Laetantesicola Maris Gen. Nov., Sp. Nov. Isolated From the Sea of Japan Shallow Sediments.

Lyudmila A. Romanenko (✉ lro@piboc.dvo.ru)

G.B. Elyakov Pacific Institute of Bioorganic Chemisrty   https://orcid.org/0000-0001-9843-3637

Valeriya V. Kurilenko

G B Elyakov Pacific Institute of Bioorganic Chemistry: Tihookeanskij institut bioorganiceskoj himii imeni G B Elakova DVO RAN

Nadezhda Yu. Chernysheva

G B Elyakov Pacific Institute of Bioorganic Chemistry: Tihookeanskij institut bioorganiceskoj himii imeni G B Elakova DVO RAN

Liudmila A. Tekutyeva

Far Eastern Federal University, Innovative Technology center

Peter V. Velansky

A V Zhirmunsky Institute of Marine Biology FEB RAS: FGBUN Nacional'nyj naucnyj centr morskoj biologii im A V Zirmunskogo Dal'nevostocnogo otdelenia Rossijskoj akademii nauk

Vassilii I. Svetashev

Zhirmunsky Institut biologii morâ imeni AV Zhirmunskogo DVO RAN: FGBUN Nacional'nyj naucnyj centr morskoj biologii im A V Zirmunskogo Dal'nevostocnogo otdelenia Rossijskoj akademii nauk

Marina P. Isaeva

G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEb RAS

Research Article

Abstract

A Gram-negative, non-motile bacterium KMM 3653^T was isolated from a sediment sample from the Sea of Japan seashore, Russia. On the basis of the 16S rRNA gene sequence analysis the strain KMM 3653^T was positioned within the family *Rhodobacteraceae* (class *Alphaproteobacteria*) forming a distinct lineage with the highest gene sequence similarities to the members of the genera *Pacificibacter* (95.2-94.7%) and *Nioella* (95.1-94.5%), respectively. According to the phylogenomic tree based on 400 conserved protein sequences, strain KMM 3653^T was placed in the cluster comprising *Vannielia litorea*, *Nioella nitratireducens*, *Litoreibacter albidus* and *Pseudoruegeria aquimaris* as a separate lineage adjacent to *Vannielia litorea* KCTC 32083^T. The average nucleotide identity values between strain KMM 3653^T and *Vannielia litorea* KCTC 32083^T, *Nioella nitratireducens* KCTC 32417^T, *Litoreibacter albidus* KMM 3851^T, and *Pseudoruegeria aquimaris* CECT 7680^T were 71.1, 70.3, 69.6, and 71.0%, respectively. Strain KMM 3653^T contained Q-10 as the predominant ubiquinone and $C_{18:1}\omega7c$ as the major fatty acid followed by $C_{16:0}$. The polar lipids were phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid, two unidentified aminolipids, and five unidentified lipids. The DNA G+C content of 61.8 % was calculated from the genome sequence. Based on the phylogenetic evidence and distinctive phenotypic characteristics, strain KMM 3653^T is proposed to be classified as a novel genus and species *Laetantesicola maris* gen. nov., sp. nov. The type strain of *Laetantesicola maris* gen. nov., sp. nov. is KMM 3653^T (=KCTC 82575^T).

Introduction

The *Roseobacter* clade (family *Rhodobacteraceae*, class *Alphaproteobacteria*) comprises a large group of bacterial genera (Garrity et al. 2005; Brinkhoff et al. 2008) which have been reported to be wide-spread microorganisms in marine environments, being isolated from seawater, sediments, polar sea ice, microbial mats, seaweeds and animals (Buchan et al. 2005). A recent phylogenomic study of Wirth and Whitman (2018) demonstrated the insufficiency of the 16S rRNA gene sequence analysis for phylogeny of the *Roseobacter* group in the family *Rhodobacteraceae*, whereas phylogeny based on a set of house-keeping core gene sequences is more reliable. As a result, applying this approach a number of genera and species of *Alphaproteobacteria* were reclassified and proposed as novel or recognized taxa (Wirth and Whitman 2018; Hördt et al. 2020). During a survey of microorganisms capable of dwelling shallow sediments of the Sea of Japan the Gram-negative, non-motile bacterium designated KMM 3653^T was found and investigated by using phenotypic and molecular methods and results obtained are reported in the present study. Based on the phylogenetic analyses data and distinctive phenotypic characteristics, a novel genus and species *Laetantesicola maris* gen. nov., sp. nov. is described to accommodate the strain KMM 3653^T (=KCTC 82575^T).

