Pacificibacter maritimus gen. nov., sp. nov., isolated from shallow marine sediment

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An aerobic, Gram-stain-negative, non-pigmented, non-motile bacterium, strain KMM 9031^T, was isolated from a sandy sediment sample collected from the shore of the Sea of Japan and subjected to phenotypic and phylogenetic analysis. Based on comparative 16S rRNA gene sequence analysis, strain KMM 9031^T constituted a separate phylogenetic line within the *Roseobacter* clade of the class *Alphaproteobacteria*, sharing highest sequence similarities with members of the genera *Roseovarius* (92.7–95.3 %), *Pseudoruegeria* (94.5 %), *Sulfitobacter* (92.7–94.4 %) and *Thalassobacter* (94.2–94.3 %). The predominant fatty acid of strain KMM 9031^T was $C_{18:1}\omega7c$, with $C_{16:0}$, $C_{10:0}$ 3-OH and $C_{12:1}$ 3-OH present in lesser amounts. The DNA G+C content of the isolate was 52.6 mol%. The major isoprenoid quinone was Q-10 and polar lipids comprised phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and two unknown lipids. On the basis of phylogenetic analysis and physiological and biochemical characterization, strain KMM 9031^T represents a novel species in a new genus, for which the name *Pacificibacter maritimus* gen. nov., sp. nov. is proposed; the type strain is KMM 9031^T (=NRIC 0785^T =JCM 17096^T).

Bacteria belonging to the *Roseobacter* clade (order *Rhodobacterales*, class *Alphaproteobacteria*; Garrity *et al.*, 2005) are probably members of one of the most abundant groups of microbial communities associated with marine sediments, water and biota (Buchan *et al.*, 2005). In recent years, the *Roseobacter* clade has expanded considerably, including the genera *Shimia* (Choi & Cho, 2006), *Phaeobacter* and *Marinovum* (Martens *et al.*, 2006), *Donghicola* (Yoon *et al.*, 2007a), *Pseudoruegeria* (Yoon *et al.*, 2007c) and *Marivita* (Hwang *et al.*, 2009). During an investigation of micro-organisms inhabiting the shallow sediments of the Sea of Japan, alphaproteobacteria-like bacteria were found as the major components in many samples. Recently, we described a new genus, *Litoreibacter*, in the class *Alphaproteobacteria* to accommodate two isolates that were recovered from a sandy snail specimen (*Umbonium costatum*) and from its surrounding sediments collected simultaneously from the Sea of Japan seashore (Romanenko *et al.*, 2011). Here, a Gram-stain-negative, aerobic, non-pigmented, non-motile bacterium, designated KMM 9031^T, which was isolated from a sandy sediment sample collected from the shore of the Sea of Japan was studied using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences confirmed that strain KMM 9031^T should be assigned to the *Roseobacter* clade of the class *Alphaproteobacteria*, where it formed a separate branch, sharing highest sequence similarity with the type strain of *Roseovarius crassostreae* (95.3 %). Based on distinctive phenotypic characteristics and phylogenetic distances, a novel species in a new genus, *Pacificibacter maritimus* gen. nov., sp. nov., is proposed.

Strain KMM 9031^T was isolated from a sandy sediment sample collected from the shore of the Sea of Japan (44° 48′ 25″ N 136° 21′ 96″ E), Russia, as described previously (Romanenko *et al.*, 2003, 2004). Strain KMM 9031^T was grown aerobically on marine agar 2216 (MA; Difco) or marine broth (MB; Difco) and stored at −80 °C in MB supplemented with 30 % (v/v) glycerol. Motility was determined by the hanging drop method as described by Gerhardt *et al.* (1994). Cells from cultures grown for 2 days on MA at 25 °C were observed by oil-immersion phase-contrast microscopy (AX70; Olympus). Gram staining,

Abbreviations: ASW, artificial seawater; ECL, equivalent chain-length; FAMEs, fatty acid methyl esters.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 9031^T is AB558927.

