Litoreibacter albidus gen. nov., sp. nov. and *Litoreibacter janthinus* sp. nov., members of the class *Alphaproteobacteria* isolated from the seashore

Lyudmila A. Romanenko,[1] Naoto Tanaka,[2] Galina M. Frolova,[1] Vassilii I. Svetashev[3] and Valery V. Mikhailov[1]

Correspondence
Lyudmila A. Romanenko
lro@piboc.dvo.ru

[1]Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, 690022 Vladivostok, Prospect 100 Let Vladivostoku, 159, Russia

[2]NODAI Culture Collection Center, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

[3]Institute of Marine Biology, Far-Eastern Branch, Russian Academy of Sciences, 690041 Vladivostok, Russia

Two Gram-negative, strictly aerobic, non-motile bacteria designated strains KMM 3851^T and KMM 3842^T were respectively isolated from a marine snail specimen (*Umbonium costatum*) and from surrounding sediments collected simultaneously from the shore of the Sea of Japan. Phylogenetic analysis based on 16S rRNA gene sequences showed that strains KMM 3851^T and KMM 3842^T were affiliated with the *Roseobacter* lineage of the class *Alphaproteobacteria* as a separate phylogenetic line adjacent to the members of the genus *Thalassobacter*. These novel isolates shared 98.5 % 16S rRNA gene sequence similarity and 15 % DNA–DNA relatedness. The major isoprenoid quinone of both strains was Q-10 and polar lipids consisted of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and aminophospholipids. In addition, strain KMM 3851^T contained two unknown lipids, whereas strain KMM 3842^T contained diphosphatidylglycerol. Fatty acid analysis revealed $C_{18:1}\omega7c$ and $C_{16:0}$ as major components and small amounts of $C_{18:2}$. The DNA G+C contents were 60.4 mol% (KMM 3851^T) and 58.5 mol% (KMM 3842^T). Based on distinctive phenotypic characteristics, DNA–DNA hybridization data and phylogenetic distance, strains KMM 3851^T and KMM 3842^T should be classified as representatives of two novel species in a new genus, *Litoreibacter* gen. nov., with the type species *Litoreibacter albidus* sp. nov. (type strain KMM 3851^T =NRIC 0773^T =JCM 16493^T) and a second species *Litoreibacter janthinus* sp. nov. (type strain KMM 3842^T =NRIC 0772^T =JCM 16492^T).

The *Roseobacter* clade (order *Rhodobacterales*, class *Alphaproteobacteria*) constitutes a large group of genera (Garrity *et al.* 2005, 2006) that have been reported to be a significant component of marine and saline environments (Buchan *et al.*, 2005). The number of genera in the *Roseobacter* clade is increasing continuously, with the recent addition of *Thalassobius* (Arahal *et al.*, 2005), *Thalassobacter* (Macián *et al.*, 2005; Pujalte *et al.*, 2005), *Shimia* (Choi & Cho, 2006), *Donghicola* (Yoon *et al.*, 2007a), *Pseudoruegeria* (Yoon *et al.*, 2007c) and *Marivita* (Hwang *et al.*, 2009). The genera *Phaeobacter* and *Marinovum* (Martens *et al.*, 2006) were recently proposed as a result of reclassification of *Roseobacter gallaeciensis* (Ruiz-Ponte *et al.*, 1998) and *Ruegeria algicola* (Lafay *et al.*, 1995; Uchino *et al.*, 1998, 1999), respectively.

Here, we report the phenotypic and phylogenetic characterization of two strains, designated KMM 3851^T and KMM 3842^T, which were isolated during a survey of the biodiversity of micro-organisms inhabiting shallow sediments of the Sea of Japan. Phylogenetic analysis based on 16S rRNA gene sequences placed strains KMM 3851^T and KMM 3842^T within the *Roseobacter* lineage as a separate branch adjacent to the members of the genus *Thalassobacter*.

Abbreviation: Bchl, bacteriochlorophyll.