Materials And Methods

Bacterial strains

Strain KMM 3653^T was isolated from a sandy sediment sample collected from the Sea of Japan seashore, Russia, in July 2002 as described in a previous paper (Romanenko et al. 2004). Strain KMM 3653^T was grown aerobically on/in Marine agar 2216 (MA) or Marine broth 2216 (MB) (both BD Difco), and stored at -70 °C in MB supplemented with 30% (v/v) glycerol. The strain KMM 3653^T has been deposited in the Collection of Marine Microorganisms (KMM), G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia, and in the Korean Collection for Type Cultures (KCTC), Korea, under number of KCTC 82575^T. The type strains, *Nioella nitratireducens* KCTC 32417^T, *Oceanicola litoreus* (*Vanniella litorea*) KCTC 32083^T, *Pacificibacter aestuarii* JCM31805^T, and *Nioella aestuarii* JCM 30752^T were purchased from the Korean Collection for Type Cultures (KCTC), Korea, and Japan Collection of Microorganisms (JCM), Japan, respectively, and used in the phenotypic and lipid analyses.

Phenotypic characterization

Gram-staining, oxidase and catalase reactions, and motility (the hanging drop method) were determined as described by Gerhardt et al. (1994). The morphology of cells grown in MB and negatively stained with a 1% phosphotungstic acid on carbon-coated 200-mesh copper grids was examined by electronic transmission microscopy [Libra 120 FE (Carl Zeiss), provided by the Far Eastern Centre of electronic microscopy, Zhirmunsky Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences]. Hydrolysis of starch, casein, gelatin, Tweens 20, 40, 80, DNA, L-tyrosine, chitin, xanthine, hypoxanthine, nitrate reduction (sulfanilic acid/α-naphthylamine test), formation of H_2S from thiosulfate, and growth at different salinities (0-12% NaCl), temperatures (4-45 °C) and pH values (4.5-10.5) were carried out using artificial sea water (ASW) as described in previous papers (Romanenko et al. 2011b, 2013). The artificial sea water (ASW) contained (per liter of distilled water): 24 g NaCl, 4.9 g $MgCl_2$, 2.0 g $MgSO_4$, 0.5 g $CaCl_2$, 1.0 g KCl, 0.01 g $FeSO_4$. Acid production from carbohydrates was examined using the oxidation/fermentation medium of Leifson (1963). Biochemical tests were performed using API 20E, API 20NE, API ID32 GN, and API ZYM test kits (bioMérieux, France) as described by the manufacturer except the cultures were suspended in ASW. Antibiotic susceptibility was examined using commercial paper discs (Research Centre of Pharmacotherapy, St. Petersburg) impregnated with the following antibiotics (mg per disc, unless otherwise indicated): ampicillin (10), benzylpenicillin (10 U), vancomycin (30), gentamicin (10), kanamycin (30), carbenicillin (100), chloramphenicol (30), neomycin (30), oxacillin (10), oleandomycin (15), lincomycin (15), ofloxacin (5), rifampicin (5), polymyxin (300 U), streptomycin (30), cephazolin (30), cephalexin (30), erythromycin (15), nalidixic acid (30), and tetracycline (30).

Chemotaxonomic characterization

Strain KMM 3653^T and type strains of related genera, *Nioella nitratireducens* KCTC 32417^T, *Nioella aestuarii* JCM 30752^T, *Oceanicola litoreus* (*Vanniella litorea*) KCTC 32083^T, *Pacificibacter aestuarii* JCM 31805^T, *Pacificibacter maritimus* KMM 9031^T were grown on MA 2216 at 28 °C. Lipids were extracted using the method of Folch et al. (1957). Two-dimensional thin layer chromatography of polar lipids was

carried out on Silica gel 60 F_{254} (10 x 10 cm, Merck, Germany) using chloroform-methanol-water (65:25:4, v/v) for the first direction, and chloroform-methanol-acetic acid-water (80:12:15:4, v/v) for the second one (Collins and Shah 1984) and spraying with specific reagents (Collins et al. 1980). Respiratory lipoquinones were analyzed by reversed-phase high performance thin-layer chromatography as described by Mitchell and Fallon (1990). Fatty acid methyl esters (FAMEs) were prepared according to the procedure of the Microbial Identification System (MIDI) (Sasser 1990). The analysis of FAMEs was performed using the GC-2010 chromatograph (Shimadzu, Kyoto, Japan) equipped with capillary columns (30 m x 0.25 mm I.D.), one coated with Supecowax-10 and the other with SPB-5. Identification of FAMEs was accomplished by equivalent chain length values and comparing the retention times of the samples to those of standards. In addition, FAMEs were analyzed using a GLC-MS Shimadzu GC-MS model QP2020 (column Shimadzu SH-Rtx-5MS, the temperature program from 160 $^{\circ}$C to 250 $^{\circ}$C, at a rate of 2 $^{\circ}$C/min). Production of bacteriochlorophyll a (Bchl a) was spectrophotometrically tested in methanolic extracts of cells grown on MA and MB in the dark as described by Lafay et al. (1995).