A supplementary figure is available with the online version of this paper.

oxidase and catalase activities, hydrolysis of gelatin, casein, DNA and Tweens 20, 40 and 80, and production of H_2S from thiosulfate were tested according to standard methods (Smibert & Krieg, 1994). Acid production from carbohydrates was examined using oxidation/fermentation medium as described by Leifson (1963). Requirements for and tolerance of sodium chloride were tested on artificial seawater (ASW)-based medium using various concentrations of NaCl in the range 0–20 %, supplemented with (per litre) 10.0 g Bacto peptone, 2.0 g yeast extract, 0.028 g $FeSO_4$ and 15.0 g agar. ASW, as described by Bruns *et al.* (2001), contained (per litre distilled water): 23.6 g NaCl, 4.53 g $MgCl_2 . 6H_2O$, 3.9 g $MgSO_4 . 7H_2O$, 0.64 g KCl and 1.3 g $CaCl_2 . 2H_2O$. In addition, the strain was tested for growth on the above medium containing NaCl alone, in the absence of any of the sea salts components (i.e. $MgCl_2$, $MgSO_4$, KCl and $CaCl_2$). Growth in the presence of organic substrates as sole carbon and energy sources was tested for 3 weeks on ASW-based medium supplemented with NH_4Cl (1 g l^{-1}), yeast extract (0.5 g l^{-1}) and 0.4 % carbon source. Growth was considered as negative if it was equal to or less than that observed in the negative control to which no carbon source had been added. Growth at different temperatures (2–45 °C) and pH (4.0–14.0) and antibiotic resistance were studied as described previously (Romanenko *et al.*, 2003, 2005). Biochemical tests were carried out using API ZYM, API 32GN and API 20NE test kits (bioMérieux) according to the manufacturer's instructions, except that the culture was suspended in ASW. Production of bacteriochlorophyll *a* was tested spectrophotometrically in methanolic extracts of cells grown on MA and MB in the dark as described by Lafay *et al.* (1995). For polar lipid, fatty acid and respiratory lipoquinone analyses, strain KMM 9031^T was cultivated on MA or MB at 28 °C for 3 days. Lipids were extracted using the method of Folch *et al.* (1957). Two-dimensional TLC of polar lipids was carried out on Silica gel 60 F_{254} (10 × 10 cm; Merck) using chloroform/methanol/water (65 : 25 : 4, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) for the second dimension (Collins & Shah, 1984). Fatty acid methyl esters (FAMEs) were prepared according to the procedure of the Microbial Identification System (MIDI; Sasser, 1990). FAME analysis was performed by GC (GC-17A; Shimadzu) equipped with a capillary column (30 m × 0.25 mm i.d.) coated with Supelcowax-10 and SPB-5 phases (Supelco). Identification of FAMEs was accomplished by determining equivalent chain-length (ECL) values and comparing the retention times of the samples to those of standards. In addition, FAMEs were analysed using a GLC-MS Shimadzu GC-MS model QP5050 (column MDM-5S; the temperature program was from 140 to 250 °C, at a rate of 2 °C min^{-1}). Isoprenoid quinones were extracted according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins & Shah (1984). The DNA G+C content was determined as described by Marmur & Doty (1962) and Owen *et al.* (1969). The 16S rRNA gene sequence of strain KMM 9031^T, consisting of 1443 nt, was determined as described by Shida *et al.* (1997). The sequence obtained was compared with 16S rRNA gene sequences retrieved from DDBJ/EMBL/GenBank by using the program FASTA (Pearson & Lipman, 1988). Phylogenetic analysis of 16S rRNA gene sequences was performed using the software package MEGA4 (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL X (version 1.83; Thompson *et al.*, 1997). Phylogenetic trees were reconstructed by the neighbour-joining and maximum-parsimony methods and distances were calculated according to the Kimura two-parameter model. The robustness of phylogenetic trees was estimated by bootstrap analysis of 1000 replicates.

