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Nioella aestuarii sp. nov., of the family *Rhodobacteraceae*, isolated from tidal flat

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Abstract

A bacterium, designated strain MME-018^T, was isolated from a tidal flat of the Muui-do in the Republic of Korea and identified within the family *Rhodobacteraceae*. The 16S rRNA gene sequence of the isolate showed the highest similarity to that of *Nioella sediminis* JS7-11^T (98.9 %), followed by *Nioella nitratireducens* SSW136^T (97.1 %). In phylogenetic analyses, these taxa formed a clade at neighbour-joining, maximum-likelihood, and maximum-parsimony algorithms, in which it was separated from other genus belonging to the family *Rhodobacteraceae*. Ubiquinone-10 (Q-10) was the major respiratory quinone. Major polar lipids included phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified phospholipids, and an unidentified lipid. Major fatty acids were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) $C_{16:0}$, cyclo $C_{19:0}\omega8c$, and 11-methyl $C_{18:1}\omega7c$. Genomic DNA G+C content was 61 mol%. Cells were Gram-stain negative, non-motile, aerobic, and rod-shaped. This strain grew in 1–4 % (w/v) NaCl, at 4–40 °C and pH 6.0–8.0, with optimal growth in 2 % (w/v) NaCl, at 25–30 °C and pH 7.0. DNA–DNA hybridization values between strain MME-018^T and *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T were 17±3 and 13±1 %, respectively. On the basis of polyphasic taxonomic analysis, strain MME-018^T is proposed to represent a novel species of the genus *Nioella*, for which the name *Nioella aestuarii* sp. nov. The type strain of *Nioella aestuarii* is MME-018^T (=KCCM 43135^T=JCM 30752^T)

The *Roseobacter* clade within the family *Rhodobacteraceae* is known to originate from marine ecosystems. Most members of this family have been isolated from saline or hypersaline environments [1, 2]. Among them, some species are moderately halophilic or halotolerant, requiring sodium ions for growth [3, 4]. The genus *Nioella* that belongs to the *Roseobacter* clade was first proposed by Rajasabapathy *et al.* [5] with the description of one species, *Nioella nitratireducens*. Recently, *Nioella sediminis* has been isolated from the surface sediment of the Jinulong River and emended to the genus *Nioella* [6]. Features of this genus are known to be Gram-stain negative, aerobic, non-spore forming, and rod shaped bacterium that requires sodium ions for growth [5, 6]. From a tidal flat of the Muui-do in Republic of Korea, we isolated a bacterium that has similar characteristics with the members of the genus *Nioella* requiring sodium ions for growth. In this study, we investigated the taxonomic position of this strain, designated MME-018^T, using a polyphasic approach.

Sample was collected from a tidal flat of the Muui-do near Incheon in the Republic of Korea, (37° 24′ 17″ N 126° 24′ 49″ E) in September of 2014. The sample was serially diluted with 3 % (w/v) NaCl. An aliquot (200 µl) of the diluted solution was spread onto the natural seawater agarose medium that contained 0.5 g yeast extract, 1.5 % (w/v) agarose, 1 ml of trace element solution SL-6 (DSM medium no. 27), and 1 ml of vitamin solution [7] per 1 l of natural seawater. The plates were incubated at 30 °C for 2 weeks. Colonies were streaked onto the same medium at least three times to obtain single and pure colonies. The pure colony designated MME-018^T was transferred onto marine agar 2216 (BD; MA) and routinely cultivated on MA at 30 °C.

To amplify the 16S rRNA gene sequence of strain MME-018^T, its genomic DNA was extracted by using G-spin Total DNA Extraction Kit (iNtRON Biotechnology) and QuickGene DNA tissue kit S (Kurabo) according to the manufacturer's instructions. The extracted genomic DNA was PCR amplified using the universal primers 27F and 1492R [8]. The PCR products were then sequenced by CosmogeneTech

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Keywords: *Rhodobacteraceae*; *Nioella aestuarii*; tidal flat; polyphasic taxonomy.
Abbreviations: CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid.
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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain MME-018^T is KP410676.
Two supplementary figures are available with the online version of this article.