The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequences of strains KMM 3842^T and KMM 3851^T are AB518880 and AB518881.

Three supplementary figures are available with the online version of this paper.

Both strains exhibited distinguishing phenotypic and chemotaxonomic traits when compared with the closest phylogenetically related bacteria. Based on distinctive phenotypic characteristics, DNA–DNA hybridization data and phylogenetic distance, isolates KMM 3851^T and KMM 3842^T represent two species of a novel genus.

Strains KMM 3851^T and KMM 3842^T were respectively isolated from an internal tissue of a marine snail (*Umbonium costatum*; Gastropoda, Trochidae) and from a sample of the surrounding sediment, collected simultaneously from shallow sediment of the Sea of Japan, Russia, as described previously (Romanenko *et al.*, 2004). Strains KMM 3851^T and KMM 3842^T were grown aerobically on marine 2216 agar (MA) or marine broth (MB) at 25–28 °C and stored at –80 °C in liquid MB supplemented with 30 % (v/v) glycerol. Strains KMM 3851^T and KMM 3842^T were deposited in the Collection of Marine Micro-organisms (KMM), Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia. Motility was determined by the hanging drop method as described by Gerhardt *et al.* (1994). Cells were observed by oil-immersion phase-contrast microscopy (AX70; Olympus) using 3-day cultures grown on MA at 25 °C. Gram staining, oxidase and catalase activities, hydrolysis of gelatin, casein, chitin, CM-cellulose, DNA and Tween 80 and H_2S production 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). Requirement for and tolerance of NaCl was tested on a medium based on artificial seawater (ASW) using various concentrations of NaCl in the range 0–20 %, supplemented with (l^{-1}) 10.0 g Bacto peptone, 2.0 g yeast extract, 0.028 g $FeSO_4$ and 15.0 g agar. The ASW contained (per litre distilled water): 24 g NaCl, 4.9 g $MgCl_2$, 3.9 g Na_2SO_4, 1.1 g $CaCl_2$, 0.66 g KCl, 0.2 g $NaHCO_3$, 0.096 g KBr, 0.026 g H_3BO_3, 0.024 g $SrCl_2$ and 0.003 g NaF (Lyman & Fleming, 1940). To test strains for sodium ion requirement, ASW was prepared without $NaNO_3$, $NaSO_4$, $NaHCO_3$ and NaF. In addition, strains were tested for growth on the above medium containing NaCl alone, without any of the sea salts components $MgCl_2$, KCl, $CaCl_2$, $NaNO_3$, K_2HPO_4, KCl, $NaSO_4$, $NaHCO_3$, NaF and $FeSO_4$. Growth at different temperatures and pH and antibiotic resistance were studied as described previously (Romanenko *et al.*, 2004, 2005). In addition, 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 cultures were suspended in ASW. Production of bacteriochlorophyll (Bchl) *a* was tested spectrophotometrically in methanolic extracts of cells grown on MA and in MB in the dark, as described by Lafay *et al.* (1995). For polar lipid and fatty acid analyses, strains KMM 3851^T and KMM 3842^T were cultivated on MA at 28 °C for 3 days and lipids were extracted using the chloroform/methanol extraction method of Bligh & Dyer (1959). Polar lipids were analysed as described by Vaskovsky & Terekhova (1979). Fatty acid methyl esters were obtained by alkaline methanolysis (15 % NaOH/methanol), extracted with hexane and analysed using a GLC-MS Hewlett Packard model 6890 gas chromatograph equipped with an HP 5 MS 5 % phenyl methyl siloxane capillary column (30 m × 250 μm × 0.25 μm) and connected to a Hewlett Packard model 5973 mass spectrometer. Cells for respiratory lipoquinone analysis were obtained from MB cultures grown at 25 °C. Isoprenoid quinones were extracted using chloroform/methanol (2 : 1, v/v), purified by preparative TLC on silica gel 60 ADAMANT plates (Fluka) and analysed by HPLC (Agilent 1100 series) using a reversed-phase column (Hypersil ODS, 5 μm; 40 × 250 mm). Methanol/2-propanol (65 : 35) was used as a mobile phase and quinones were detected at 270 nm. The DNA base composition was determined as described by Marmur & Doty (1962) and Owen *et al.* (1969). The photobiotin-labelled DNA probe microplate method described by Ezaki *et al.* (1989) was applied to determine DNA–DNA relatedness between strains KMM 3851^T and KMM 3842^T. The 16S rRNA gene sequences of strains KMM 3851^T and KMM 3842^T, respectively containing 1440 and 1437 nt, were determined as described by Shida *et al.* (1997). The sequences obtained were compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DDBJ databases by using the FASTA program (Pearson & Lipman, 1988). Phylogenetic analysis of 16S rRNA gene sequences was performed using the software package MEGA 4 (Tamura *et al.*, 2007) after multiple alignment of the data by CLUSTAL_X (version 1.83; Thompson *et al.*, 1997). Phylogenetic trees were constructed by the neighbour-joining and maximum-parsimony methods and distances were calculated according to Kimura's two-parameter model. The robustness of phylogenetic trees was estimated by bootstrap analysis of 1000 replicates.

