Antonie van Leeuwenhoek (2021) 114:2147–2162
https://doi.org/10.1007/s10482-021-01669-6

ORIGINAL PAPER

Roseitranquillus sediminis gen. nov., sp. nov. a novel genus and species of the family *Rhodobacteraceae*, isolated from sediment of an Arctic fjord

Md. Umar · Kottekkatu Padinchati Krishnan · Rupesh Kumar Sinha · Thasreefa Kannukkarathi · Titus Susan Merlin · Jeslin Illiparambil Johnson · Valsamma Joseph · Sajeevan Thavarool Puthiyedathu

Received: 3 May 2021 / Accepted: 29 September 2021 / Published online: 13 October 2021

Abstract A Gram-negative, aerobic, non-motile, oxidase-positive, catalase-positive, rod-shaped bacterium, designated strain MCCB 386^T was isolated from sediment samples collected from Kongsfjorden, an Arctic fjord. The strain MCCB 386^T showed growth at 4–37 °C (optimum 27°C) in the presence of 1–8% NaCl (w/v, optimum 3.5%) and at pH 6.0–8.0 (optimum pH 7.0). The major fatty acids were $C_{18:1}\omega7c$ (54.0%) and 11-methyl $C_{18:1}\omega7c$ (22.6%). The dominant respiratory quinone was Q-10. The major polar lipids comprised of phosphatidylcholine (PC), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphoglycolipid (PGL), one unidentified aminolipid, two glycolipids and two unidentified lipids. The genomic G+C content of the strain MCCB 386^T was 68.1 mol%. The 16 S rRNA gene sequences based phylogenetic analysis of MCCB 386^T showed that *Psychromarinibacter halotolerans* YBW34^T (95.88%) is the most closely related species. In addition, overall genome relatedness indices (OGRI) of MCCB 386^T with closely related strains were lower than threshold level for species and genus delineation. The analysis of Biosynthetic Gene clusters (BGCs) revealed the potential of this strain for production of novel bioactive secondary metabolites. As per polyphasic taxonomic characterisation, strain MCCB 386^T represents a novel species of a novel genus for which the name *Roseitranquillus sediminis* gen. nov., sp. nov. is suggested. The type strain of the species is MCCB 386^T (= JCM 33,538^T= KACC 21,531^T).

Keywords Arctic fjord · Draft genome · *Roseitranquillus sediminis* gen. nov., sp. nov. · *Rhodobacteraceae*

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10482-021-01669-6.

Md. Umar · T. Kannukkarathi · T. S. Merlin · J. I. Johnson · V. Joseph · S. T. Puthiyedathu (✉)
National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Fine Arts Avenue, Kochi, Kerala 682 016, India
e-mail: sajeev@cusat.ac.in

K. P. Krishnan · R. K. Sinha
National Centre for Polar and Ocean Research, Ministry of Earth Sciences, Headland Sada, Vasco da Gama, Goa 403 804, India

K. P. Krishnan
CUSAT-NCPOR Centre for Polar Sciences, Cochin University of Science and Technology (CUSAT), Kochi 682 016, India

Introduction

The family *Rhodobacteraceae*, is one of the morphologically, physiologically and ecologically diverse subgroups of *Alphaproteobacteria*. They are

characterised with phototrophy, methylotrophy, organic sulfur metabolism, carbon monoxide oxidation, presence of poly-β-hydroxybutyrate (PHB) granules, and the production of secondary metabolites (Pujalte et al. 2014). Members of *Rhodobacteraceae* inhabit a wide range of ecological niches including water, sediments, sea ice etc. (Lai et al. 2013). In recent years, many novel genera belonging to the family *Rhodobacteraceae* have been described such as *Albirhodobacter* (Nupur et al. 2013), *Aliiroseovarius* (Park et al. 2015b), *Cereibacter* (Suresh et al. 2015), *Frigidibacter* (Li and Zhou 2015), *Halovulum* (Sun et al. 2015), *Nioella* (Rajasabapathy et al. 2015), *Pontivivens* (Park et al. 2015c), *Pseudohalocynthiibacter* (Won et al. 2015), *Pseudooceanicola* (Lai et al. 2015), *Pseudooctadecabacter* (Billerbeck et al. 2015), *Pseudoseohaeicola* (Park et al. 2015a) and *Thiobacimonas* (Li et al. 2015). At the time of writing this manuscript nearly 121 genera have been reported under the *Rhodobacteraceae* family.

Here we describe a pink-pigmented bacterial strain designated MCCB 386^T, isolated from the sediment collected from an Arctic fjord, Kongsfjorden, Svalbard. Through polyphasic taxonomic approach, which included determination of its phenotypic and chemotaxonomic characteristics along with genomic analysis, we confirmed that, the strain MCCB 386^T represents a novel genus and species under *Rhodobacteraceae* family for which the name *Roseitranquillus sediminis* gen. nov., sp. nov. is proposed. The genomic analysis was performed to reveal the biosynthetic potential of the strain MCCB 386^T for the production of novel secondary metabolites.

Materials and methods

Strain isolation

Sediment samples were collected from Kongsfjorden (78°57′31.2″N and 11°49′20.5″E), an Arctic fjord in Ny-Ålesund, Svalbard in June 2014 using a Van Veen grab sampler. The samples were shipped to National Centre for Aquatic Animal Health, India under cold conditions. Isolation of bacteria was done by spread plate method using Nutrient Agar (NA) media prepared in seawater (35 ppt) and incubated at 20 °C for 14 days. Gram straining was performed using standard methods. Motility was determined by microscopic examinations of wet mounts of strains and stab inoculation on a semisolid agar medium (0.3% agar) in a test tube using 24 h old culture and incubated at 24 °C. Cell morphology was observed under scanning electron microscope after cells were negatively stained with 1% (w/v) phosphotungstic acid. To test growth under anaerobic conditions, bacterial strains were cultured on nutrient broth with cysteine (0.01%, w/v) and overlaid with paraffin oil and incubated at 24°C for one month. The temperature range for growth was determined in nutrient broth by incubating the cultures at different temperatures ranging from 4 to 45°C.

Phenotypic characterisation and Chemotaxonomic characterisation

Growth at different salt concentrations (1–12% NaCl) and pH ranges (4.0–11.0 at an interval of 0.5) were investigated. The following phenotypic tests were carried out according to standard procedures using sterile seawater as basal medium: catalase and oxidase activities, DNase activity, urease, MOF, alkaline phosphatase, nitrate reduction, Voges-Proskauer test, methyl-red test, indole production, citrate utilisation, ONPG, acid production from carbohydrates and hydrolysis of compounds including starch, CM-cellulose, casein and gelatin were recorded. Degradation of chitin was examined on chitin agar with sterile seawater (Hsu and Lockwood 1975). Biochemical tests were performed using Biolog GN2 (Biolog, Inc., USA) microplate following the manufacturer's protocol. Substrates were categorised as carbohydrates, amino acids, esters, fatty acids, alcohols, amines, amides, polymers and carboxylic acids. Analysis of fatty acids, respiratory

quinones and polar lipids were carried out at DSMZ, Germany.

16 S rRNA gene sequence and phylogeny analysis

Genomic DNA (gDNA) of strain MCCB 386^T was extracted using PureLink^{TM} Genomic DNA Mini Kit (Thermo Fisher Scientific, USA). PCR amplification of 16 S rRNA gene was carried out using universal primers 16S1 (GAGTTTGATCCTGGCTCA) and 16S2 (ACGGCTACCTTGTTACGACTT). Sequencing was performed on Genetic Analyzer 3500 (Applied Biosystems, Carlsbad, CA, USA). An almost-complete 16 S rRNA gene sequence (1439 nt) obtained was submitted to the GenBank database (Accession Number: MK417831). Pairwise similarity values between strain MCCB 386^T and the closely related type strains were calculated using the EzTaxon-e server (Yoon et al. 2017). In addition, the taxonomic assignment of strain MCCB 386^T was performed using the RDP naive Bayesian rRNA Classifier tool based on 80% confidence threshold (Wang et al. 2007).