Co. Ltd. using the bacterial primers 27F, 337F, 518R, 785F and 1492R [9]. These sequences were assembled using the SeqMan software (DNAStar) according to the methods of Roh *et al.* [10]. To find closely related taxa, the 16S rRNA gene sequence of strain MME-018^T was aligned by using SILVA (http://www.arb-silva.de/aligner) [11]. To analyze a phylogenetic tree, based on the 16S rRNA gene sequence, the nearest phylogenetic neighbours of the isolated strain were identified using the BLAST (http://www.ncbi.nlm.nih.gov/blast/) and the EzBioCloud database (http://www.ezbiocloud.net/) [12]. The sequences of related taxa were acquired from the same websites. The Kimura two-parameter model [13] was used to calculate evolutionary distances between the isolated strain and reference taxa. The phylogenetic tree of the 16S rRNA gene sequences of the strain MME-018^T and related taxa was constructed using MEGA 6 program [14] and three algorithms, neighbour-joining [15], maximum-likelihood [16], and maximum-parsimony [17]. In this study, the bootstrap values were calculated based on 1000 random replications. The 16S rRNA gene sequence of strain MME-018^T was determined to be 1392 bp in length. Levels of 16S rRNA gene sequence similarity between the strain MME-018^T and others were as follows: *Nioella sediminis* JS7-11^T (98.9 %), *Nioella nitratireducens* SSW136^T (97.1 %), *Aestuariivita atlantica* 22II-SI1-z3^T (95.8 %), *Marivita geojedonensis* DPG-138^T (95.7 %), and *Roseovarius aestuarii* SMK-122^T (95.5 %). In the construction of phylogenetic trees, the strain MME-018^T clustered with the members of the genus *Nioella* which includes *Nioella sediminis* JS7-11^T and *Nioella nitratireducens* SSW136^T (Fig. 1). This is suggested that strain MME-018^T belongs to the genus *Nioella*.

To further characterize and compare the isolated strain MME-018^T and the reference strains, *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T, were purchased from Korean Collection for Type Cultures (Korea). The isolate and both references were routinely cultivated on MA at 30 °C. The respiratory quinones were extracted using chloroform/methanol (2 : 1, v/v) [18] and identified using an HPLC system (YL9100; Younglin). The polar lipids were isolated and examined by two-dimensional TLC using the method of Minnikin *et al.* [19]. The lipid spots were sprayed according to the protocols described by Minnikin *et al.* [19] and Komagata and Suzuki [20]. The isolated strains MME-018^T, *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T were cultivated on MA at 30 °C for 3 days to compare the compositions of cellular fatty acid. The cells were harvested and freeze-dried. The cellular fatty acids were saponified, methylated, and extracted as described by Sasser [21]. The extracted cellular fatty acids were analysed according to Miller [22] using an Agilent 6890 gas chromatography system and a cross-linked methyl siloxane column (HP-1; A30 m×0.320 mm×0.25 μm). Analysis of the cellular fatty acids was determined by using the Sherlock MIS Software ver. 6.2, based on the TSBA6 database [21]. The genomic DNA G+C content was determined by using a reverse-phase HPLC [23].

The respiratory quinone of strain MME-018^T was ubiquinone-10 (Q-10), which was related to the family *Rhodobacteraceae*. In this study, the major common polar lipids of the isolated strain MME-018^T and the related taxa *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T were phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), two unidentified phospholipids, and an unidentified lipid (Fig. S1, available in the online version of this article). Although two unidentified aminolipids had been slightly detected from *Nioella nitratireducens* SSW136^T [5], they were not detected along with strain MME-018^T in the current study. The predominant (>5 %) cellular fatty acids of strain MME-018^T were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{16:0}$, cyclo $C_{19:0}\omega8c$, and 11-methyl $C_{18:1}\omega7c$. The cellular fatty acid composition was similar to that of the related taxa *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T (Table 1). The genomic DNA G+C content of the isolated strain was 61.5 mol%.