Comparative 16S rRNA gene sequence analysis showed that isolates KMM 3851^T and KMM 3842^T were members of the class *Alphaproteobacteria* and were closely related to each other, with a sequence similarity of 98.5 %. Their closest phylogenetic neighbours were *Thalassobacter arenae* GA2-M15^T (96.0 and 96.1 % sequence similarity, respectively), *Roseovarius crassostreae* CV919-312^T (95.1 and 95.8 %), *Pseudoruegeria aquimaris* SW-255^T (94.7 and 95.6 %) and *Sulfitobacter marinus* SW-265^T (94.2 and 94.7 %). Different treeing algorithms (neighbour-joining and maximum-parsimony) positioned strains KMM 3851^T and KMM 3842^T within the *Roseobacter* lineage of the *Alphaproteobacteria* as a separate branch adjacent to the members of the genus *Thalassobacter* (Fig. 1 and Supplementary Fig. S1). Low DNA–DNA relatedness of 15 % was determined between strains KMM 3851^T and KMM 3842^T, indicating that they belong to separate species (Wayne *et al.*, 1987). As shown in Table 1, there are sufficient differences between these two genospecies in physiological and metabolic traits to allow their phenotypic identification.

Physiological, biochemical and chemotaxonomic characteristics of strains KMM 3851^T and KMM 3842^T are given in Tables 1 and 2, Supplementary Fig. S2 and in the genus and species descriptions. Strains KMM 3851^T and KMM 3842^T were Gram-negative, strictly aerobic, oxidase- and