The 16 S rRNA gene sequences of closely related type strains were retrieved from the NCBI database and aligned with strain MCCB 386^T using Clustal W (Larkin et al. 2007). Phylogenetic trees based on the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms were reconstructed using the software package MEGA version X (Stecher et al. 2020). The genetic distances were calculated by Kimura's two-parameter model (Kimura 2020) form the NJ and ML trees. Bootstrap analysis of the trees topologies were performed by using 1000 replicates (Felsenstein 1985).

Whole Genome sequencing and Genomic analysis

The whole genome of strain MCCB 386^T was sequenced using pair-end sequencing on Illumina HIseq 2500. The quality of genome was analysed using FastQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). The assembly was performed using Velvet v. 1.2.10 (Zerbino and Birney 2008) and the quality of the assembled genome was evaluated using combination of QUAST version 5.0.0 (Gurevich et al. 2013) and the DFAST web server. Gene search and annotation were performed using the Prokka package v 1.14.5 (Seemann 2014) and RAST server (Overbeek 2005). For analysis of genome relatedness, the whole genome sequences of closely related species like *Rhodovulum adriaticum* DSM 2781^T, *Rhodovulum marinum* DSM 18,063^T, *Rhodovulum strictum* DSM 11,289^T, *Rhodovulum steppense* DSM 21,153^T, *Rhodovulum sulfidophilum* DSM 1374^T, *Tranquillimonas alkanivorans* DSM 19,547^T, *Dinoroseobacter shibae* DSM 16,493^T, *Roseivivax marinus* DSM 27,511^T and *Tropicimonas isoalkanivorans* DSM 19,548^T were retrieved from the GenBank database. The *in silico* DNA-DNA hybridisation (dDDH) was calculated with the formula 2 model on the Genome-to-Genome Distance Calculator (GGDC 2.1) web tool (http://ggdc.dsmz.de/distcalc2.php) (Auch et al. 2010; Meier-Kolthoff et al. 2013) and the Average Nucleotide Identity (ANI) based on BLAST (OrthoANIb) was calculated and heatmap generated using OAT software (Lee et al. 2016). Tetra-nucleotide signatures correlation indexes (Tetra) were calculated using JSpecies (Richter and Rosselló-Móra 2009) and Jspecies WS (http://jspecies.ribohost.com/jspeciesws/#Analyse) (Richter et al. 2016). CompareM tool (https://github.com/dparks1134/CompareM) was used for calculating the Average Amino acid Identity (AAI) (Konstantinidis and Tiedje 2005). POCP (Qin et al. 2014) was calculated using GET_HOMOLOGUES tool version 3.3.4 (Contreras-Moreira and Vinuesa 2013). Heatmaps visualising the dDDH values (%), Tetra, AAI and POCP were rendered in R programme version 4.0.2 (packages 'ggplot2' (Wickham 2016) and 'pheatmap' (Kolde 2015). The potential for the production of secondary metabolites by MCCB 386^T was analysed using antiSMASH 5.0 (Blin et al. 2019).

Phylogenomic analysis

The Genome Taxonomy Database toolkit (GTDB-Tk) V 1.4.1) (Chaumeil et al. 2019), release 05-RS95

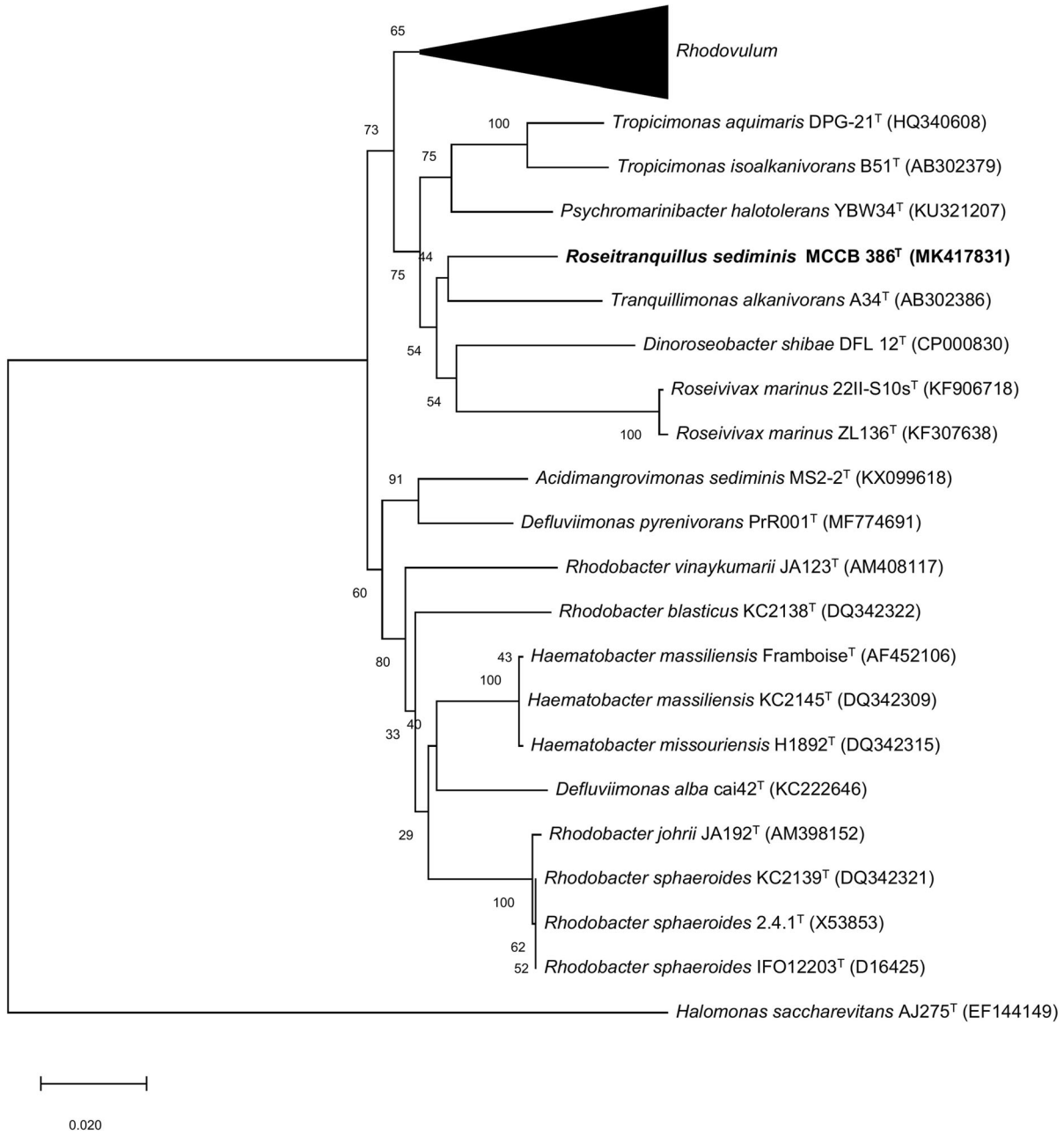


Fig. 1 Neighbour-joining phylogenetic tree showing the relationships between strain MCCB 386^T and representatives of the family *Rhodobacteraceae* based on 16 S rRNA gene sequences similarity. Bootstrap values were derived from 1000 replicates. *Halomonas saccharevitans* AJ275^T was used as an outgroup. GenBank accession numbers are given in parentheses. Bar, 0.020 substitutions per nucleotide position

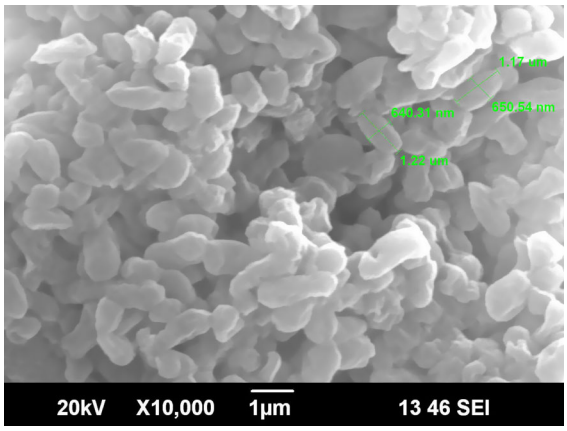


Fig. 2 Scanning Electron Microscopic Image of MCCB 386^T grown in Nutrient Broth at 27 °C for 7 days

(https://github.com/Ecogenomics/GTDB-Tk) (Parks et al. 2020), was used to identify the 120 conserved single-copy genes in strain MCCB 386^T and phylogenetically related *Rhodobacteraceae*. These multiple alignment of concatenated single-copy gene sequences built in GTDB-Tk was used to construct a phylogenetic tree in IQ-Tree (Nguyen et al. 2015) and the best-fit model LG + F + R4 as selected by the ModelFinder (Kalyaanamoorthy et al. 2017) in IQ-Tree. Node support was tested using the ultrafast bootstrapping (Hoang et al. 2018) based on 1000 trials. The resulting Newick tree was imported into MEGA X (Stecher et al. 2020) for visualisation.