16S rRNA gene sequence and phylogenetic analysis

Genomic DNA of the strain KMM 3653[T] was extracted using a commercial genomic DNA extraction kit (Fermentas, EU) following the manufacturer's instruction. The universal bacterial primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1522R (5'-AAGGAGGTGATCCAGCCGCA-3') (Edwards et al. 1989) were used for amplification of the 16S rRNA gene. The 16S rRNA gene was PCR-amplified and sequenced as described in a previous paper (Romanenko et al. 2019). The 16S rRNA gene sequence obtained for strain KMM 3653[T] was compared with those of the closest relatives using the BLAST (http://www.ncbi.nlm.nih.gov/blast/) and EzBioCloud service (Yoon et al. 2017). Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis (MEGA X) (Kumar et al. 2018). Phylogenetic trees were constructed by the neighbor-joining and the maximum-likelihood and the distances were calculated according to the Kimura two-parameter model (Kimura 1980). The robustness of phylogenetic trees was estimated by the bootstrap analysis of 1000 replicates.

Whole-genome sequencing and genome-based phylogenetic analysis

The genomic DNA was obtained from the strain KMM 3653[T] using the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). The quantity and quality of the genomic DNA was measured using DNA gel electrophoresis and the Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA). Preparation of the DNA sequencing library was carried out using Nextera DNA Flex kits (Illumina, San Diego, CA, USA) and whole-genome sequencing was performed subsequently using paired-end runs on an Illumina MiSeq platform with a 150-bp read length. The reads were trimmed using Trimmomatic (Bolger et al. 2014) and their quality assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Filtered reads were assembled into contigs with SPAdes version 3.11.1 (Bankevich et al. 2012) and genome metrics were calculated with the help of QUAST (Galaxy version 5.0.2+galaxy0) (Gurevich et al. 2013). The draft genome assembly was annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016). Comparisons of the Average Nucleotide Identity (ANI) and *in silico* DNA-

DNA hybridization (dDDH) values of the strain KMM 3653^T and its closest neighbors were performed with the JSpecies Web Server (Richter et al. 2016), and TYGS platform (Meier-Kolthoff and Göker 2019), respectively. The phylogenomic analysis was performed using PhyloPhlAn 3.0 software based on a set of 400 conserved bacterial protein sequences (Asnicar et al. 2020).

Results And Discussion

16S rRNA genesequencing and genome-based phylogenetic analysis

Comparative 16S rRNA gene sequence analysis showed that the novel strain KMM 3653^T belongs to the family *Rhodobacteraceae* (class *Alphaproteobacteria*) and its closest phylogenetic neighbours were found to be members of the genera *Pacificibacter* (95.2-94.7% gene sequence similarity) and *Nioella* (95.1-94.5%), respectively. Different treeing algorithms (neighbor-joining, Fig. 1, and maximum-likelihood, Supplementary Figure S1) revealed that strain KMM 3653^T formed a separate branch located between *Nioella* and *Pacificibacter* clusters. The low values of 16S rRNA gene sequence similarities with related alphaproteobacteria demonstrated that strain KMM 3653^T can be representative a novel genus in the family *Rhodobacteraceae*. The phylogenetic tree based on concatenated 400 protein sequences from whole genome sequences supported the placement of the novel strain KMM 3653^T in the family *Rhodobacteraceae* as a separate line adjacent to *Vannielia litorea* KCTC 32083^T [former *Oceanicola litoreus* (Park et al. 2013; Hördt et al. 2020)] (Fig. 2). The ANI and dDDH values between strain KMM 3653^T and *Vannielia litorea* KCTC 32083^T, *Nioella nitratireducens* KCTC 32417^T, *Pseudoruegeria aquimaris* CECT 7680^T, and *Litoreibacter albidus* KMM 3851^T were 71.1, 70.3, 71.0, and 69.6 %, and values of 18.5 % to each of the first three strains and 19.5 % to the last, respectively. These values obtained were significantly lower than the ANI and dDDH values of 95-96% and 70%, respectively, which have been accepted for bacterial species discrimination (Chun et al. 2018). The draft genome features of the strain KMM 3653^T are summarized in Table S1. The phylogenomic analysis data evidence that the strain KMM 3653^T does not belong to any of recognized genera and could be classified as an individual genus and species of the family *Rhodobacteraceae*.