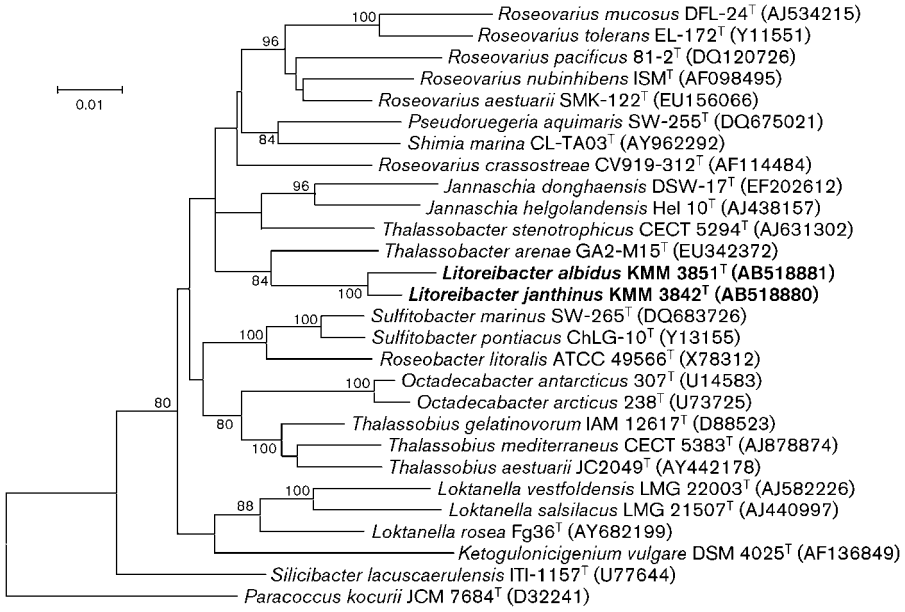

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers in parentheses) showing the relationship of isolates KMM 3851^T and KMM 3842^T and related genera of the class *Alphaproteobacteria*. Phylogenetic analysis was performed using the software package MEGA 4 (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL_X (version 1.83; Thompson *et al.*, 1997). Bootstrap values greater than 70 % based on 1000 replications are given at branching points. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of strains KMM 3851^T and KMM 3842^T and related members of the *Alphaproteobacteria*

Taxa: 1, KMM 3851^T; 2, KMM 3842^T; 3, *Thalassobacter stenotrophicus* (unless indicated, data from Macián *et al.*, 2005); 4, *Thalassobacter arenae* (Kim *et al.*, 2009); 5, *Jannaschia helgolandensis* (Wagner-Döbler *et al.*, 2003); 6, *Jannaschia donghaensis* (Yoon *et al.*, 2007d); 7, *Roseovarius crassostreae* (Boettcher *et al.*, 2005); 8, *Pseudoruegeria aquimaris* (Yoon *et al.*, 2007c); 9, *Sulfitobacter marinus* (Yoon *et al.*, 2007b). All bacteria are negative for gelatin hydrolysis. +, Positive; −, negative; W, weak reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Pigmentation*	WH	GV	SP	BR	WH	O	PB	GY	C
Bchl *a*	−	−	+	ND	ND	−	−	ND	ND
Motility	−	−	+	+	−	−	+	−	−
Growth at:									
4 °C	+	+	−	−	−	+	−	−	+
37 °C	+	W	+	−	−	−	+	+	−
>37 °C	−	−	−	−	−	−	−	+	−
NaCl range for growth (%)	0.5–8	0.5–6	0.85–7	0.85–8	1–7	ND	ND	ND	ND
Nitrate reduction	−	−	−	−	−	−	+	−	−
H_2S production	+	−	−	ND	ND	−	ND	−	−
D-Glucose assimilation	−	−	+†	+	+	−	−	+	−
β-Galactosidase	+	+	−	+	ND	−	−	+	−
Hydrolysis of:									
Aesculin	+	+	+	+	−	−	−	+	−
Tween 80	+	−	−	ND	−	W	ND	−	+
Tyrosine	+	−	−	W	ND	−	ND	−	+
DNA G+C content (mol%)	60.4	58.5	59	56	63.0–63.1	65.2	59	67.0	57.8

*BR, Brown; C, cream; GV, greyish violet; GY, greyish yellow; O, orange; PB, pinkish beige; SP, salmon pink; WH, whitish.
†Data from Kim *et al.* (2009).

Table 2. Fatty acid and polar lipid compositions of strains KMM 3851^T and KMM 3842^T and related members of the *Alphaproteobacteria*

Taxa: 1, KMM 3851^T; 2, KMM 3842^T; 3, *Thalassobacter arenae* (data from Kim *et al.*, 2009); 4, *Thalassobacter stenotrophicus* (fatty acids, Macián *et al.*, 2005; polar lipids, Kim *et al.*, 2009); 5, *Jannaschia helgolandensis* (Wagner-Döbler *et al.*, 2003); 6, *Jannaschia donghaensis* (Yoon *et al.*, 2007d); 7, *Roseovarius crassostreae* (Boettcher *et al.*, 2005); 8, *Pseudoruegeria aquimaris* (Yoon *et al.*, 2007c); 9, *Sulfitobacter marinus* (Yoon *et al.*, 2007b). Values for fatty acids are percentages of total fatty acids; −, not detected. Polar lipids are indicated as present (+) or absent (−). ND, No data available.