Results

Strain identification and taxonomic identification

A pink pigmented colony of strain MCCB 386^T was isolated from the sediment samples collected from an Arctic fjord Kongsfjorden (78°57′31.2″N and 11°49′20.5″E), in Ny-Ålesund, Svalbard. Pairwise comparison of the 16 S rRNA gene sequence with the GenBank database using the BLASTN program (Altschul et al. 1990) revealed that the new isolate MCCB 386^T was closely related to species within the *Rhodobacteraceae* family, showing sequence similarities with genera such as *Psychromarinibacter halotolerans* YBW34T (95.88%), *Tranquillimonas* (92.72–94.81%), *Rhodovulum* (93.03–95.44%), *Roseivivax* (94.05–94.23%) and *Tropicimonas* (92.90–94.20%). The 16 S rRNA gene sequences based neighbour-joining phylogenetic tree showed that the strain MCCB 386^T formed a distinct lineage among the most closely related genera of the family *Rhodobacteraceae* (Fig. 1). Phylogenetic analysis based on the NJ method (Fig. 1) showed that strain MCCB 386^T joined *Tranquillimonas alkanivorans* A34^T with a quite low bootstrap resampling value of 44%. While ML and MP trees (Fig. S1 and Fig. S2) showed that strain MCCB 386^T formed a distinct lineage within the clade of family *Rhodobacteraceae*, without joining any genera in this family. The taxonomic analysis using the RDP (Ribosomal Data Project) Classifier tool (Maidak 2000) also indicated that strain MCCB 386^T should be classified as an unclassified member under the family *Rhodobacteraceae*. Hence, the strain MCCB 386^T cannot be assigned to any of the validly published taxa at both the species and genus levels.

Physiological and chemotaxonomic characteristics

MCCB 386^T is a Gram-negative, aerobic, non-motile, both oxidase and catalase positive, rod-shaped bacterium and producing pink colour colony on Nutrient agar (NA) medium. Scanning electron microscopy of the culture showed that cells of the strain MCCB 386^T are short, straight rods and measured approximately 0.64–0.65 µm in diameter and 1.17–1.22 µm in length (Fig. 2). Comparison of physiological and biochemical characteristics between MCCB 386^T and closely related reference genera were given in Table 1. Growth occurs at 4–37 °C (optimum 27 °C), pH 6–8 (optimum pH 7) and NaCl 1–8% (w/v) (optimum 3.5%). Tests for catalase, oxidase, nitrate reduction, ONPG, alkaline phosphatase are positive. Tests for

Table 1 Differential characteristics of strain MCCB 386^T and the type strains of closely related genera in the family *Rhodobacteraceae* Taxa: 1-MCCB 386^T (data from this study); 2 - *Psychromarinibacter halotolerans* YBW34^T (Qiao et al. 2017); 3-*Tranquillimonas alkanivorans* A34^T (Harwati et al. 2008); 4-*Rhodovulum adriaticum* strain DSM 2781^T (Neutzling et al. 1984); 5-*Roseivivax marinus* strain ZL136^T (Dai et al. 2014) ; 6- *Tropicimonas isoalkanivorans* B51^T (Harwati et al. 2009), optimum values are given in brackets, positive reaction (+); negative reaction (-); AL-unidentified aminolipid; GL-unidentified glycolipid; L-unidentified lipid; PC-phosphatidylcholine; PE-phosphatidylethanolamine; PG-phosphatidylglycerol; PL-unidentified phospholipid; SL-sulpholipid; ND-Not Detected

Characteristic	1	2	3	4	5	6
Diameter (μm)	0.64–0.65	0.7–1.5	0.2–0.5	0.5–0.8	0.3–0.4	0.3–0.5
Length (μm)	1.17–1.22	2.4–4.4	1.7–2.8	1–2	1.2–1.3	0.5–1.0
Flagellation	ND	Lateral flagellum	−	−	−	Peritrichous flagella
Temperature (°C)	4–37 (27)	10–45 (28)	10–15	25–30	4–37 (32)	10–46 (37)
NaCl (%)	1–8 (3.5)	1.0–2.0 (4.0)	1–13 (2)	1–10	0–12 (3–4)	1.0–6.0 (3.0)
pH	6–8 (7.0)	6.0–8.0 (7.0)	6.5–9.5 (8.5)	6.0–8.5	6.0–10.0 (8.0)	5.5–8.0 (7.6)
Catalase	+	+	+	ND	+	−
Nitrate reduction	+	+	+	−	ND	+
Assimilation of:						
D-Mannose	+	−	+	ND	+	+
D-Cellobiose	+	−	ND	ND	+	+
α-D-Glucose	−	−	−	+	+	+
Maltose	−	−	+	+	+	+
D-Trehalose	−	−	ND	ND	−	+
Succinic acid	−	−	ND	+	+	−
D-Galactose	+	+	−	ND	−	+
L-Glutamic acid	+	−	+	ND	+	+
D-Fructose	−	+	+	+	+	+
Acetic acid	−	−	−	+	−	−
Tween 20	+	+	ND	ND	−	ND
Tween 40	+	−	ND	ND	−	ND
Major quinone	Q-10 (97.3%)	Q-10	Q-10	Q-10	Q-10	Q-10
Polar lipids	PC, DPG, PG, PGL, AL, GL1, GL2, L1, L2	PC, PG, PE, AL1, AL2, AL3	ND	SQD, PG, AL	PG, PC, PE, DPG, PL1-PL3, L1	GL1, GL2, PC, PG, AL, L1, L2, L3
G+C (mol%)	68.1	64.2	69.1	64.9-66.7	67.0	66.5

Table 2 Cellular fatty acid content (% of total) of strain MCCB 386^T and the reference strains are detailed in the table. Strains: 1-MCCB 386^T (data from this study); 2-*Psychromarinibacter halotolerans* YBW34^T (Qiao et al. 2017); 3-*Tranquillimonas alkanivorans* A34^T (Harwati et al. 2008); 4-*Rhodovulum adriaticum* strain DSM 2781^T (Neutzling et al. 1984, Imhoff 1991); 5-*Roseivivax marinus* strain ZL136^T (Dai et al. 2014); 6-*Tropicimonas isoalkanivorans* B51^T(Harwati et al. 2009). Values are percentages of total fatty acids; Major fatty acid (<10%) are highlighted in bold, TR (<1%); "-" indicates not detected/not reported

Fatty acid	1	2	3	4	5	6
$C_{10:0}$ 3-OH	2.1	–	–	–	TR	–
$C_{12:0}$ 3-OH	1.6	4.9	–	–	3.6	3.6
$C_{14:0}$	TR	–	–	TR	TR	–
$C_{16:0}$	6.7	**18.3**	9.1	3.8	8.0	8.7
Summed feature 3	TR	–	–	–	–	–
$C_{17:0}$	2.0	–	TR	TR	TR	1.7
$C_{17:1}\omega 8c$	TR	–	–	–	–	–
Summed feature 8	**54.0**	**56.8**	**56.2**	**67.2**	**64.5**	**34.9**
$C_{18:0}$	3.1	4.5	1.6	**19.8**	9.5	6.4
$C_{18:1}\omega 9c$	TR	–	–	–	–	**13.8**
11-methyl $C_{18:1}\omega 7c$	**22.6**	1.7	0.8	–	7.3	2.7
Cyclo- $C_{19:0}\omega 8c$	4.5	2.6	**26.0**	–	–	2.1
$C_{20:1}\omega 7c$	1.1	–	TR	4.6	–	–