Based on 16S rRNA gene sequence analysis, strain KMM 9031^T was assigned to the *Roseobacter* clade of the class *Alphaproteobacteria* where it formed a separate phylogenetic line; different treeing algorithms, neighbour-joining (Fig. 1) and maximum-parsimony, showed the same tree topology. The novel strain shared highest sequence similarities with the type strain of *Roseovarius crassostreae* (95.3 %) and with members of the genera *Roseovarius* (92.7–95.3 %), *Pseudoruegeria* (94.5 %), *Sulfitobacter* (92.7–94.4 %) and *Thalassobacter* (94.2–94.3 %). The low sequence similarity values found with recognized species of the *Roseobacter* clade demonstrated that KMM 9031^T can be considered to be a representative of a novel genus. The phylogenetic distinctiveness found for strain KMM 9031^T was supported by a combination of phenotypic characteristics that enabled the novel strain to be differentiated from its close relatives. Physiological, morphological, biochemical and chemotaxonomic characteristics of strain KMM 9031^T are given in Table 1, Table 2, Fig. 2, Fig. 3, Supplementary Fig. S1 (available in IJSEM Online) and in the genus and species descriptions. Strain KMM 9031^T was characterized by the ability to grow between 2 and 36 °C and at pH 5.5–9.5. It required sodium chloride, but not sea salts for growth. Strain KMM 9031^T gave negative results for carbon source assimilation in the API 20NE tests, but could utilize a number of substrates during growth on ASW-based media containing carbohydrates or organic or amino acids as sole carbon and energy sources. Interestingly, on L-tyrosine-containing medium, strain KMM 9031^T produced grey-brownish colonies and exhibited remarkable hydrolytic activity, producing a clearance zone around the colonies, but no diffusible pigments. Similar activity was observed previously for *Litoreibacter albidus* KMM 3851^T (Romanenko *et al.*, 2011) and confirmed in the present study for *Thalassobacter arenae* GA2-M15^T (Supplementary Fig. S1), which had previously been reported as having a weak reaction for hydrolysis of L-tyrosine (Kim *et al.*, 2009). It is worth noting that strain KMM 9031^T was susceptible to 18 of the 20 antibiotics that were tested as listed in the species description. Chemotaxonomic properties of strain KMM 9031^T (ubiquinone Q-10, the predominance of $C_{18:1}\omega7c$, the presence of the hydroxy acids $C_{10:0}$ 3-OH and $C_{12:1}$ 3-OH, and the presence of phosphatidylcholine,

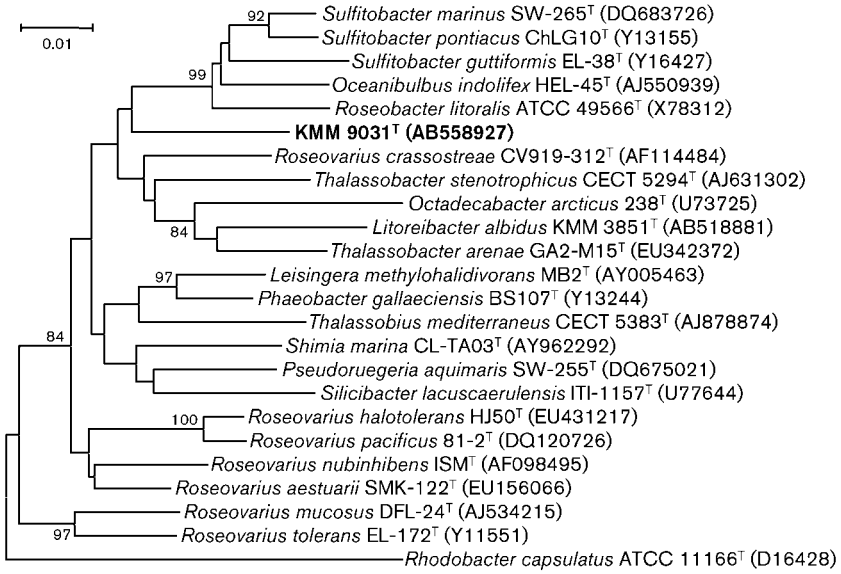

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences available from DDBJ/GenBank/EMBL (accession numbers are given in parentheses) showing the relationship between strain KMM 9031^T and members of related genera of the class *Alphaproteobacteria*. Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 70 % are shown. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of strain KMM 9031^T and type strains of related members of the class *Alphaproteobacteria*

Taxa: 1, *Pacificibacter maritimus* gen. nov., sp. nov. KMM 9031^T (data from present study); 2, *Roseovarius crassostreae* (Boettcher *et al.*, 2005; Wang *et al.*, 2009); 3, *Thalassobacter stenotrophicus* (Macián *et al.*, 2005; Kim *et al.*, 2009); 4, *Thalassobacter arenae* (Kim *et al.*, 2009); 5, *Pseudoruegeria aquimaris* (Yoon *et al.*, 2007c); 6, *Sulfitobacter marinus* (Yoon *et al.*, 2007b). ND, Not determined.

