUS *AESTUARIICOCCUS* GEN. NOV.

Aestuariicoccus (Aes.tu.a.ri.i.coc′cus. L. neut. n. *aestuarium* mud flat; Gr. masc. n. *coccus* a grain or berry; N.L. masc. n. *Aestuariicoccus* a coccus-shaped bacterium from mud flat).

Cells are Gram-stain-negative, strictly aerobic and non-motile cocci without flagella. Oxidase and catalase are positive. Nitrate is reduced to nitrite. The major isoprenoid quinone is ubiquinone-10. The major cellular fatty acids are summed feature 8 (comprising $C_{18:1}\omega7c$/$C_{18:1}\omega6c$) and $C_{18:0}$. The major polar lipids are phosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine. The genus is a member of the family *Rhodobacteraceae* of the

Table 2. Cellular fatty acid compositions (%) of strain NAP41^T and closely related taxa of the family *Rhodobacteraceae*

Taxa: 1, strain NAP41^T; 2, *P. insulae* KCTC 42602^T; 3, *T. lentus* KCTC 32084^T; 4, *R. lentus* KCTC 22708^T, 5; *M. aquimaris* KCTC 42721^T. All data were obtained from this study. Data are expressed as percentages of the total fatty acids, and fatty acids amounting to less than 0.5 % in all strains are not shown. Major components (>5.0 %) are highlighted in bold. TR, Trace amount (<0.5 %); –, not detected.

Fatty acid	1	2	3	4	5
Saturated:					
$C_{12:0}$	–	0.5	–	–	TR
$C_{16:0}$	3.8	**10.2**	2.0	**7.1**	5.5
$C_{17:0}$	0.6	0.5	–	1.2	1.1
$C_{18:0}$	**8.2**	**5.1**	**10.5**	4.6	**11.6**
Unsaturated:					
$C_{18:1}\omega7c$ 11-methyl	3.7	0.9	3.5	**7.1**	3.9
Hydroxy:					
$C_{10:0}$ 3-OH	–	0.6	0.6	1.1	TR
$C_{12:0}$ 3-OH	–	–	–	0.6	–
$C_{12:1}$ 3-OH	1.6	3.0	3.0	0.7	1.8
Branched:					
anteiso-$C_{14:0}$	1.1	–	–	–	–
anteiso-$C_{15:0}$	0.7	–	–	TR	–
Summed feature*:					
3	–	1.1	–	0.5	TR
8	**78.6**	**78.1**	**80.5**	**72.0**	**74.4**

*Summed features represent groups of two fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

phylum *Proteobacteria*. The type species is *Aestuariicoccus marinus.*

DESCRIPTION OF *AESTUARIICOCCUS MARINUS* SP. NOV.

Aestuariicoccus marinus (ma.ri′nus. L. masc. adj. *marinus* of the sea).

In addition to the characteristics given in the genus description above, this species has the following properties. Growth occurs at 15–40 °C (optimum, 37 °C), at pH 6.5–9.0 (optimum, pH 7.0–7.5) and in the presence of 0.5–12.0 % (w/v) NaCl (optimum, 2 %). Hydrolyses Tween 80, aesculin and tyrosine, but not casein, Tween 20 and starch. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are positive, but lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase and β-glucosidase activities are negative. Assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, maltose and malic acid is positive, but assimilation of *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid is negative. The major polar lipids consist of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, an unidentified aminolipid and three unidentified lipids.

The type strain is NAP41^T (KACC 18431^T=JCM 30739^T), isolated from sea-tidal flat sediment in South Korea. The DNA G+C content is 56.0 mol%.

Funding information
This work was supported by the Program for Collection of Domestic Biological Resources from the National Institute of Biological Resources (NIBR no. 2017-02-001) of Ministry of Environment (MOE) and the National Research Foundation of Korea (2017R1A2B4004888) of MEST, Republic of Korea.