*Summed features are group of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprised (16:1ω7c/16:1ω6c), Summed feature 8 (C18:1 ω7c)

DNase activity, indole production, citrate utilisation, hydrolysis of starch, CM-cellulose, casein, gelatin, chitin, MOF, and urease were negative. The strain was observed to utilises D-arabitol, D-cellobiose, D-fucose, D-galactose, D-mannitol, D-mannose, D-melibiose, turanose, α-D-glucose, L-threonine, L-glutamic acid, L-proline, L-pyroglutamic acid, Tween 40, Tween 80, putrescine, dextrin, α-cyclodextrin, D-saccharic acid, mono-methyl-succinate, α-hydroxy butyric acid and γ-amino butyric acid under aerobic conditions at pH 7 and 27 °C in GN2 Biolog Microplate whereas rest are negative. It shows negative reactions for Voges-Proskauer reaction. Detailed physiological characteristics of strain MCCB 386^T are summarised in Table S2 and Table S3.

The fatty acid profile of strain MCCB 386^T is listed in Table 2. The predominant constituents in the cellular fatty acid profile of MCCB 386^T were $C_{18:1}\omega 7c$ (54.0%) and 11-methyl $C_{18:1}\omega 7c$ (22.6%). Fatty acid profile of MCCB 386^T was found similar to those of closely related type strains. Moreover, 11-methyl $C_{18:1}\omega 7c$ was detected in MCCB 386^T in higher proportion (22.6%), which distinguishes MCCB 386^T from other genera despites sharing $C_{18:1}\omega 7c$ as their predominant cellular fatty acid. The presence of cis-11 octadecenoic acid (i.e. $C_{18:1}\omega 7c$) as the principal fatty acid is a characteristic feature of taxa within the *Alphaproteobacteria* (Martínez-Checa et al. 2005).

The major polar lipids comprised of phosphatidylcholine (PC), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphoglycolipid (PGL), one unidentified aminolipid, two glycolipids and two unidentified lipids (Supplementary Fig. S3). The respiratory quinones found in MCCB 386^T were ubiquinone-9 (0.2%), ubiquinone-10 (97.3%) and ubiquinone-11 (2.5%). The presence of ubiquinone-10 as the dominant respiratory lipoquinone is characteristic of the member of the class *Alphaproteobacteria* (Yokota et al. 1992; Lechner et al. 1995; Busse et al. 1999).

Genome characterisation

The genome sequencing generated forward 1.52 Gb and reverse 1.52 Gb of clean data (approximately 570-fold genome depth coverage). The genome completeness of the strain MCCB 386^T was 99.12% with 0.15% contamination. The whole genome assemblies of strain MCCB 386^T contain 3,732,706-bp with 37 contigs contained 3696 genes, of which 1615 encode hypothetical gene, 3629 protein-coding genes, 67 RNAs (one complete 16 S rRNA operon, one 5 S rRNAs, one 23 S rRNA, 47 tRNAs and 17 misc

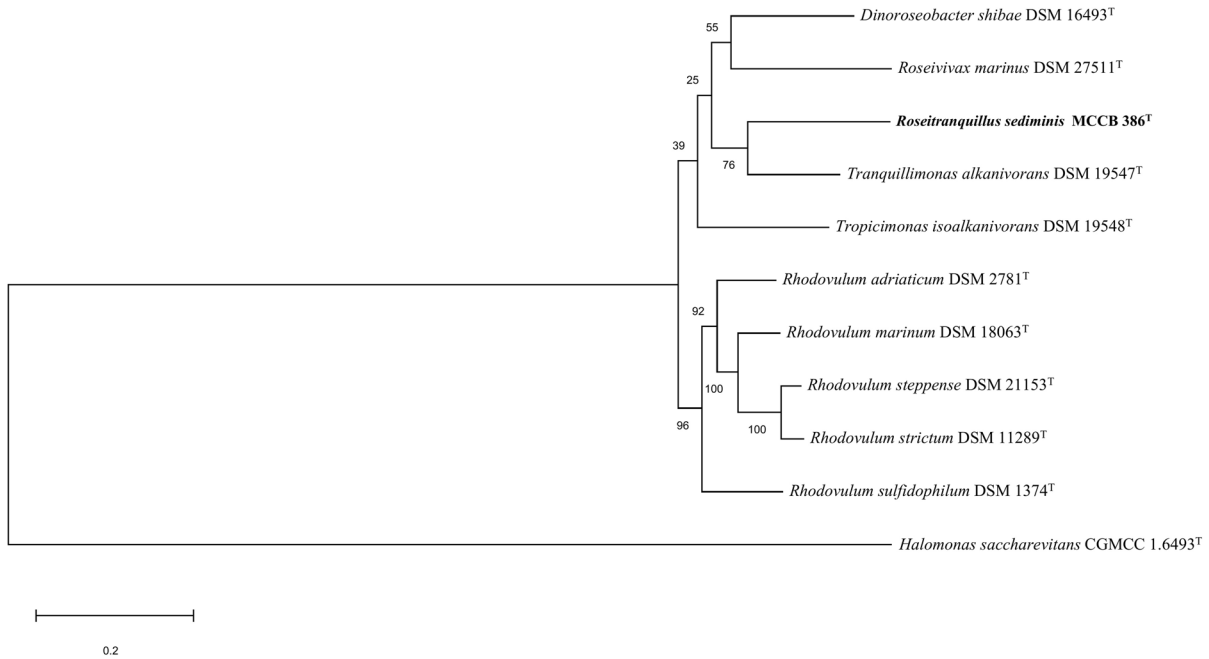


Fig. 3 Phylogenomic tree inferred from the concatenation of 120 single-copy, phylogenetically informative bacterial marker genes showing the phylogenetic position of strain MCCB 386^T. The tree was reconstructed using the Genome Taxonomy Database toolkit (Parks et al. 2018), release 05-RS95. The significance levels of interior branch points obtained in maximum-likelihood analysis were determined by bootstrap analysis (1000 data re-samplings). *Halomomas saccharevitans* CGMCC 1.6493^T was used as a outgroup. Bar, 0.2 substitutions per amino acid position

RNAs) predicted by Prokka v1.14.5 summarised in Table S1. The full-length 16 S rRNA gene extracted from genome was completely consistent with the sequence obtained by conventional Sanger sequencing. These values correspond to 985 protein-coding genes per Mb and a coding density of 90.6%.

The phylogenomic analysis based on 120 conserved single copy marker genes of strain MCCB 386^T and the closest species with standing in nomenclature are presented in Fig. 3, where it confirmed that strain MCCB 386^T is most closely related to members of the genus *Tranquillimonas* and its independent position within genus *Rhodovulum* and other closest genera. The genomic DNA G+C content of strain MCCB 386^T was 68.1 mol%, which is slightly lower than that of *Tranquillimonas alkanivorans* DSM 19,547^T (69.1 mol%) but higher than other closely related species (Table 1).