Morphological, physiological and chemotaxonomic characteristics

Morphological, physiological, biochemical and chemotaxonomic characteristics of strain KMM 3653^T are given in Table 1, Table 2, Supplementary Figure S2, Supplementary Figure S3, and in the genus and species descriptions. Strain KMM 3653^T was ovoid or rod-shaped bacteria dividing by the budding process and capable of producing extracellular material (Supplementary Figure S2). The novel bacterium KMM 3653^T was not able to hydrolyse a number of polymeric substrates (Table 1) and assimilate most carbon sources in API 32GN, API 20E and API 20NE tests (Table S2). Strain KMM 3653^T contained ubiquinone Q-10 as the major respiratory quinone and $C_{18:1}\omega7c$ as the major fatty acid followed by $C_{16:0}$ (Table 2). Fatty acid profiles found in all related type strains were similar in a large proportion of $C_{18:1}\omega7c$ that is characteristic for the members of the family *Rhodobacteraceae* although strain KMM 3653^T

differed in content of some fatty acids, such as $C_{14:1}$ 3-OH, $C_{12:0}$ 3-OH, $C_{16:0}$ 2-OH or $C_{19:0}$ cyclo (Table 2). The polar lipids of the strain KMM 3653[T] were found to be phosphatidylcholine (PC), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an unidentified phospholipid (PL), two unidentified aminolipids (AL) and five unidentified lipids (L) (Fig. S3). The polar lipids profile of the strain KMM 3653[T] was similar to those of *Pacificibacter* species (Romanenko 2020) and differed from the other related genera in the absence of PE. As reported members of *Vannielia* (Park et al. 2013), *Litoreibacter* (Romanenko et al. 2011a; Kim et al. 2012) and *Nioella* (Rajasabapathy et al. 2015; Cha et al. 2017; Liu et al. 2017; Yang et al. 2019) contained PE in their lipid profiles (Table 1). In the present study PE was present in *V.litorea* KCTC 32083[T], but was not found in *N. nitratireducens* KCTC 32417[T] and *N. aestuarii* JCM 30752[T] (Fig. S3). The DNA G+C content of 61.8 % was calculated from the genome sequence. The value obtained for the strain KMM 3653[T] is close to some extent to those of *Nioella* and *Litoreibacter* members but significantly differed from those of *Pacificibacter* and *Vannielia* (Table 1). The phylogenetic relationships observed on the basis of 16S rRNA gene and whole genome sequences, and genetic distinctness as revealed by ANI and dDDH analyses were supported by phenotypic differences of the novel isolate KMM 3653[T] in its growth temperature and salinity ranges, and substrate hydrolysis. Differential phenotypic characteristics are indicated in Table 1. Based on the combined phylogenetic evidence, phenotypic and biochemical characteristics, it is proposed to classify strain KMM 3653[T] as a novel genus and species, *Laetantesicola maris* gen. nov., sp. nov., with the type strain of the type species KMM 3653[T] (=KCTC 82575[T]).

Description of *Laetantesicola* gen. nov.

Laetantesicola (Lae.tan.te.si.co'la L. masc. n. *laetantes*, seashore; L. masc./fem. suff. *-cola*, inhabitant; N.L. n. *Laetantesicola*, inhabitant of seashore, referring to the isolation source of the type species).

Gram-negative, aerobic, oxidase- and catalase positive, ovoid or rod-shaped bacteria enlarged at one pole due to the cell division by budding. Chemoorganoheterotrophic. Sodium ions are essential for growth. The predominant isoprenoid quinone is Q-10. Polar lipids include phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, two unidentified aminolipids, an unidentified phospholipid, and five unidentified lipids. The major fatty acid is $C_{18:1}\omega 7c$ followed by $C_{16:0}$. Isolated from the marine environments. On the basis of the 16S rRNA gene sequence analysis the genus represents a separate branch within the family *Rhodobacteraceae* of the class *Alphaproteobacteria*. The type species of the genus is *Laetantesicola maris*.

Description of *Laetantesicola maris* sp. nov.

Laetantesicola maris (ma'ris. L. neut. n. *mare* (gen. *maris*), *maris,* of the sea).