To investigate the morphological, physiological and phenotypic properties, the strains MME-018^T, *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T were routinely cultivated on MA at 30 °C, but conditions were changed slightly to fit the experimental needs. Cell motility was determined as described by Tittsler and Sandholzer [24] using semi-solid agar medium (containing 0.3 % agarose). To observe the flagella and the cell morphology, microscopic test was performed using phase contrast microscopy (Primo Star; Carl Zeiss) and transmission electron microscopy (JEM-1010; JEOL). Gram staining was carried out using Gram staining kit (Bioworld), according to the manufacturer's instructions. Catalase- and oxidase-tests were performed according to the procedures previously described by Kovacs [25] and Smibert and Krieg [26]. Growth at temperature ranges of 0, 5, 10, 15, 20, 25, 30, 37, 40, 45, and 50 °C was assessed on MA for 4 weeks. The NaCl concentration for growth of the strain MME-018^T was investigated on artificial seawater (ASW) agar medium [27] of which the range of NaCl concentrations was 0–10 % (w/v) with 1 % increments. The pH for growth was investigated at pH 5.0–11.0 with 1.0 pH unit increment by using 10 mM MES (pH 5.0 and 6.0), 10 mM bis-Tris propane (pH 7.0–9.0), or 10 mM CAPS (pH 10.0 and 11.0). Anaerobic growth was determined by incubation on MA with 10 mM nitrate, 10 mM $FeCl_3$, and 10 mM thiosulfate as electron acceptors in a GasPak EZ anaerobic gas-generating pouch system with indicator (BD) at 30 °C for 4 weeks. Starch and casein hydrolysis were tested as per the methods provided by Benson [28], while hydrolysis of Tween 20, 40, and 80 was determined as described by Gonzáles *et al.* [29]. Gelatin and L-tyrosine hydrolysis were evaluated according to the methods of Smibert and Krieg [26]. Substrate utilization was assessed using the modified MA according to methods previously described by Park *et al.* [30] supplemented with followings at 1 % (w/v): acetate, L-arabinose, benzoate, cellobiose, citrate, formate, D-fructose, D-galactose, D-glucose, L-glutamate, glycerol, inositol, lactose, malate, maltose, D-