Characteristic	1	2	3	4	5	6
Pigmentation	Whitish	Pinkish-beige	Salmon-pink	Pinkish-beige†	Greyish-yellow	Cream
Motility	−	+	+	+	−	−
Growth at:						
4 °C	+	−	−	+†	−	+
37 °C	−	+	+	−	+	−
>37 °C	−	−	−	−	+	−
Nitrate reduction	−	+	−	−	−	−
Utilization of:						
Glucose	+	−	+*	+	+	−
Sucrose	−	ND	−*	−	+	ND
Maltose	+	ND	−*	−	+	ND
Xylose	−	ND	−	+	+	ND
Cellobiose	+	ND	−*	−	+	ND
D-Galactose	−	ND	−	+	+	ND
Melibiose	+	ND	−	+	ND	ND
Glycerol	−	+	−	+	ND	ND
Citrate	+	−	−*	+	+	−
Acetate	+	ND	+	ND	+	−
Lactate	+	ND	−	ND	ND	ND
β-Galactosidase	+	−	−*	+	+	−
Hydrolysis of:						
Aesculin	+	−	+	+	+	−
Tween 80	−	ND	−	−†	−	+
Tyrosine	+	ND	−	+†	−	+
DNA G+C content (mol%)	52.6	59	59	56	67	57.8

*Data from Kim *et al.* (2009).
†Data obtained from the present study.

Table 2. Fatty acid composition (%) and polar lipids of strain KMM 9031^T and related members of the class *Alphaproteobacteria*

Taxa: 1, *Pacificibacter maritimus* gen. nov., sp. nov. KMM 9031^T (data from the present study); 2, *Roseovarius crassostreae* (Boettcher *et al.*, 2005); 3, *Thalassobacter stenotrophicus* (Macián *et al.*, 2005; Kim *et al.*, 2009); 4, *Thalassobacter arenae* (Kim *et al.*, 2009); 5, *Pseudoruegeria aquimaris* (Yoon *et al.*, 2007c); 6, *Sulfitobacter marinus* (Yoon *et al.*, 2007b). −, Not detected; ND, not determined.

Component	1	2	3	4	5	6
Fatty acid						
$C_{10:0}$ 3-OH	6.45	2.1±0.3	3.15	3.7	2.9	3.6
$C_{12:1}$ 3-OH	6.93	−	−	−	−	−
$C_{16:1}$	3.90	−	−	−	−	−
$C_{16:0}$	8.67	3.6±0.9	0.42	10.4	1.4	8.3
$C_{17:0}$	0.55	−	0.33	−	0.9	0.7
$C_{18:1}\omega7c$	66.03	85.2±1.7	78.09	74.3	72.9	77.1
11-Methyl $C_{18:1}\omega7c$	4.76	0.8±0.2	6.96	5.9	2.8	6.9
$C_{18:0}$	2.17	0.7±0.3	2.59	1.2	6.6	0.6
$C_{19:1}\omega6c$/cyclo $C_{19:0}$	−	−	1.47	−	−	−
Cyclo $C_{19:0}\omega8c$	−	−	1.47‡	−	5.9	−
$C_{20:1}\omega7c$	−	−	1.04	−	0.9	−
ECL 11.799	−	−	3.55	3.0	2.8	−
Polar lipid*						
PC	+	ND	+†	+	−	ND
PE	−	ND	+	+	+	ND
PG	+	ND	+	+	+	ND
DPG	+	ND	+	+	+	ND
APL	−	ND	−	−	−	ND
AL	−	ND	−	−	−	ND
PL	−	ND	+	−	+	ND
L	+	ND	−	−	−	ND
GL	−	ND	−	−	+	ND

*AL, Unknown aminolipid; APL, unknown aminophospholipid; DPG, diphosphatidylglycerol; GL, unknown glycolipid; L, unknown lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid.
†Data for polar lipids from Kim *et al.* (2009).
‡Detected as part of a summed feature.