In addition to properties given in the genus description the species is characterized as follows:

cells are ovoid or rod-shaped 0.9-1.4 µm in diameter and 1.0-2.7 µm in length. Cells are capable producing of capsular material. Non-motile. Whitish-pigmented, smooth and shiny colonies with the regular edges of 2-3 mm in diameter are produced on MA. Growth occurs at 7-36 °C (optimum, 28-30 °C) and in the presence of 1-5% (w/v) NaCl (optimum, 2-3% NaCl). The pH range for growth is 6.0-10.0 with an optimum of 7.0-8.0. Negative for hydrolysis of gelatin, casein, tyrosine, starch, Tweens 20, 40, 80, chitin, xanthine and hypoxanthine, and H_2S production. Positive for DNA and urea hydrolysis. Negative for acid production from D-glucose, D-fructose, maltose, lactose, D-galactose, cellobiose, sucrose, D-xylose, D-raffinose, and L-arabinose. According to the API 20NE, positive for aesculin hydrolysis and PNPG test. In the API 20E tests positive for urease production and oxidation of L-rhamnose, D-sucrose (weakly), and amygdalin. In the API ZYM tests positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase. Susceptible to (content per disc): ampicillin (10 mg), benzylpenicillin (10 U), vancomycin (30 mg), kanamycin (30 mg), carbenicillin (100 mg), chloramphenicol (30 mg), lincomycin (15 mg), neomycin (30 mg), oxacillin (10 mg), oleandomycin (15 mg), ofloxacin (5 mg), rifampicin (5 mg), streptomycin (30 mg), tetracycline (30 mg), cephazolin (30 mg), cephalexin (30 mg), erythromycin (15 mg); and resistant to gentamicin (10 mg), nalidixic acid (30 mg), and polymyxin B (300 U). The major isoprenoid quinone is ubiquinone Q-10. Major fatty acid is $C_{18:1}\omega7c$ followed by $C_{16:0}$. The polar lipids include phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid, two unidentified aminolipids and five unidentified lipids. The DNA G+C content of 61.8 % is calculated from the genome sequence.

The type strain of the species is strain KMM 3653[T] (=KCTC 82575[T]) isolated from a shallow sediment sample collected from the Sea of Japan, Russia.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals.

References

1. Asnicar F, Thomas AM, Beghini F, Mengoni C, Manara S, Manghi P, Zhu Q, Bolzan M, Cumbo F, May U, Sanders JG, Zolfo M, Kopylova E, Pasolli E, Knight R, Mirarab S, Huttenhower C, Segata N (2020) Precise phylogenetic analysis of microbial isolates and genomes from metagenomes using PhyloPhlAn 3.0. Nat Commun 11:1-10

2. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Bioinform Comput Biol 19:455-477

3. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 15:2114-2120

4. Brinkhoff T, Giebel H-A, Simon M (2008) Diversity, ecology, and genomics of the *Roseobacter* clade: a short overview. Arch Microbiol 189: 531-539

5. Buchan A, Gonzalez JM, Moran MA (2005) Overview of the marine *Roseobacter* lineage. Appl Environ Microbiol 71:5665-5677

6. Cha IT, Cho ES, Park JM, Yeh JY, Seo MJ (2017) *Nioella aestuarii* sp. nov., of the family *Rhodobacteraceae*, isolated from tidal flat. Int J Syst Evol Microbiol 67:5205-5210

7. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, Da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461-468

8. Collins MD, Goodfellow M, Minnikin DE (1980) Fatty acid, isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. J Gen Microbiol 118:29-37

9. Collins MD, Shah HN (1984) Fatty acid, menaquinone and polar lipid composition of *Rothia dentosacariosa*. Arch Microbiol 137:247-249

10. Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC (1989) Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843-7853

11. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509

12. Garrity GM, Bell JA, Lilburn T (2005) Family I. *Rhodobacteraceae* fam. nov. In: Brenner DJ, Krieg NR, Staley JT and Garrity GM (eds) Bergey's Manual of Systematic Bacteriology, 2nd ed, vol. 2, part C. New York: Springer; pp. 161

13. Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) (1994) Methods for General and Molecular Bacteriology. Washington, DC, American Society for Microbiology

14. Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: Quality assessment tool for genome assemblies. Bioinformatics 29:1072-1075

15. Hördt A, Lopez MG, Meier-Kolthoff JP, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, Goker M (2020) Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of *Alphaproteobacteria*. Front Microbiol 11:468.