Component	1	2	3	4	5	6	7	8	9
Fatty acids									
C_{10} 3-OH	2.2	3.2	3.7	3.2	5.6	1.5	2.1±0.3	2.9	3.6
$C_{12:1}$	3.2	3.0	−	−	4.9	−	−	−	−
$C_{16:0}$	10.3	11.9	10.4	0.4	−	−	3.6±0.9	1.4	8.3
$C_{17:0}$	1.0	−	−	0.3	1.0	1.8	−	0.9	0.7
$C_{18:2}$	2.3	2.8	−	−	−	−	−	−	−
$C_{18:1}\omega7c$	77.1	65.2	74.3	78.1	45.1	69.2	85.2±1.7	72.9	77.1
11-Methyl $C_{18:1}\omega7c$	−	−	5.9	7.0	5.7	6.9	0.8±0.2	2.8	6.9
$C_{18:0}$	1.0	1.4	1.2	2.6	10.8	5.2	0.7±0.3	6.6	0.6
$C_{19:1}$	1.3	10.5	−	−	−	−	−	−	−
$C_{19:0}$	−	−	−	−	−	5.9	−	−	−
$C_{19:1}/C_{19:0}$ cyclo	−	−	−	1.5	2.9	−	−	−	−
$C_{19:0}$ cyclo $\omega8c$	−	−	−	−	−	25.1	−	5.9	−
$C_{20:1}\omega7c$	−	−	−	1.0	−	2.8	−	0.9	−
Unknown ECL 11.799	−	−	3.0	3.6	−	2.1	−	2.8	−
Polar lipids									
Phosphatidylcholine	+	+	+	+	+	+	ND	−	ND
Phosphatidylethanolamine	+	+	+	+	+	+	ND	+	ND
Phosphatidylglycerol	+	+	+	+	+	+	ND	+	ND
Diphosphatidylglycerol	−	+	+	+	+	−	ND	+	ND
Unidentified components									
Aminophospholipid(s)	+	+	−	−	−	−	ND	−	ND
Aminolipid(s)	−	−	−	−	+	−	ND	−	ND
Phospholipid(s)	−	−	−	+	−	−	ND	+	ND
Lipid(s)	+	−	−	−	−	−	ND	−	ND
Glycolipid(s)	−	−	−	−	−	+	ND	+	ND

catalase-positive, non-fermentative, heterotrophic, non-motile and rod-shaped bacteria that were enlarged at one pole, indicating cell division by budding (Supplementary Fig. S3). Strains KMM 3842^T and KMM 3851^T differed from each other in colony pigmentation, as they formed greyish-violet and whitish colonies, respectively, when grown on MA at 4–30 °C. However, strain KMM 3851^T was able to produce diffusible greenish-violet pigment during cultivation at 35 °C on MA and other media. In addition, the strains could be distinguished by growth in 8 % NaCl, hydrolysis of Tween 80 and L-tyrosine and H_2S production (Table 1). They also yielded different results in carbon source assimilation tests (API 32GN): strain KMM 3851^T assimilated acetate, D-mannitol, melibiose, propionic acid, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline, whereas KMM 3842^T could not assimilate any compounds that were included on the API 32GN strip.

The major isoprenoid quinone was determined to be Q-10 in both strains. Polar lipids of both strains consisted of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminophospholipid. In addition, strain KMM 3851^T contained two unknown lipids, whereas KMM 3842^T contained diphosphatidylglycerol (Supplementary Fig. S2). Fatty acid analysis revealed $C_{18:1}\omega7c$ and $C_{16:0}$ as dominant components. The chemotaxonomic properties of strains KMM 3851^T and KMM 3842^T (ubiquinone Q-10, the predominance of $C_{18:1}\omega7c$ and the presence of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol) are similar to those reported for phylogenetically related genera of the *Roseobacter* clade. At the same time, strains KMM 3851^T and KMM 3842^T differed substantially from their phylogenetic relatives in the presence of an unknown aminophospholipid and the absence of 11-methyl $C_{18:1}\omega7c$, $C_{20:1}\omega7c$ and $C_{19:0}$ cyclo in their fatty acid and polar lipid profiles (Table 2).