The *in silico* DDH values between strain MCCB 386^T and *Rhodovulum adriaticum* DSM 2781^T, *Rhodovulum marinum* DSM 18,063^T, *Rhodovulum strictum* DSM 11,289^T, *Rhodovulum steppense* DSM 21,153^T, *Rhodovulum sulfidophilum* DSM 1374^T, *Tranquillimonas alkanivorans* DSM 19,547^T, *Dinoroseobacter shibae* DSM 16,493^T, *Roseivivax marinus* DSM 27,511^T and *Tropicimonas isoalkanivorans* DSM 19,548^T were 18.8%, 19.1%, 19.3%, 18.7%, 18.9%, 19.7%, 19.4%, 19.8%, and 23.3% respectively (Fig. 4a) which are lower than 70% cut-off used for delineating species (Chun et al. 2018). Ortho Average nucleotide Identity (orthoANI) between strain MCCB 386^T and *Rhodovulum adriaticum* DSM 2781^T, *Rhodovulum marinum* DSM 18,063^T, *Rhodovulum strictum* DSM 11,289^T, *Rhodovulum steppense* DSM 21,153^T, *Rhodovulum sulfidophilum* DSM 1374^T, *Tranquillimonas alkanivorans* DSM 19,547^T,

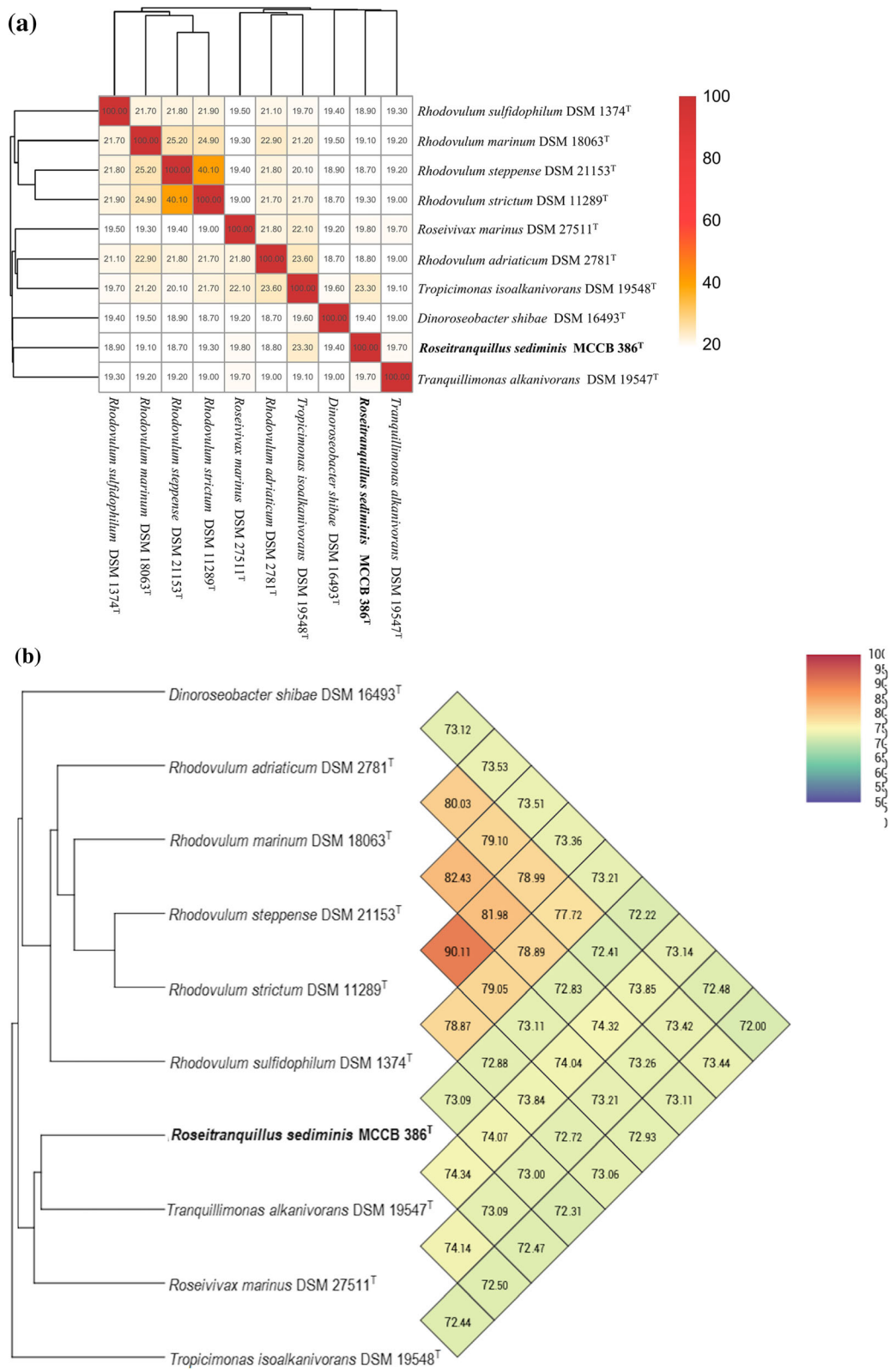
Dinoroseobacter shibae DSM 16,493^T, *Roseivivax marinus* DSM 27,511^T and *Tropicimonas isoalkanivorans* DSM 19,548^T were 72.41%, 72.83%, 72.88%, 73.11%, 73.09%, 74.34%, 72.22%, 73.09% and 72.47% respectively, which were below the threshold value of 95% (Chun et al. 2018), thus confirming that MCCB 386^T is distinct from these phylogenetically close bacterial taxa (Fig. 4b). Further Tetra value range from 0.69 to 0.95 (Fig. 4c) is well below the accepted cut-off values of 0.989 for species delineation (Teeling et al. 2004; Richter and Rosselló-Móra 2009). The AAI values ranging from 65.14 to 68.21% with the reference genomes (Fig. 4d) which is below the threshold value of 80–85% proposed for classification of a novel genus (Konstantinidis and Tiedje 2005; Luo et al. 2014) while the POCP values for MCCB 386^T ranged from 51.13 to 56.26% (Fig. 4e). Based on physiological, chemotaxonomic, phylogenetic, and genotypic data we conclude that MCCB 386^T represents a novel genus and species of the family *Rhodobacteraceae* for which the name *Roseitranquillus sediminis* gen. nov., sp. nov. is proposed. The type strain MCCB 386^T (=JCM 33,538^T = KACC 21,531^T) is isolated from the sediment sample collected Kongsfjorden, Ny-Ålesund, Svalbard, Arctic.

Secondary metabolite gene cluster analysis The secondary metabolite gene cluster analysis of MCCB 386^T showed eight putative biosynthetic gene clusters (BGCs) (Fig. S4). These clusters were predicted to encode terpenes (2), RiPP like (ribosomally synthesized and post translationally modified peptides) such as lassopeptides and lantipeptides (1), RRE containing Lassopeptide (1), haserlactone (2) redox co-factor (1) and ectoin (1). Two biosynthetic gene cluster (BGCs) (cluster 5 terpene and cluster 8 ectoin) code for the metabolic compound that exhibit relative similarity to already known clusters (16–80%), all other six clusters showed very less relative similarity (0–8% relative similarity) to any existing clusters, which indicates the possibility that MCCB 386^T may encode for novel chemical scaffold.

Discussion

Through genomic and phenotypic approach, we have characterised a novel bacterial species MCCB 386^T representing a novel genus under the family *Rhodobacteraceae* isolated from the sediments collected from an Arctic Fjord. The DNA G+C content of strain MCCB 386^T was calculated from the draft genome and found to be 68.1 mol%. Phylogenomic approaches such as dDDH, ANI and Tetra are often used for microbial taxonomy and give more brevity for species identification compared to 16 S rRNA gene sequence similarity. Recently, additional parameters such as AAI (Konstantinidis and Tiedje 2005) and the POCP (Qin et al. 2014) were proposed to delineate organisms at the genus level. Bacterial strains below 70% threshold value for dDDH, 95% for ANI and 0.989 for Tetra were considered as different species (Teeling et al. 2004; Richter and Rosselló-Móra 2009). The *in silico* DDH values between strain MCCB 386^T and closely related strains well below the cut-off (70%) used for delineating species.