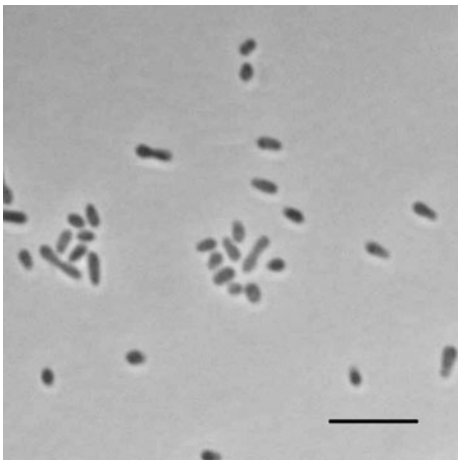

Fig. 2. Phase-contrast micrograph showing cell morphology of strain KMM 9031^T grown on MA for 2 days at 25 °C. Bar, 10 μm.

phosphatidylglycerol and diphosphatidylglycerol) are in agreement with characteristics reported for phylogenetically related bacteria of the *Roseobacter* clade. At the same time, the presence of $C_{16:1}$, the absence of phosphatidylethanolamine and an unknown aminolipid and phospholipid, and the low DNA G+C value of 52.6 mol% distinguished strain KMM 9031^T from its phylogenetic relatives. In addition, strain KMM 9031^T could be phenotypically distinguished from related species by the lack of pigmentation, immobility, growth at 2–4 °C and carbon source utilization pattern (Table 1). The following characteristics, shown by strain KMM 9031^T, enabled this strain to be differentiated from its closest relative, *Roseovarius crassostreae* (Boettcher *et al.*, 2005): the absence of growth at 37 °C; positive reactions for β-galactosidase, aesculin hydrolysis, and utilization of glucose and citrate; a negative reaction for nitrate reduction; and a low DNA G+C content (Table 1). Based on its distinctive phenotypic characteristics and phylogenetic distances, strain KMM 9031^T is considered to represent a novel species in a new genus, *Pacificibacter maritimus* gen. nov., sp. nov.

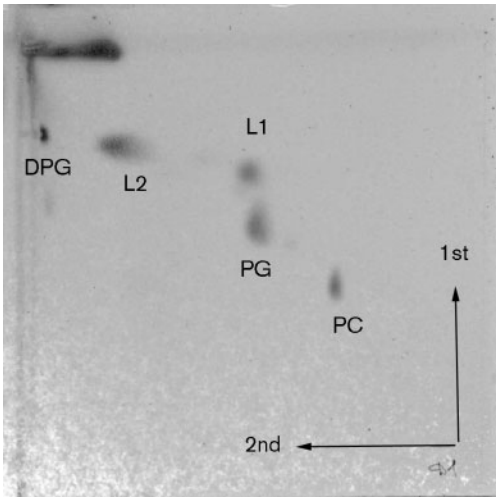

Fig. 3. Two-dimensional TLC of polar lipids of strain KMM 9031^T. DPG, Diphosphatidylglycerol; L1 and L2, unknown lipids; PC, phosphatidylcholine; PG, phosphatidylglycerol.

Description of *Pacificibacter* gen. nov.

Pacificibacter [Pa.ci.fi.ci.bac′ter. N.L. n. (*oceanus*) *pacificus* the Pacific Ocean; N.L. n. *bacter* (from Greek *baktron*) a rod; N.L. masc. n. *Pacificibacter* a rod isolated from the Pacific Ocean].