16. Kim YO, Park S, Nam BH, Kang SJ, Hur YB, Kim DG, Oh TK, Yoon JH (2012) Description of *Litoreibacter meonggei* sp. nov., isolated from the sea squirt *Halocynthia roretzi*, reclassification of *Thalassobacter arenae* as *Litoreibacter arenae* comb. nov. and emended description of the genus *Litoreibacter* Romanenko et al. 2011. Int J Syst Evol Microbiol 62:1825-183

17. Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346-351

18. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120

19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547-1549

20. Lafay B, Ruimy R, Rausch de Traubenberg C, Breittmayer V, Gauthier MJ, Christen R (1995) *Roseobacter algicola* sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate *Prorocentrum lima*. Int J Syst Bacteriol 45:290-296

21. Leifson E (1963) Determination of carbohydrate metabolism of marine bacteria. J Bacteriol 85:1183-1184

22. Liu Y, Du J, Lai Q, Dong C, Shao Z (2017) *Nioella sediminis* sp. nov., isolated from surface sediment and emended description of the genus *Nioella*. Int J Syst Evol Microbiol 67:1271-1274

23. Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 1:1-10

24. Mitchell K, Fallon RJ (1990) The determination of ubiquinone profiles by reversed-phase high performance thin-layer chromatography as an aid to the speciation of *Legionellaceae*. J Gen Microbiol 136:2035-2041

25. Park S, Lee MH, Yoon JH (2013) *Oceanicola litoreus* sp. nov., an alphaproteobacterium isolated from the seashore sediment. Antonie Van Leeuwenhoek 103:859-866

26. Rajasabapathy R, Mohandass C, Yoon J, Dastager SG, Liu Q, Khieu TN, Son CK, Li WJ, Colaco A. (2015) *Nioella nitratireducens* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae* isolated from Azorean Island. Antonie van Leeuwenhoek 107:589-595

27. Richter M, Rosselló-Móra R, Glöckner FO, Peplies J (2016) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929-931

28. Romanenko LA, Schumann P, Rohde M, Mikhailov VV, Stackebrandt E (2004) *Reinekea marinisedimentorum* gen. nov., sp. nov., a novel gammaproteobacterium from marine coastal sediments. Int J Syst Evol Microbiol 54:669-673

29. Romanenko LA, Tanaka N, Frolova GM, Svetashev VI, Mikhailov VV (2011a) *Litoreibacter albidus* gen. nov., sp. nov. and *Litoreibacter janthinus* sp. nov., members of the class *Alphaproteobacteria* isolated from the seashore. Int J Syst Evol Microbiol 61:148-154

30. Romanenko LA, Tanaka N, Svetashev VI, Kalinovskaya NI (2011b) *Pacificibacter maritimus* gen. nov., sp. nov., isolated from shallow marine sediment. Int J Syst Evol Microbiol 61:1375-1381

31. Romanenko LA, Tanaka N, Svetashev VI (2013) *Devosia submarina* sp. nov., isolated from deep sea surface sediments. Int J Syst Evol Microbiol 63:3079-3085

32. Romanenko LA, Kurilenko VV, Guzev KV, Svetashev VI (2019) Characterization of *Labrenzia polysiphoniae* sp. nov. isolated from red alga *Polysiphonia* sp. Arch Microbiol 201:705-712

33. Romanenko LA (2020) *Pacificibacter*. In Bergey's Manual of Systematics of Archaea and Bacteria (eds W.B. Whitman, F.A. Rainey, P. Kämpfer, M.E. Trujillo, P. DeVos, B. Hedlund and S. Dedysh).

doi:10.1002/9781118960608.gbm01815

34. Sasser M (1990) Microbial identification by gas chromatographic analysis of fatty acid methyl esters (GC-FAME). Technical Note 101. Newark, DE: MIDI

35. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 14:6614-6624

36. Wirth JS, Whitman WB (2018) Phylogenomic analyses of a clade within the roseobacter group suggest taxonomic reassignments of species of the genera *Aestuariivita, Citreicella, Loktanella, Nautella, Pelagibaca, Ruegeria, Thalassobius, Thiobacimonas* and *Tropicibacter*, and the proposal of six novel genera. Int J Syst Evol Microbiol 68:2393-2411

37. Yang Q, Jiang Z, Zhou X, Zhang R, Wu Y, Lou L, Ma Z, Wang D, Ge Y, Zhang X, Yu X (2020) *Nioella ostreopsis* sp. nov., isolated from toxic dinoflagellate, *Ostreopsis lenticularis*. Int J Syst Evol Microbiol 70:759-765

38. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613

Tables

Table 1. Differential characteristics of strain KMM 3653^T and related genera.