OrthoANI values among closely related species ranged from 72.00 to 90.11% (Fig. 4b). When strain MCCB 386^T was compared with these closely related species, values ranged from 72.22% with *Dinoroseobacter shibae* DSM 16,493^T to 74.07% with *Tranquillimonas alkanivorans* 19,547^T. Stephan et al. (2014) recently proposed ANIb based delineation among genera of the family *Enterobacteriaceae* which is ranged from 75 to 80%. Several other studies for novel genus delineation such as description of the new genus *Endobacterium* (Menéndez et al. 2020) and *Georhizobium* (Cao et al. 2020) were also supported by the results of ANIb calculation, with values lower than 76%. The ANI values obtained for MCCB 386^T within the closest genera of the family *Rhodobactereceaea* were found to be lower than 76%, which support


(a)
Rhodovulum sulfidophilum DSM 1374T
Rhodovulum marinum DSM 18063T
Rhodovulum steppense DSM 21153T
Rhodovulum strictum DSM 11289T
Roseivivax marinus DSM 27511T
Rhodovulum adriaticum DSM 2781T
Tropicimonas isoalkanivorans DSM 19548T
Dinoroseobacter shibae DSM 16493T
Roseitranquillus sediminis MCCB 386T
Tranquillimonas alkanivorans DSM 19547T
100
80
60
40
20
(b)
Dinoroseobacter shibae DSM 16493T
Rhodovulum adriaticum DSM 2781T
Rhodovulum marinum DSM 18063T
Rhodovulum steppense DSM 21153T
Rhodovulum strictum DSM 11289T
Rhodovulum sulfidophilum DSM 1374T
Roseitranquillus sediminis MCCB 386T
Tranquillimonas alkanivorans DSM 19547T
Roseivivax marinus DSM 27511T
Tropicimonas isoalkanivorans DSM 19548T

◀**Fig. 4** Heat maps indicating the genetic relatedness between *MCCB 386*^{T} and closely related taxa displayed as a cladogram (**a**) digital DNA–DNA hybridization (dDDH), (**b**) OrthoANI (**c**) Tetra, (**d**) average amino acid identity (AAI) and (**e**) Percentage of conserved protein (POCP)

the affiliation of this strain to a new genus within *Rhodobactereceaea* family.

Furthermore AAI analysis between strain MCCB 386^T and related strains were below the threshold value proposed for classification of a novel genus, thus confirming the distinctive taxonomic status of MCCB 386^T. However, the POCP value for MCCB 386^T ranged from 51.13 to 56.26% which is slightly above the cut-off (50%) prescribed for genus delineation. However, POCP is affected by genome size and hence cannot be used for the species undergoing extreme genome reduction (Hayashi Sant'Anna et al. 2019). Henceforth, applying this threshold for delineating genera within the family *Rhodobacteraceae* (Wirth and Whitman 2018), *Methylococcaceae* (Orata et al. 2018) and *Neisseriaceae* (Li et al. 2017) are contested, thus suggesting a necessity to describe appropriate POCP cut-off values for various families (Suresh et al. 2019).

Analysis of Biosynthetic Gene clusters (BGCs) revealed the potential of this strain for the production of novel secondary metabolites which warrants further study, including the purification and characterisation of the bioactive lead compounds. This study emphasises the importance of polar microbial diversity and their bioprospecting potential for the discovery of novel secondary metabolites. We recently reported novel actinomycetes from arctic environments with the potential to produce secondary metabolites (Dhaneesha et al. 2017, 2021).

In summary, based on physiological, chemotaxonomy, phylogenetic, and genotypic data we conclude that MCCB 386^T represents a novel genus and species of the family *Rhodobacteraceae* for which the name *Roseitranquillus sediminis* gen. nov., sp. nov. is proposed, which having potential for production of novel secondary metabolites.

Description of *Roseitranquillus* gen. nov.

Roseitranquillus (Ro.se.i.tran.quil′lus. L. masc. adj. roseus rose-coloured, pink; L. masc. adj. tranquillus quiet, calm, still; N.L. masc. n. Roseitranquillus a pink-coloured non-motile organism).

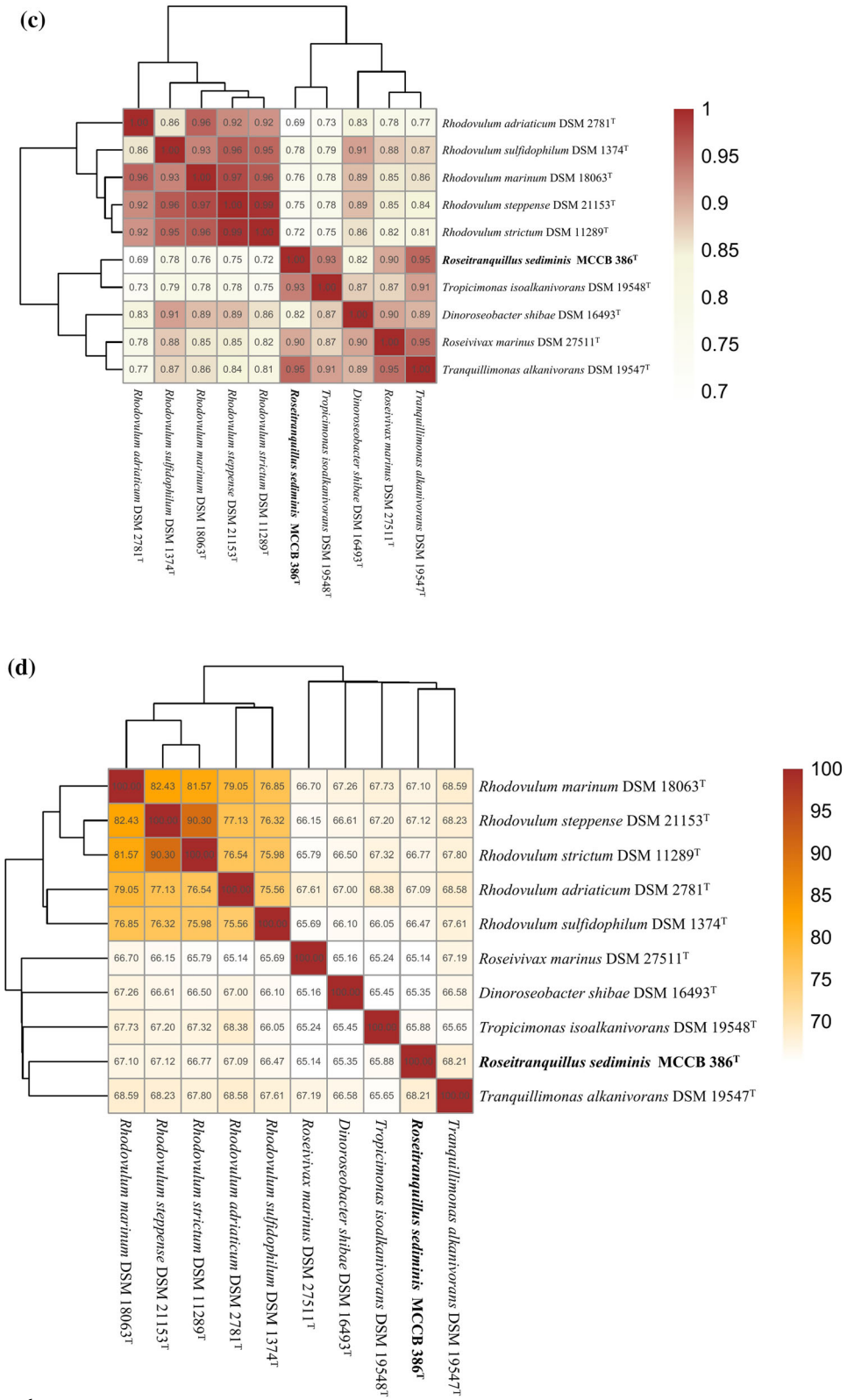
Cells are Gram-negative, aerobic, non-motile, short bacilli and non-spore forming. The organism is positive for catalase and oxidase activities. Respiratory lipoquinones Q-10 is the major ubiquinone (97.3%) and $C_{18:1}\omega7c$ (54.0%), 11-methyl $C_{18:1}\omega7c$ (22.6%) are the main fatty acids. The major polar lipids are phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, one unidentified aminolipid, two glycolipids and two unidentified lipids. Phylogenetically, 16 S rRNA gene sequence analysis classifies strain MCCB 386^T to the family *Rhodobacteraceae* of the class *Alphaproteobacteria*. The type species of the genus is *Roseitranquillus sediminis.*

Description of *Roseitranquillus sediminis* sp. nov.