The DNA G+C contents determined for strains KMM 3851^T and KMM 3842^T (60.4 and 58.5 mol%, respectively) were close to the values reported for *Thalassobacter*

stenotrophicus and *Roseovarius crassostreae* (both 59 mol%) and *Sulfitobacter marinus* (57.8 mol%) and slightly higher than the value of 56 mol% reported for *Thalassobacter arenae*, but were clearly distinct from values reported for *Jannaschia* species (63.0–65.2 mol%) and *Pseudoruegeria aquimaris* (67.0 mol%) (Table 1).

The phylogenetic distinctiveness found for strains KMM 3851^T and KMM 3842^T was supported by a combination of phenotypic characteristics that allowed them to be differentiated from closely related bacteria. Both isolates could be distinguished from their closest relative *Thalassobacter arenae* KACC 12675^T in morphological characteristics, including cell dimensions (0.4–0.6 μm wide and 1.8–2.0 μm long, against 0.7–1.2 μm wide and 1.2–2.4 μm long for *Thalassobacter arenae*), immobility, the presence of budding cell division, colony pigmentation, growth at 4 and 37 °C and the inability to assimilate D-glucose and most carbohydrates (Table 1) and in fatty acid and polar lipid profiles (Table 2). Based on their distinctive phenotypic characteristics and phylogenetic distance, strains KMM 3851^T and KMM 3842^T are considered to represent a new genus, *Litoreibacter* gen. nov., with two novel species, the type species *Litoreibacter albidus* sp. nov. (type strain KMM 3851^T) and a second species, *Litoreibacter janthinus* sp. nov. (type strain KMM 3842^T).

Description of *Litoreibacter* gen. nov.

Litoreibacter (Li.to.re′i.bac′ter. L. adj. *litoreus* belonging to the seashore; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Litoreibacter* rod from the seashore).

Gram-negative, strictly aerobic, oxidase- and catalase-positive, rod-shaped bacteria that are 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, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, aminophospholipids and unknown lipids. The major fatty acid is $C_{18:1}\omega7c$, followed by $C_{16:0}$. On the basis of 16S rRNA gene sequence analysis, the genus represents a separate branch within the *Alphaproteobacteria*, closely related to the genera *Thalassobacter*, *Jannaschia* and *Pseudoruegeria*. Known strains have been isolated from marine environments. The type species is *Litoreibacter albidus*.

Description of *Litoreibacter albidus* sp. nov.

Litoreibacter albidus (al′bi.dus. L. masc. adj. *albidus* white).