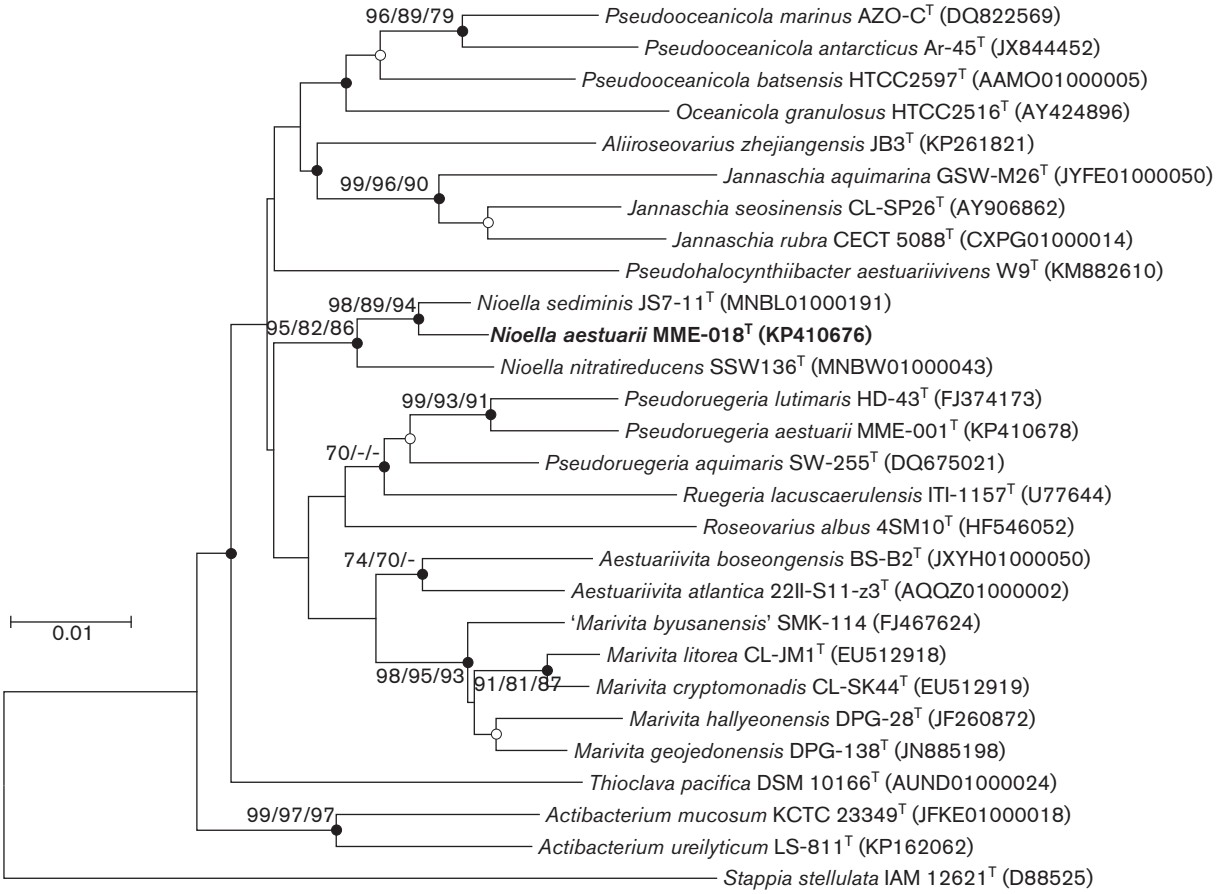

Fig. 1. Neighbour-joining (NJ) phylogenetic tree based on the 16S rRNA gene sequences showing the position and relationship between strain MME-018^T and *Nioella nitratireducens* and other members of the *Roseobacter* clade. Numbers at nodes indicate bootstrap values (>70 %) calculated based on the NJ/maximum-likelihood (ML)/maximum-parsimony (MP) algorithms. Closed circles indicate that the corresponding nodes are also recovered by the ML and MP. Open circles indicate that the corresponding nodes are also recovered by the ML or MP. *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

mannitol, D-mannose, melibiose, L-ornithine, pyruvate, raffinose, L-rhamnose, D-ribose, D-sorbitol, succinate, sucrose, trehalose and D-xylose. Acid production from carbohydrates were detected as described by Park *et al.* [30] and Leifson [31] supplemented with 1 % (w/v) cellobiose, D-galactose, D-mannose, melibiose, raffinose, L-rhamnose, and trehalose. The enzyme activities of strain MME-018^T were determined using the API 20NE and API ZYM strips.

After 3 days on MA at 30 °C, colonies of strain MME-018^T were circular, smooth, convex, white in colour, and 0.5–1.0 mm in diameter. These isolates were Gram-negative and rod-shaped bacteria 0.5–0.8 µm wide and 1.6–2.0 µm long (Fig. S2). The flagella were not observed. Catalase- and oxidase-reactions were positive. Strain MME-018^T grew in the presence of 1–4 % (w/v) NaCl at 4–40 °C and pH 6.0–8.0, and grew optimally in the presence of 2 % (w/v) NaCl, at 25–30 °C and pH 7.0. Strain MME-018^T required sodium ions for growth, which is consistent with *Nioella nitratireducens* KCTC 32417^T [5]. Anaerobic growth was not observed. Hydrolysis of starch, casein, L-tyrosine, gelatin, and Tween 20, 40, and 80 was not detected in this study. Nitrate was aerobically reduced to nitrite, but reduction of nitrite to nitrogen was not observed. In the API 20NE, arginine dihydrolyase and β-galactosidase were positive, whereas indole production, glucose fermentation, and activities of urease, β-glucosidase (aesculin), and protease (gelatin) were negative. In API ZYM tests, activities of alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β-galactosidase were positive. Other characteristics are provided in Table 2 along with the species description.

To investigate the requirement of cationic ions for the growth of strain MME-018^T, each of 0.2 % (w/v) $CaCl_2 \cdot 2H_2O$, 0.05 % (w/v) KCl and 0.6 % (w/v) $MgCl_2 \cdot 6H_2O$ was added to modified ASW containing 5 g yeast extract, 1 g peptone, 25 g NaCl and 0.01 g $FePO_4$ per litre of distilled water as described by Seo *et al.* [32], followed by the

Table 1. Cellular fatty acid compositions (%) of strain MME-018^T and related taxa

Taxa: 1, strain MME-018^T; 2, *Nioella sediminis* KCTC 42144^T; 3, *Nioella nitratireducens* KCTC 32417^T. All data are from this study. Fatty acids that represented ≥1 % are given. TR, trace amount (<1 %). All strains were cultivated on MA at 30 °C for 3 days.