Gram-stain-negative, strictly aerobic, oxidase- and catalase-positive, rod-shaped bacteria enlarged at one pole due to cell division by budding. Chemo-organoheterotrophic. Sodium ions are essential for growth. The predominant isoprenoid quinone is Q-10. Polar lipids include phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and unknown lipids. The major fatty acid is $C_{18:1}\omega7c$, followed by $C_{16:0}$, $C_{10:0}$ 3-OH and $C_{12:1}$ 3-OH. Isolated from marine environments. Based on 16S rRNA gene sequence analysis, the genus represents a separate branch within the *Alphaproteobacteria*, close to the genera *Roseovarius*, *Pseudoruegeria*, *Sulfitobacter* and *Thalassobacter*. The type species of the genus is *Pacificibacter maritimus*.

Description of *Pacificibacter maritimus* sp. nov.

Pacificibacter maritimus (ma.ri.ti′mus. L. masc. adj. *maritimus* maritime, marine).

In addition to properties given in the genus description, the species can be characterized as follows. Cells are rods, 0.4–0.6 μm in diameter and 1.8–2.0 μm in length, which are enlarged at one pole due to the budding process. Non-motile. Colonies are whitish, translucent, smooth, shiny with regular edges and 2–3 mm in diameter on MA. Bacteriochlorophyll α is not produced. Requires NaCl for growth; growth occurs with 0.5–6.0 % (w/v) NaCl; growth is weak with 6.0 % NaCl and optimal with 2–3 %. Grows in/on basal media containing only NaCl in the absence of any other sea salts components ($MgCl_2$, KCl, $CaCl_2$ and $MgSO_4$). The temperature range for growth is 2–36 °C, with optimum growth at 25–30 °C and no growth at 37 °C. Grows at pH 5.5–9.5, with optimum growth at pH 6.5–8.5. Negative for nitrate reduction, H_2S production, and hydrolysis of casein, gelatin, DNA, chitin and Tween 80. Positive for hydrolysis of Tweens 40 and 20 and L-tyrosine. Weakly positive for hydrolysis of starch. On L-tyrosine-containing medium, produces grey-brownish colonies and forms a clearance zone, but not diffusible brown pigments. No acid is produced from D-glucose, maltose, D-galactose, lactose, D-mannose, cellobiose, D-xylose, L-arabinose, L-rhamnose, melibiose, D-ribose, fructose, L-sorbose, raffinose, *N*-acetylglucosamine, glycerol, D-*myo*-inositol or D-mannitol. According to the API 20NE test kit, positive for the PNPG (β-galactosidase) test and aesculin hydrolysis, but negative for nitrate reduction, indole production, glucose acidification under anaerobic conditions, gelatin hydrolysis, arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate. The API 32GN test kit yielded positive results for assimilation of inositol, lactic acid, melibiose and L-proline, but negative results for assimilation of L-rhamnose, *N*-acetylglucosamine, D-ribose, sucrose, maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicin, L-fucose, D-sorbitol, L-arabinose, propionic acid, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid. Positive API ZYM test results were obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase; negative results were obtained for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. Utilizes D-glucose, maltose, cellobiose, L-tyrosine, citrate, acetate, fumarate and malate as carbon and energy sources; weakly utilizes inositol and L-arginine; and does not utilize L-rhamnose, L-arabinose, D-galactose, D-mannose, L-xylose, sucrose, fructose, raffinose, D-mannitol, glycerol, aminoacetic acid, ornithine, DL-leucine, L-α-alanine, DL-β-phenylalanine, DL-lysine, L-asparagine or L-methionine. The major fatty acids are $C_{18:1}\omega7c$, followed by $C_{16:0}$, $C_{10:0}$ 3-OH and $C_{12:1}$ 3-OH. Detailed fatty acid composition and polar lipids are shown in Table 2 and Fig. 3. Susceptible to antibiotics (content per disc): ampicillin (10 μg), benzylpenicillin (10 U), carbenicillin (100 μg), gentamicin (10 μg), rifampicin (5 μg), streptomycin (30 μg), vancomycin (30 μg), nalidixic acid (30 μg), ofloxacin (5 μg), oxacillin (10 μg), neomycin (30 μg), kanamycin (30 μg), oleandomycin (15 μg), erythromycin (15 μg), cephazolin (30 μg), cephalexin (30 μg), tetracycline (30 μg) and chloramphenicol (30 μg). Resistant to lincomycin (15 μg) and polymyxin (300 U).