In addition to the properties given in the genus description, the species is characterized as follows. Cells are 0.4–0.6 μm in diameter and 1.8–2.0 μm long. Non-motile. Produces whitish-pigmented, smooth, shiny colonies with regular edges, 2–3 mm in diameter, on MA. Greenish-violet diffusible pigment is observed during cultivation at 35 °C. Bchl *a* is not produced. Requires NaCl for growth; growth occurs at 0.5–8 % NaCl (w/v) and is optimal in 2–3 % NaCl (w/v). Grows in/on basal medium containing NaCl alone without any of the sea salts components $MgCl_2$, KCl, $CaCl_2$, $NaNO_3$, K_2HPO_4, KCl, $NaSO_4$, $NaHCO_3$, NaF and $FeSO_4$. Psychrotolerant; the temperature range for growth is 4–37 °C with an optimum at 25–28 °C. No growth is observed above 37 °C. Grows at pH 6.0–10.0 with an optimum at pH 7.5–8.5. Negative for hydrolysis of casein, gelatin, DNA, chitin and starch. Positive for H_2S production and hydrolysis of Tween 80 and L-tyrosine. On L-tyrosine-containing medium, melanin-like pigments are not produced but a clearance zone is formed. No acid production is observed from D-glucose, maltose, sucrose, lactose, D-galactose, D-mannose, cellobiose, D-xylose, L-arabinose, L-rhamnose, D-sorbitol or D-mannitol. In API 20NE tests, positive for β-galactosidase (PNPG test) and aesculin hydrolysis and 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. In ID32 GN tests, assimilates sodium acetate, D-mannitol, melibiose, propionic acid, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline and does not assimilate L-rhamnose, *N*-acetylglucosamine, D-ribose, inositol, sucrose, maltose, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-glucose, salicin, L-fucose, D-sorbitol, L-arabinose, capric acid, valeric acid, trisodium citrate, L-histidine or 4-hydroxybenzoic acid. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. The detailed compositions of fatty acids and polar lipids are given in Table 2. Susceptible to (μg per disc unless indicated) ampicillin (10), benzylpenicillin (10 U), carbenicillin (100), gentamicin (10), rifampicin (5), streptomycin (30), vancomycin (30), kanamycin (30), nalidixic acid (30), ofloxacin (5), polymyxin (300 U), oxacillin (10), erythromycin (15), cephazolin (30), cephalexin (30), tetracycline (30) and chloramphenicol (30) and resistant to lincomycin (15), neomycin (30) and oleandomycin (15). The DNA G+C content of the type strain is 60.4 mol% (thermal denaturation method).

The type strain is KMM 3851^T (=NRIC 0773^T =JCM 16493^T), isolated from the internal tissues of a marine snail specimen (*Umbonium costatum*) collected from shallow sediments of the Sea of Japan, Russia.

Description of *Litoreibacter janthinus* sp. nov.

Litoreibacter janthinus (jan′thi.nus. L. masc. adj. *janthinus* violet–blue).

In addition to the properties given in the genus description, the species is characterized as follows. Cells are 0.4–0.6 μm in diameter and 1.8–2.0 μm long. Non-motile. Produces greyish-violet-pigmented colonies with regular edges, 2–3 mm in diameter, on MA. Bchl *a* is not produced. Requires NaCl for growth; growth occurs at 0.5–6 % NaCl (w/v) and is optimal in 2–3 % NaCl (w/v). Grows in/on basal medium containing NaCl alone without any of the sea salts components $MgCl_2$, KCl, $CaCl_2$, $NaNO_3$, K_2HPO_4, KCl, $NaSO_4$, $NaHCO_3$, NaF and $FeSO_4$. Psychrotolerant; temperature range for growth is 4–37 °C, with an optimum at 25–28 °C. No growth is observed above 37 °C. Grows at pH 6.0–10.0, with an optimum at pH 7.0–8.5. Negative for H_2S production and hydrolysis of casein, gelatin, DNA, Tween 80, chitin, starch and L-tyrosine. On L-tyrosine-containing medium, melanin-like pigments and clearance zones are not produced. No acid production from D-glucose, maltose, sucrose, lactose, D-galactose, D-mannose, cellobiose, D-xylose, L-arabinose, L-rhamnose, D-sorbitol or D-mannitol. In API 20NE tests, positive for gelatin hydrolysis and β-galactosidase (PNPG test) and negative for nitrate reduction, indole production, glucose acidification under anaerobic conditions, arginine dihydrolase, urease production, gelatin hydrolysis and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate. In ID32 GN tests, does not assimilate L-rhamnose, *N*-acetylglucosamine, D-ribose, inositol, sucrose, maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicin, melibiose, L-fucose, D-sorbitol, L-arabinose, propionic acid, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline. In API ZYM tests, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, α-glucosidase and β-glucosidase, weakly positive for naphthol-AS-BI-phosphohydrolase and negative for esterase (C4), lipase (C14), trypsin, α-chymotrypsin, cystine arylamidase, *N*-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. Susceptible to (μg per disc unless indicated) ampicillin (10), benzylpenicillin (10 U), carbenicillin (100), gentamicin (10), lincomycin (15), oleandomycin (15), rifampicin (5), streptomycin (30), vancomycin (30), kanamycin (30), nalidixic acid (30), neomycin (30), ofloxacin (5), polymyxin (300 U), erythromycin (15), cephazolin (30), cephalexin (30), chloramphenicol (30), oxacillin (10) and tetracycline (30). Detailed fatty acid and polar lipid compositions are listed in Table 2. The DNA G+C content of the type strain is 58.5 mol% (thermal denaturation method).