Fatty acid (%)	1	2	3
$C_{10:0}$ 3-OH	1.8	2.2	2.4
$C_{12:0}$ 3-OH	1.9	1.3	2.5
$C_{16:0}$	10.4	10.5	14.4
$C_{16:0}$ 2-OH	4.9	4.3	4.5
$C_{18:0}$	2.4	4.2	2.3
11-methyl $C_{18:1}\omega7c$	8.9	9.2	4.9
$C_{18:1}$ 2-OH	TR	TR	1.3
Cyclo $C_{19:0}\omega8c$	10.1	5.3	9.7
Summed features*			
3	1.5	TR	TR
8	54.7	58.0	55.4

*As indicated by Montero-Calasanz *et al.* [37] summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

incubation at 30 °C for 7 days. The strain MME-018^T required Ca^{2+} and Mg^{2+} ions for growth. *Nioella sediminis* KCTC 42144^T required Mg^{2+} ion for growth, while *Nioella nitratireducens* KCTC 32417^T required none of the cationic ions.

To test antibiotic susceptibility, strain MME-018^T was inoculated onto MA plates containing discs with the following antibiotics (μg ml^{-1} unless indicated): ampicillin (10), carbenicillin (100), cephalothin (30), ciprofloxacin (10), erythromycin (25), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), norfloxacin (20), novobiocin (10), penicillin G (20 UI), polymyxin B (100 UI), streptomycin (50) and tetracycline (30). Strain MME-018^T was resistant to gentamicin, but susceptible to other antibiotics.

DNA–DNA hybridization (DDH) was conducted fluorometrically by the membrane filter method using a DIG High Prime DNA Labelling and Detection Starter kit II (Roche Applied Science) to identify the genetic relationship of strain MME-018^T and reference strains, *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T [33, 34]. The DDH values of strain MME-018^T with *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T were 17±3 and 13±1 %, respectively. According to current prokaryotic systematics defining DDH values of <70 % as indicative of a distinct species [35, 36], the determined DDH values indicated that the strain MME-018^T were distinguished from *Nioella sediminis* KCTC 42144^T and *Nioella nitratireducens* KCTC 32417^T.

Table 2. Differential phenotypic characteristics of strain MME-018^T and related taxa

Taxa: 1, strain MME-018^T; 2, *Nioella sediminis* KCTC 42144^T; 3, *Nioella nitratireducens* KCTC 32417^T. All data were obtained from this study, Liu *et al.* [6] and Rajasabapathy *et al.* [5]. +, positive; −, negative. All strains are Gram-stain negative, non-motile, aerobic, and rod-shaped. All strains are: positive for catalase- and oxidase-reaction, reduction of nitrate to nitrite, enzyme activities of alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase, utilization of acetate, citrate, L-glutamate, inositol, malate, D-mannitol, L-ornithine, pyruvate, D-sorbitol, succinate, and trehalose; negative for hydrolysis of starch, casein, and Tween 20, 40, and 80, indole production, glucose fermentation, enzyme activity of lipase (C_{14}), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, and utilization of L-arabinose, benzoate, formate, D-ribose, and D-xylose.

Characteristic	1	2	3
Growth at/in			
4 °C	+	−	−
40 °C	+	+	−
1 % (w/v) NaCl	+	+	−
11 % (w/v) NaCl	−	+	+
Optimal growth pH	7.0	7.0	6.0
Hydrolysis of:*			
Gelatin	−	+	−
L-Tyrosine	−	+	−
Enzyme activity of:*			
Arginine dihydrolyase	+	−	−
Cystine arylamidase	−	+	+
Acid phosphatase	+	+	−
α-Glucosidase	+	+	−
β-Glucosidase	−	+	+
Utilization of:*			
Cellobiose	+	−	+
D-Fructose	–	−	+
D-Galactose	+	−	+
D-Glucose	–	−	+
Glycerol	+	−	+
Lactose	+	−	−
Maltose	−	−	+
D-Mannose	+	−	−
Melibiose	+	−	−
Raffinose	+	−	−
L-Rhamnose	+	−	−
Sucrose	−	−	+
Requirement of cations:*			
Ca^{2+}	+	+	–
K^+	–	+	–
Mg^{2+}	+	–	–
Genomic DNA G+C content (mol%)	61.5	63.4	63.5

*Data from this study.