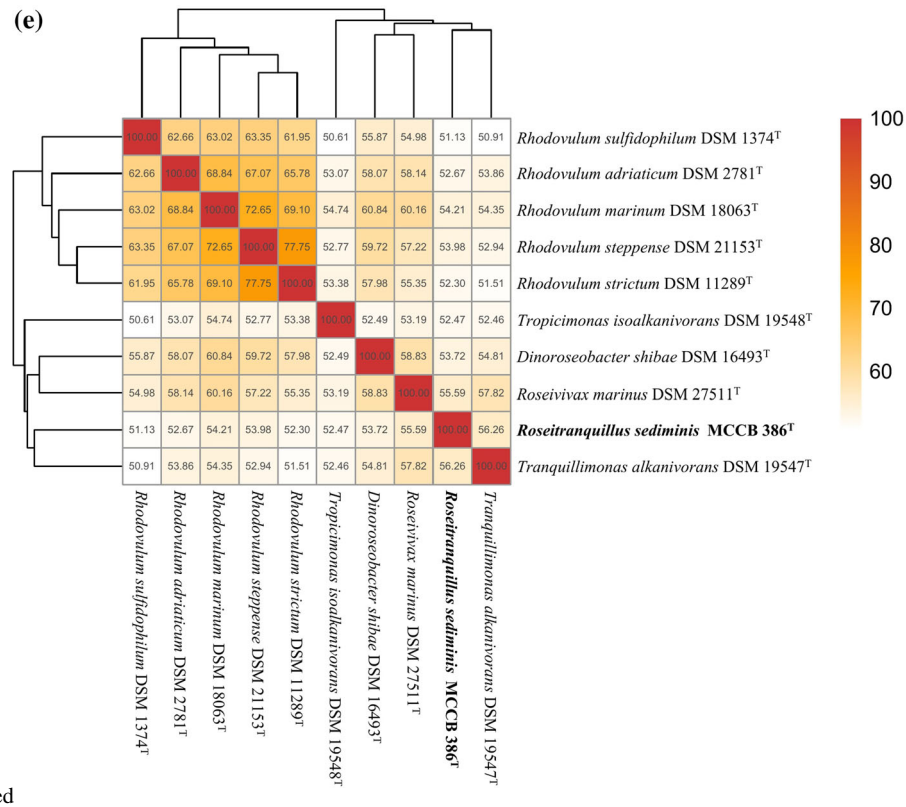
Roseitranquillus sediminis (se.di′mi.nis. L. gen. n. *sediminis* of a sediment).

In addition to the characteristics that define the genus, the following characteristics are observed. Cells are 0.64–0.65 µm width and 1.17–1.22 µm in length. Colonies on nutrient agar are pink in colour, circular, convex, smooth and opaque with entire margins. Growth occurs at an optimum concentration 3.5% NaCl (w/v) and at a temperature of 27 °C. Nitrate is reduced to nitrite. Growth under anaerobic conditions is not observed. The genomic DNA G+C content is 68.1 mol%.

The type strain MCCB 386^T was isolated from the sediment collected from Kongsfjorden, Ny-Ålesund, Svalbard was deposited in the Japan Collection of Microorganisms (=JCM 33,538^T) and Korean Agricultural Culture Collection (=KACC 21,531^T). The GenBank/EMBL/DDBJ accession numbers for the draft genome and 16 S rRNA gene sequences of strain MCCB 386^T are JAFFOB000000000 and MK41783, respectively.



◀**Fig. 4** continued



◀**Fig. 4** continued

Acknowledgements The authors wish to acknowledge the Director, National Centre for Polar and Ocean Research, Ministry of Earth Sciences (NCPOR, MoES) Govt. of India for the logistic support for the field access to the Arctic. MU would like to thank University Grant Commission (UGC), Govt. of India for providing Junior Research Fellowship.

Authors' contribution MU isolated the bacterium, performed the genomic and phenotypic test, RKS and TK contributed in genomic and phenotypic analysis, MTS and JIJ performed the electron microscopy, STP, KP and VJ planned the experiments including sample collection. MD and STP wrote the manuscript. All authors reviewed the manuscript.

Funding MU and TSM would like to thank University Grant Commission (UGC), Govt. of India for providing Junior Research Fellowships.

Declarations

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

References

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Auch AF, von Jan M, Klenk H-P, Göker M (2010) Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2:117–134. https://doi.org/10.4056/sigs.531120

Billerbeck S, Orchard J, Tindall BJ et al (2015) Description of *Octadecabacter temperatus* sp. nov., isolated from the southern North Sea, emended descriptions of the genus *Octadecabacter* and its species and reclassification of *Octadecabacter jejudonensis* Park and Yoon 2014 as *Pseudooctadecabacter jejudonensis* gen. nov., comb. nov . Int J Syst Evol Microbiol 65:1967–1974. https://doi.org/10.1099/ijs.0.000205

Blin K, Shaw S, Steinke K et al (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87. https://doi.org/10.1093/nar/gkz310

Busse H-J, Kämpfer P, Denner EBM (1999) Chemotaxonomic characterisation of *Sphingomonas*. J Ind Microbiol Biotechnol 23:242–251. https://doi.org/10.1038/sj.jim.2900745

Cao J, Wei Y, Lai Q et al (2020) *Georhizobium profundi* gen. nov., sp. nov., a piezotolerant bacterium isolated from a deep-sea sediment sample of the New Britain Trench. Int J

Syst Evol Microbiol 70:373–379. https://doi.org/10.1099/ijsem.0.003766

Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH (2019) GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics. https://doi.org/10.1093/bioinformatics/btz848

Chun J, Oren A, Ventosa A et al (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.002516

Contreras-Moreira B, Vinuesa P (2013) GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. Appl Environ Microbiol 79:7696–7701. https://doi.org/10.1128/AEM.02411-13

Dhaneesha M, Benjamin Naman C, Krishnan KP et al (2017) *Streptomyces artemisiae* MCCB 248 isolated from Arctic fjord sediments has unique PKS and NRPS biosynthetic genes and produces potential new anticancer natural products. 3 Biotech 7:32. https://doi.org/10.1007/s13205-017-0610-3

Dhaneesha M, Umar M, Merlin TS et al (2021) *Pseudonocardia cytotoxica* sp. nov., a novel actinomycete isolated from an Arctic fjord with potential to produce cytotoxic compound. Antonie Van Leeuwenhoek 114:23–35. https://doi.org/10.1007/s10482-020-01490-7

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086

Hayashi Sant'Anna F, Bach E, Porto RZ et al (2019) Genomic metrics made easy: what to do and where to go in the new era of bacterial taxonomy. Crit Rev Microbiol 45:182–200. https://doi.org/10.1080/1040841X.2019.1569587

Hoang DT, Chernomor O, von Haeseler A et al (2018) UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol Biol Evol 35:518–522. https://doi.org/10.1093/molbev/msx281

Hsu SC, Lockwood JL (1975) Powdered Chitin Agar as a Selective Medium for Enumeration of Actinomycetes in Water and Soil1. Appl Microbiol. https://doi.org/10.1128/aem.29.3.422-426.1975

Kalyaanamoorthy S, Minh BQ, Wong TKF et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285

Kimura M (2020) The Neutral Theory and Molecular Evolution. In: Journal of Molecular Evolution. Cambridge University Press, pp 119–138

Kolde R (2015) Pretty Heatmaps in R Package. R Package, Version 1.0.8. 2015.Available online: https://CRAN.R-project.org/package=pheatmap

Konstantinidis KT, Tiedje JM (2005) Towards a genome-based taxonomy for prokaryotes. J Bacteriol 187:6258–6264. https://doi.org/10.1128/JB.187.18.6258-6264.2005

Lai PY, Miao L, Lee OO et al (2013) *Profundibacterium mesophilum* gen. nov., sp. nov., a novel member in the family *Rhodobacteraceae* isolated from deep-sea sediment in the Red Sea, Saudi Arabia. Int J Syst Evol Microbiol 63:1007–1012. https://doi.org/10.1099/ijs.0.041525-0