The type strain, KMM 3842^T (=NRIC 0772^T =JCM 16492^T), was isolated from a shallow sediment sample collected from the Sea of Japan, Russia.

Acknowledgements

This study was supported by a grant from the Presidium of RAS 'Molecular and Cell Biology', by the Federal Agency for Science and Innovations of the Russian Federation (State Contract 02.518.11.7169) and by a grant from Presidium Far-Eastern Branch of Russian Academy of Sciences (09-III-A-06_227).

References

Arahal, D. R., Macián, M. C., Garay, E. & Pujalte, M. J. (2005). *Thalassobius mediterraneus* gen. nov., sp. nov., and reclassification of *Ruegeria gelatinovorans* as *Thalassobius gelatinovorans* comb. nov. *Int J Syst Evol Microbiol* **55**, 2371–2376.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.

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.

Buchan, A., Gonzalez, 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.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2005). Order III. *Rhodobacterales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2C, p. 161. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2006). Order III. *Rhodobacterales* ord. nov. In *List of New Names and New Combinations Previously Effectively, but not Validly, Published*, Validation list no. 107. *Int J Syst Evol Microbiol* **56**, 1–6.

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.

Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 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.

Owen, R. J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base composition 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.

Pujalte, M. J., Macián, M. C., Arahal, D. R. & Garay, E. (2005). *Thalassobacter stenotrophicus* Macián *et al.* 2005 is a later synonym of *Jannaschia cystaugens* Adachi *et al.* 2004, with emended description of the genus *Thalassobacter*. *Int J Syst Evol Microbiol* **55**, 1959–1963.

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.

Ruiz-Ponte, C., Cilia, V., Lambert, C. & Nicolas, J. L. (1998). *Roseobacter gallaeciensis* sp. nov., a new marine bacterium isolated from rearings and collectors of the scallop *Pecten maximus*. *Int J Syst Bacteriol* **48**, 537–542.

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). MEGA 4: 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.

Uchino, Y., Hirata, A., Yokota, A. & Sugiyama, J. (1998). Reclassification of marine *Agrobacterium* species: proposals of *Stappia stellulata* gen. nov., comb. nov., *Stappia aggregata* sp. nov., nom. rev., *Ruegeria atlantica* gen. nov., comb. nov., *Ruegeria gelatinovora* comb. nov., *Ruegeria algicola* comb. nov., and *Ahrensia kieliense* gen. nov., sp. nov., nom. rev. *J Gen Appl Microbiol* **44**, 201–210.

Uchino, Y., Hirata, A., Yokota, A. & Sugiyama, J. (1999). *Ruegeria algicola* comb. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 68. *Int J Syst Bacteriol* **49**, 1–3.

Vaskovsky, V. E. & Terekhova, T. A. (1979). HPTLC of phospholipid mixtures containing phosphatidylglycerol. *J High Resol Chromatogr Chromatogr Comm* **2**, 671–672.

Wagner-Döbler, I., Rheims, H., Felske, A., Pukall, R. & Tindall, B. J. (2003). *Jannaschia helgolandensis* gen. nov., sp. nov., a novel abundant member of the marine *Roseobacter* clade from the North Sea. *Int J Syst Evol Microbiol* **53**, 731–738.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

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.

Yoon, J. H., Kang, S. J., Park, S. & Oh, T. K. (2007d). *Jannaschia donghaensis* sp. nov., isolated from seawater of the East Sea, Korea. *Int J Syst Evol Microbiol* **57**, 2132–2136.