The results of phenotypic, genotypic, and chemotaxonomic analyses revealed that the strain MME-018^T shares common taxonomic properties with the members of the genus

Nioella. According to our phylogenetic analysis, the strain MME-018^T was the most closely related to *Nioella sediminis*, but some aspects of the polyphasic taxonomic properties of strain MME-018^T were different from those of the reference species. Therefore, strain MME-018^T is considered to represent a novel species of the genus *Nioella*, for which the name *Nioella aestuarii* sp. nov. is proposed.

DESCRIPTION OF *NIOELLA AESTUARII* SP. NOV.

Nioella 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 0.8–1.0 µm in width by 1.8–2.0 µm in length. Colonies on MA are circular, smooth, convex, and white in colour and 0.5–1.0 mm in diameter after 3 days incubation at 30 °C. Growth occurs at 4–40 °C (optimum 25–30 °C) in presence of 1–4 % NaCl (optimum 2 %, w/v) and at pH 6.0–8.0 (optimum pH 7.0). Requires Na^+, Ca^{2+}, or Mg^{2+} ions for growth. Nitrate, $FeCl_2$, and thiosulfate are not reduced under anaerobic conditions. Catalase and oxidase are positive. Hydrolysis of starch, casein, L-tyrosine, gelatin, and Tween 20, 40, and 80 does not occur. Nitrate is aerobically reduced to nitrite in API 20NE kit. Arginine dihydrolyase and β-galactosidase are positive, while urease, β-glucosidase (aesculin), and protease (gelatin) are negative. Indole is not produced. Glucose is not fermented. In the API ZYM strips, alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, valine arylamidase, acid phosphatase, α-glucosidase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase activities are positive, whereas cystine arylamidase, lipase (C_{14}), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are negative. Acetate, cellobiose, citrate, D-galactose, L-glutamate, glycerol, inositol, lactose, malate, D-mannitol, D-mannose, melibiose, L-ornithine, pyruvate, raffinose, L-rhamnose, D-sorbitol, succinate, and trehalose are utilized, while L-arabinose, benzoate, formate, D-fructose, D-glucose, maltose, D-ribose, sucrose, and D-xylose are not utilized. Acid is produced from D-galactose, D-mannose, L-rhamnose, but not cellobiose, melibiose, raffinose, and trehalose. The dominant respiratory quinone is Q-10. The major polar lipids are PC, PG PE, two unidentified phospholipids, and an unidentified lipid. The major fatty acids are summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{16:0}$, cyclo $C_{19:0}\omega8c$, and 11-methyl $C_{18:1}\omega7c$.

The type strain, MME-018^T (=KCCM 43135^T=JCM 30752^T) was isolated from the tidal flat sediment of Muui-do in Incheon, the Republic of Korea. The genomic DNA G+C content is 61.6 mol%.