Acknowledgements
The authors thank a nomenclature reviewer for the support regarding the nomenclature of the microorganism.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Pujalte MJ, Lucena T, Ruvira MA, Arahal DR, Macián MC *et al.* The family *Rhodobacteraceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F *et al.* (editors). *The Prokaryotes, Alphaproteobacteria and Betaproteobacteria*, 4th ed. Berlin: Springer; 2014. pp. 545–577.
2. Buchan A, González JM, Moran MA. Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* 2005;71:5665–5677.
3. Romanenko LA, Tanaka N, Svetashev VI, Mikhailov VV. *Primorskyibacter sedentarius* gen. nov., sp. nov., a novel member of the class *Alphaproteobacteria* from shallow marine sediments. *Int J Syst Evol Microbiol* 2011;61:1572–1578.
4. Jin HM, Jeong HI, Jeon CO. *Aliiglaciecola aliphaticivorans* sp. nov., an aliphatic hydrocarbon-degrading bacterium, isolated from a sea-tidal flat and emended description of the genus *Aliiglaciecola* Jean *et al.* 2013. *Int J Syst Evol Microbiol* 2015;65:1550–1555.
5. Baek K, Jeon CO. *Rheinheimera gaetbuli* sp. nov., a marine bacterium isolated from a tidal flat. *Curr Microbiol* 2016;72:344–350.
6. Kim KH, Jin HM, Jeong HI, Jeon CO. *Maribacter lutimaris* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2016;66:1773–1778.
7. Jeong HI, Jin HM, Jeon CO. *Confluentimicrobium naphthalenivorans* sp. nov., a naphthalene-degrading bacterium isolated from sea-tidal-flat sediment, and emended description of the genus *Confluentimicrobium* Park *et al.* 2015. *Int J Syst Evol Microbiol* 2015;65:4191–4195.
8. Kim JM, Le NT, Chung BS, Park JH, Bae JW *et al.* Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes by the newly isolated bacterium *Pseudoxanthomonas spadix* BD-a59. *Appl Environ Microbiol* 2008;74:7313–7320.
9. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
10. Nawrocki EP, Eddy SR. Query-dependent banding (QDB) for faster RNA similarity searches. *PLoS Comput Biol* 2007;3:e56.
11. Felsenstein J. *PHYLIP (Phylogeny Inference Package), Version 3.6a*. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA: ; 2002.
12. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.

13. **Wang Q, Garrity GM, Tiedje JM, Cole JR.** Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;73:5261–5267.
14. **Gomori G.** Preparation of buffers for use in enzyme studies. *Methods Enzymol* 1955;1:138–146.
15. **Smibert RM, Krieg NR.** Phenotypic characterization. In: Gerhardt P (editor). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
16. **Lányi B.** Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 1987;19:1–67.
17. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.*** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
18. **Komagata K, Suzuki K.** Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–208.
19. **Gonzalez JM, Saiz-Jimenez C.** A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.
20. **Sasser M.** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
21. **Minnikin DE, Patel PV, Alshamaony L, Goodfellow M.** Polar lipid composition in the classification of *nocardia* and related bacteria. *Int J Syst Bacteriol* 1977;27:104–117.
22. **Park S, Park JM, Jung YT, Won SM, Yoon JH.** *Primorskyibacter insulae* sp. nov., isolated from the junction between the ocean and a freshwater spring. *Int J Syst Evol Microbiol* 2015;65:3971–3976.
23. **Park S, Jung YT, Kim SI, Yoon JH.** *Thalassococcus lentus* sp. nov., an alphaproteobacterium isolated from seawater of a seaweed farm. *Antonie van Leeuwenhoek* 2013;103:465–473.
24. **Park S, Kang SJ, Oh TK, Yoon JH.** *Roseivivax lentus* sp. nov., isolated from a tidal flat sediment, and emended description of the genus *Roseivivax* Suzuki *et al*. 1999. *Int J Syst Evol Microbiol* 2010;60:1113–1117.
25. **Jung YT, Lee JS, Yoon JH.** *Maliponia aquimaris* gen. nov., sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 2016;66:2271–2277.