Feature	KMM 3653[T]	*Pacificibacter*	*Nioella*	*Vannielia*	*Litoreibacter*
DNA G+C content (mol%)	61.8	52.05-53.9	63.4-63.6	67.6	56.0-60.4
Colonies pigmentation	Whitish	Whitish, creamy	White, cream, orange	Greyish yellow	Whitish, creamy, greyish-violet
Motility	-	-	-	-	V(-)
Budding	+	+	-	-	+
Growth at 4 °C	-	V(+)	V(-)	+	+
Maximal growth temperature (°C)	36	36	40	40	37
Maximal NaCl concentration (%)	5	10	12	9	8
Nitrate reduction	-	-	V(+)	-	-
Hydrolysis of:					
Gelatin	-	V(-)	V(-)	-	V(-)
Starch	-	V(+)	-	-	-
DNA	+	-	V(-)	+	-
Casein	-	V(-)	-	-	-
Tyrosine	-	V(+)	V(+)	-	V(+)
Tween-80	-	V(-)	-	+	V(+)
Polar lipids	PC, PG, DPG, AL, L, PL	PC, PG, DPG, AL, L, PL	PC, PE, PG, DPG, AL, L, PL	PC, PG, DPG, PE, AL, L, PL	PC, PG, PE, AL, L

Data from present study and from: Romanenko et al. (2011b) and Romanenko (2020) for *Pacificibacter*; Park et al. (2013) for *Vannielia*; Rajasabapathy et al. (2015), Cha et al. (2017), Liu et al. (2017) and Yang et al. (2020) for *Nioella*; Romanenko et al. (2011a) and Kim et al. (2012) for *Litoreibacter*.

+, positive; -, negative; V(-), variable reaction between species, reaction of the type strain of the type species is negative; V(+), variable reaction between species, reaction of the type strain of the type species is positive.

Table 2. Fatty acid composition (%) of strain KMM 3653[T] and the type strains of related genera of the family *Rhodobacteraceae*.

Fatty acid	1	2	3	4	5	6	7
$C_{10:0}$ 3-OH	2.26	2.26	2.11	9.68	1.48	2.94	2.2
$C_{12:1}$	-	-	-	1.65	2.89	-	3.2
$C_{12:1}$ 3-OH	3.77	1.88	1.72	-	-	-	-
$C_{12:0}$ 3-OH	-	-	-	-	2.00	3.04	-
$C_{14:1}$ 3-OH	-	4.11	3.50	-	0.97	3.91	-
$C_{16:1}\omega 7c$	0.55	3.53	3.51	1.07	0.65	1.72	-
$C_{16:0}$	13.16	7.64	4.83	5.20	11.75	6.40	10.3
$C_{17:0}$	0.50	0.51	1.00	3.00	0.47	-	1.0
$C_{16:0}$ 2-OH	-	-	-	-	4.47	10.92	-
$C_{16:0}$ 3-OH	0.23	0.56	0.25	1.57	-	-	-
$C_{18:1}\omega 7c$	74.28	58.95	66.04	72.91	55.72	54.85	77.1
$C_{18:0}$	2.44	2.41	3.51	0.82	1.59	1.94	1.0
11-Methyl $C_{18:1}\omega 7c$	1.74	7.90	5.95	-	6.87	9.91	-
$C_{19:0}$ cyclo	-	-	-	2.30	8.70	1.98	-
$C_{16:0}$ 2-OH	-	-	-	-	2.26	2.40	-
$C_{18:1}$ 3-OH	0.58	9.81	6.55	1.10	-	-	-

1, KMM 3653[T]; 2, *Pacificibacter maritimus* KMM 9031[T]; 3, *Pacificibacter aestuarii* JCM 31805[T]; 4, *Vannielia litorea* KCTC 32083[T]; 5, *Nioella nitratireducens* KCTC 32417[T]; 6, *Nioella aestuarii* JCM 30752[T] (data from present study); 7, *Litoreibacter albidus* KMM 3851[T] (data from Romanenko et al. 2011a). Fatty acids representing < 1% in all strains tested were not shown; -, not detected.