Lai Q, Li G, Liu X et al (2015) *Pseudooceanicola atlanticus* gen. nov. sp. nov., isolated from surface seawater of the Atlantic Ocean and reclassification of *Oceanicola batsensis, Oceanicola marinus, Oceanicola nitratireducens, Oceanicola nanhaiensis, Oceanicola antarcticus* and *Oceanicol flagellatus*, as *Pseudooceanicola batsensis* comb. nov., *Pseudooceanicola marinus* comb. nov., *Pseudooceanicola nitratireducens* comb. nov., *Pseudooceanicola nanhaiensis* comb. nov., *Pseudooceanicola antarcticus* comb. nov., and *Pseudooceanicola flagellatus* comb. nov. Antonie Van Leeuwenhoek 107:1065–1074. https://doi.org/10.1007/s10482-015-0398-2

Larkin MA, Blackshields G, Brown NP et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Lechner U, Baumbach R, Becker D et al (1995) Degradation of 4-chloro-2-methylphenol by an activated sludge isolate and its taxonomic description. Biodegradation 6:83–92. https://doi.org/10.1007/BF00695339

Lee I, Ouk Kim Y, Park S-C, Chun J (2016) OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760

Li A-H, Zhou Y-G (2015) *Frigidibacter albus* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae* isolated from lake water. Int J Syst Evol Microbiol 65:1199–1206. https://doi.org/10.1099/ijs.0.000080

Li S, Tang K, Liu K, Jiao N (2015) *Thiobacimonas profunda* gen. nov., sp. nov., a member of the family *Rhodobacteraceae* isolated from deep-sea water. Int J Syst Evol Microbiol 65:359–364. https://doi.org/10.1099/ijs.0.066449-0

Li Y, Xue H, Sang S et al (2017) Phylogenetic analysis of family *Neisseriaceae* based on genome sequences and description of *Populibacter corticis* gen. nov., sp. nov., a member of the family *Neisseriaceae*, isolated from symptomatic bark of Populus × euramericana canker. PLoS One 12:e0174506. https://doi.org/10.1371/journal.pone.0174506

Luo C, Rodriguez-R LM, Konstantinidis KT (2014) MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. Nucleic Acids Res 42:e73–e73. https://doi.org/10.1093/nar/gku169

Maidak BL (2000) The RDP (Ribosomal Database Project) continues. Nucleic Acids Res 28:173–174. https://doi.org/10.1093/nar/28.1.173

Martínez-Checa F, Quesada E, Martínez-Cánovas MJ et al (2005) *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 55:2525–2530. https://doi.org/10.1099/ijs.0.63906-0

Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471-2105-14-60

Menéndez E, Flores-Félix JD, Ramírez-Bahena MH et al (2020) Genome analysis of *Endobacterium cerealis*, a novel genus

and species isolated from zea mays roots in North Spain. Microorganisms 8:939. https://doi.org/10.3390/microorganisms8060939

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300

Nupur VB, Tanuku NRS, Pinnaka AK (2013) *Albirhodobacter marinus* gen. nov., sp. nov., a member of the family *Rhodobacteraceae* isolated from sea shore water of Visakhapatnam, India. Antonie Van Leeuwenhoek 103:347–355. https://doi.org/10.1007/s10482-012-9814-z

Orata FD, Meier-Kolthoff JP, Sauvageau D, Stein LY (2018) Phylogenomic analysis of the gammaproteobacterial methanotrophs (order *Methylococcales*) calls for the reclassification of members at the genus and species levels. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.03162

Overbeek R (2005) The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res 33:5691–5702. https://doi.org/10.1093/nar/gki866

Park S, Park J-M, Kang C-H et al (2015a) *Pseudoseohaeicola caenipelagi* gen. nov., sp. nov., isolated from a tidal flat. Int J Syst Evol Microbiol 65:1819–1824. https://doi.org/10.1099/ijs.0.000182

Park S, Park J-M, Kang C-H, Yoon J-H (2015b) *Aliiroseovarius pelagivivens* gen. nov., sp. nov., isolated from seawater, and reclassification of three species of the genus *Roseovarius* as *Aliiroseovarius crassostreae* comb. nov., *Aliiroseovarius halocynthiae* comb. nov. and *Aliiroseovarius sediminilitori* comb. nov. Int J Syst Evol Microbiol 65:2646–2652. https://doi.org/10.1099/ijs.0.000315

Park S, Won S-M, Park J-M et al (2015c) *Pontivivens insulae* gen. nov., sp. nov., isolated from seawater. Int J Syst Evol Microbiol 65:2896–2902. https://doi.org/10.1099/ijs.0.000352

Parks DH, Chuvochina M, Chaumeil P-A et al (2020) A complete domain-to-species taxonomy for Bacteria and Archaea. Nat Biotechnol 38:1079–1086. https://doi.org/10.1038/s41587-020-0501-8

Parks DH, Chuvochina M, Waite DW et al (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36:996–1004. https://doi.org/10.1038/nbt.4229

Pujalte MJ, Lucena T, Ruvira MA et al (2014) The family *Rhodobacteraceae*. In: The Prokaryotes. Springer Berlin Heidelberg, Berlin, pp 439–512

Qin Q-L, Xie B-B, Zhang X-Y et al (2014) A proposed genus boundary for the prokaryotes based on genomic insights. J Bacteriol 196:2210–2215. https://doi.org/10.1128/JB.01688-14

Rajasabapathy R, Mohandass C, Yoon J-H et al (2015) *Nioella nitratireducens* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae* isolated from Azorean Island. Antonie Van Leeuwenhoek 107:589–595. https://doi.org/10.1007/s10482-014-0355-5

Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci 106:19126–19131. https://doi.org/10.1073/pnas.0906412106

Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J (2016) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929–931. https://doi.org/10.1093/bioinformatics/btv681

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Stecher G, Tamura K, Kumar S (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Mol Biol Evol 37:1237–1239. https://doi.org/10.1093/molbev/msz312

Stephan R, Grim CJ, Gopinath GR et al (2014) Re-examination of the taxonomic status of *Enterobacter helveticus*, *Enterobacter pulveris* and *Enterobacter turicensis* as members of the genus *Cronobacter* and their reclassification in the genera *Franconibacter* gen. nov. and *Siccibacter* gen. nov. as *Franconibacter helveticus* comb. nov., *Franconibacter pulveris* comb. nov. and *Siccibacter turicensis* comb. nov., respectively. Int J Syst Evol Microbiol 64:3402–3410. https://doi.org/10.1099/ijs.0.059832-0

Sun F, Du Y, Liu X et al (2015) *Halovulum dunhuangense* gen. nov., sp. nov., isolated from a saline terrestrial spring. Int J Syst Evol Microbiol 65:2810–2816. https://doi.org/10.1099/ijs.0.000332

Suresh G, Lodha TD, Indu B et al (2019) Taxogenomics resolves conflict in the genus *Rhodobacter*: a two and half decades pending thought to reclassify the genus *Rhodobacter*. Front Microbiol 10. https://doi.org/10.3389/fmicb.2019.02480

Suresh G, Sasikala C, Ramana CV (2015) Reclassification of *Gemmobacter changlensis* to a new genus as *Cereibacter changlensis* gen. nov., comb. nov. Int J Syst Evol Microbiol 65:794–798. https://doi.org/10.1099/ijs.0.000016

Teeling H, Meyerdierks A, Bauer M et al (2004) Application of tetranucleotide frequencies for the assignment of genomic fragments. Environ Microbiol 6:938–947. https://doi.org/10.1111/j.1462-2920.2004.00624.x

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07

Wickham HC (2016) ggplot2-Elegant Graphics for Data Analysis. Springer Int Publ New York

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. https://doi.org/10.1099/ijsem.0.002833

Won S-M, Park S, Park J-M et al (2015) *Pseudohalocynthiibacter aestuariivivens* gen. nov., sp. nov., isolated from a tidal flat. Int J Syst Evol Microbiol 65:1509–1514. https://doi.org/10.1099/ijs.0.000128

Yokota A, Akagawa-Matsushita M, Hiraishi A et al (1992) Distribution of quinone system in microorganisms: Gram-negative eubacteria. Bull Jpn Fed Cult Coll 8:136–171

Yoon S-H, Ha S-M, Kwon S et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol

Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755

Zerbino DR, Birney E (2008) Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107