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

References

1. Labrenz M, Lawson PA, Tindall BJ, Collins MD, Hirsch P. *Roseisalinus antarcticus* gen. nov., sp. nov., a novel aerobic bacteriochlorophyll α-producing α-proteobacterium isolated from hypersaline Ekho Lake, Antarctica. *Int J Syst Evol Microbiol* 2005;55:41–47.
2. Martínez-Checa F, Quesada E, Martínez-Cánovas MJ, Llamas I, Béjar V. *Palleronia marisminoris* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium belonging to the '*Alphaproteobacteria*', isolated from a saline soil. *Int J Syst Evol Microbiol* 2005;55:2525–2530.
3. Petursdottir SK, Kristjansson JK. *Silicibacter lacuscaerulensis* gen. nov., sp. nov., a mesophilic moderately halophilic bacterium characteristic of the Blue Lagoon geothermal lake in Iceland. *Extremophiles* 1997;1:94–99.
4. Pujalte MJ, Macián MC, Arahal DR, Ludwig W, Schleifer KH *et al.* *Nereida ignava* gen. nov., sp. nov., a novel aerobic marine alphaproteobacterium that is closely related to uncultured *Prionitis* (alga) gall symbionts. *Int J Syst Evol Microbiol* 2005;55:631–636.
5. Rajasabapathy R, Mohandass C, Yoon JH, Dastager SG, Liu Q *et al.* *Nioella nitratireducens* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae* isolated from Azorean Island. *Antonie van Leeuwenhoek* 2015;107:589595.
6. Liu Y, Du J, Lai Q, Dong C, Shao Z. *Nioella sediminis* sp. nov., isolated from surface sediment and emended description of the genus *Nioella*. *Int J Syst Evol Microbiol* 2017;67:12711274.
7. Wolin EA, Wolin MJ, Wolfe RS. Formation of methane by bacterial extracts. *J Biol Chem* 1963;238:2882–2886.
8. Lane DJ. 16S/23S rRNA sequencing. In: Stackbrandt E and Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. Chichester, UK: Jon Wiley and Sons; 1991. pp. 115–175.
9. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173: 697703.
10. Roh SW, Sung Y, Nam YD, Chang HW, Kim KH *et al.* *Arthrobacter soli* sp. nov., a novel bacterium isolated from wastewater reservoir sediment. *J Microbiol* 2008;46:4044.
11. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
12. 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.
13. Kimura M. *The Neutral Theory of Molecular Evolution*. Cambridge, UK: Cambridge University Press; 1983.
14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:27252729.
15. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
16. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
17. Kluge AG, Farris JS. Quantitative phyletics and the evolution of anurans. *Syst Biol* 1969;18:1–32.
18. Collins MD, Jones D. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* 1981;45:316354.
19. 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.

20. **Komagata K, Suzuki K.** Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161207.
21. **Sasser M.** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
22. **Miller LT.** Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584586.
23. **Mesbah M, Whitman WB.** 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* 1989;479:297306.
24. **Tittsler RP, Sandholzer LA.** The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* 1936;31:575–580.
25. **Kovacs N.** Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 1956;178:703.
26. **Smibert RM, Krieg NR.** Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA and Krieg NR (editors). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
27. **Cha IT, Park SJ, Kim SJ, Kim JG, Jung MY *et al.*** *Marinoscillum luteum* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2013;63:3475–3480.
28. **Benson HJ.** *Microbiological Application: Laboratory Manual in General Microbiology*, 8th ed. NY: McGraw-Hill; 2002.
29. **Gonzalez C, Gutierrez C, Ramirez C.** *Halobacterium vallismortis* sp. nov. An amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* 1978;24:710–715.
30. **Park I, Cha IT, Seo MJ.** *Thalassobius litorarius* sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol* 2016;66:1666–1672.
31. **Leifson E.** Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* 1963;85:1183–1184.
32. **Seo HS, Kwon KK, Yang SH, Lee HS, Bae SS *et al.*** *Marinoscillum* gen. nov., a member of the family *'Flexibacteraceae'*, with *Marinoscillum pacificum* sp. nov. from a marine sponge and *Marinoscillum furvescens* nom. rev., comb. nov. *Int J Syst Evol Microbiol* 2009;59:1204–1208.
33. **de Ley J, Cattoir H, Reynaerts A.** The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* 1970; 12:133–142.
34. **Huss VA, Festl H, Schleifer KH.** Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* 1983;4:184–192.
35. **Wayne LG, Moore WEC, Stackebrandt E, Kandler O, Colwell RR *et al.*** Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
36. **Stackebrandt E, Goebel BM.** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 1994;44:846–849.
37. **Montero-Calasanz M, Göker M, Rohde M, Spröer C, Schumann P *et al.*** *Chryseobacterium hispalense* sp. nov., a plant-growth-promoting bacterium isolated from a rainwater pond in an olive plant nursery, and emended descriptions of *Chryseobacterium defluvii*, *Chryseobacterium indologenes*, *Chryseobacterium wanjuense* and *Chryseobacterium gregarium*. *Int J Syst Evol Microbiol* 2013;63:4386–4395.