Figures
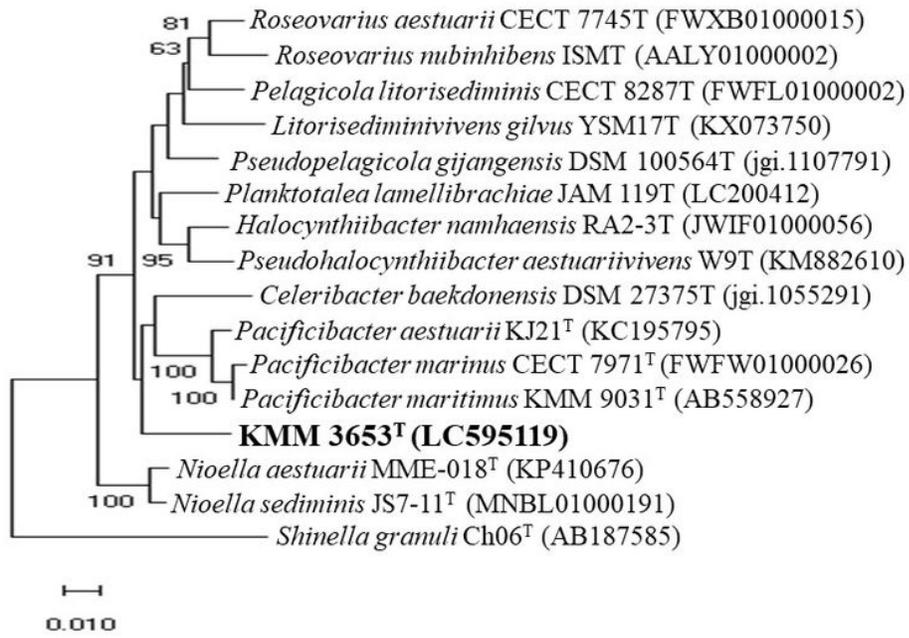
Figure 1

Neighbor-joining tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing relationships of strain KMM 3653T and related taxa. Bootstrap values based on 1000 replications are given as percentages at the branching points and numbers indicate percentages greater than 60%. Bar, 0.01 substitutions per nucleotide position.
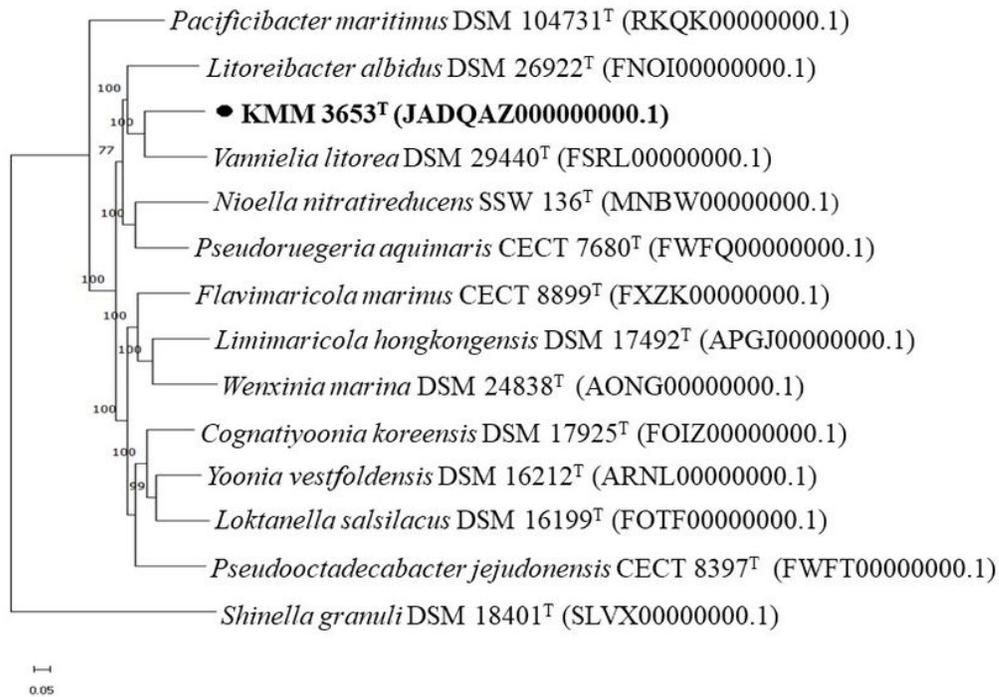
Figure 2

Maximum-likelihood tree based on concatenated 400 protein sequences translated from genome sequences showing phylogenetic position of strain KMM 3653T among related members of the family Rhodobacteraceae. Bootstrap values are based on 100 replicates. Bar, 0.05 substitutions per amino acid position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarydataKMM3653.pdf