The type strain is strain KMM 9031^T (=NRIC 0785^T =JCM 17096^T), isolated from a sandy sediment sample collected from the Sea of Japan seashore, Russia. The DNA G+C content of the type strain is 52.6 mol% (T_m).

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References

Boettcher, K. J., Geaghan, K. K., Maloy, A. P. & Barber, B. J. (2005). *Roseovarius crassostreae* sp. nov., a member of the *Roseobacter* clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. *Int J Syst Evol Microbiol* **55**, 1531–1537.

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

Buchan, A., González, J. M. & Moran, M. A. (2005). Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* **71**, 5665–5677.

Choi, D. H. & Cho, B. C. (2006). *Shimia marina* gen. nov., sp. nov., a novel bacterium of the *Roseobacter* clade isolated from biofilm in a coastal fish farm. *Int J Syst Evol Microbiol* **56**, 1869–1873.

Collins, M. D. & Shah, H. N. (1984). Fatty acid, menaquinone and polar lipid composition of *Rothia dentosacariosa*. *Arch Microbiol* **137**, 247–249.

Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**, 497–509.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2005). *Rhodobacterales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2 (*The Proteobacteria*), part C (*The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*), pp. 161–224. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

Hwang, C. Y., Bae, G. D., Yih, W. & Cho, B. C. (2009). *Marivita cryptomonadis* gen. nov., sp. nov. and *Marivita litorea* sp. nov., of the family *Rhodobacteraceae*, isolated from marine habitats. *Int J Syst Evol Microbiol* **59**, 1568–1575.

Kim, B. Y., Weon, H. Y., Son, J. A., Lee, C. M., Hong, S. B., Jeon, Y. A., Koo, B. S. & Kwon, S. W. (2009). *Thalassobacter arenae* sp. nov., isolated from sea sand in Korea. *Int J Syst Evol Microbiol* **59**, 487–490.

Lafay, B., Ruimy, R., Rausch de Traubenberg, C., Breittmayer, V., Gauthier, M. J. & 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.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

Macián, M. C., Arahal, D. R., Garay, E., Ludwig, W., Schleifer, K. H. & Pujalte, M. J. (2005). *Thalassobacter stenotrophicus* gen. nov., sp. nov., a novel marine α-proteobacterium isolated from Mediterranean sea water. *Int J Syst Evol Microbiol* **55**, 105–110.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

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

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

Owen, R. J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base compositions from melting profiles in dilute buffers. *Biopolymers* **7**, 503–516.

Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.

Romanenko, L. A., Schumann, P., Zhukova, N. V., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2003). *Oceanisphaera litoralis* gen. nov., sp. nov., a novel halophilic bacterium from marine bottom sediments. *Int J Syst Evol Microbiol* **53**, 1885–1888.

Romanenko, L. A., Schumann, P., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2004). *Reinekea marinisedimentorum* gen. nov., sp. nov., a novel gammaproteobacterium from marine coastal sediments. *Int J Syst Evol Microbiol* **54**, 669–673.

Romanenko, L. A., Uchino, M., Falsen, E., Frolova, G. M., Zhukova, N. V. & Mikhailov, V. V. (2005). *Pseudomonas pachastrellae* sp. nov., isolated from a marine sponge. *Int J Syst Evol Microbiol* **55**, 919–924.

Romanenko, L. A., Tanaka, N., Frolova, G. M., Svetashev, V. I. & Mikhailov, V. V. (2011). *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.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–655. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Wang, B., Tan, T. & Shao, Z. (2009). *Roseovarius pacificus* sp. nov., isolated from deep-sea sediment. *Int J Syst Evol Microbiol* **59**, 1116–1121.

Yoon, J. H., Kang, S. J. & Oh, T. K. (2007a). *Donghicola eburneus* gen. nov., sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* **57**, 73–76.

Yoon, J. H., Kang, S. J. & Oh, T. K. (2007b). *Sulfitobacter marinus* sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* **57**, 302–305.

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