Salinimicrobium marinum sp. nov., a halophilic bacterium of the family *Flavobacteriaceae*, and emended descriptions of the genus *Salinimicrobium* and *Salinimicrobium catena*

Olga I. Nedashkovskaya,[1] Marc Vancanneyt,[2] Seung Bum Kim,[3] Jihye Han,[3] Natalia V. Zhukova[4] and Lyudmila S. Shevchenko[1]

Correspondence
Olga I. Nedashkovskaya
olganedashkovska@yahoo.com

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

[2]BCCM/LMG Bacteria Collection, and Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

[3]Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

[4]Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, Pal'chevskogo St 17, 690032, Vladivostok, Russia

Two novel heterotrophic, facultatively anaerobic, gliding and yellow-pigmented bacteria, designated strains KMM 6270^T and KMM 6320, were isolated from different marine environments and studied using a polyphasic taxonomic approach. 16S rRNA gene sequence analysis placed the strains within the family *Flavobacteriaceae*. Strains KMM 6270^T and KMM 6320 were most closely related to the type strains of recognized species of the genus *Salinimicrobium* (95.0–96.6 % 16S rRNA gene sequence similarity). The G+C content of the genomic DNA was 40–41 mol%. The strains grew with 0.5–15 % (w/v) NaCl (optimum 4 % NaCl) and at 4–41 °C (optimum 28–32 °C). Aesculin and gelatin were hydrolysed, but agar, casein, DNA and chitin were not. The phylogenetic data taken together with the results of the genotypic and phenotypic studies permit the classification of strains KMM 6270^T and KMM 6320 as members of a novel species of the genus *Salinimicrobium*, for which the name *Salinimicrobium marinum* sp. nov. is proposed. The type strain is KMM 6270^T (=KCTC 12719^T=LMG 25395^T).

The genus *Salinimicrobium*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), was proposed for the reclassification of the species *Salegentibacter catena* (Ying *et al.*, 2007) and the accommodation of newly isolated heterotrophic, facultatively anaerobic, yellow-pigmented, non-gliding and oxidase-negative bacteria in a novel species, *Salinimicrobium xinjiangense* (Lim *et al.*, 2008). Since then, the genus description has been emended because of additional phenotypic findings (Chen *et al.*, 2008). At the time of writing, the genus *Salinimicrobium* comprised three recognized species: *Salinimicrobium catena*, isolated from sediment collected from Xijiang oilfield in the South China Sea (Ying *et al.*, 2007; Lim *et al.*, 2008) and *S. xinjiangense* and *Salinimicrobium terrae*, isolated from soil samples of the salt lakes in China (Chen *et al.*, 2008; Lim *et al.*, 2008).

The present study examined the taxonomic position of two heterotrophic, facultatively anaerobic, motile by gliding, yellow-pigmented and Gram-negative bacterial strains, designated KMM 6270^T and KMM 6320, that were isolated from different marine environments. Strain KMM 6270^T was isolated from a sediment sample collected at a depth of 210 m in Rudnaya Bay, the East Sea (also known as the Sea of Japan) during cruise 29 of R/V *Academician Oparin*. Strain KMM 6320 was isolated from an unidentified ascidian collected at a depth of 39 m in Aniva Bay, the Okhotsk Sea, during cruise 31 of R/V *Academician Oparin*. Both strains were isolated by the direct plating technique on a medium containing (per litre natural seawater/distilled water, 50 : 50) 5.0 g Bacto peptone, 1.0 g glucose, 2.5 g yeast extract (Difco), 0.1 g KH_2PO_4, 0.1 g $MgSO_4$ and 15.0 g Bacto agar. After primary isolation and purification, the strains were cultivated at 28 °C on the same medium or on marine agar 2216 (Difco; MA) and stored at −80 °C in marine broth 2216 (Difco) supplemented with 20 % (v/v) glycerol.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KMM 6270^T and KMM 6320 are GQ866112 and GQ866113, respectively.

Downloaded from www.microbiologyresearch.org by
IP: 193.191.134.1
On: Thu, 09 Jun 2016 12:10:24

The almost-complete 16S rRNA gene sequences (1474 nt) of strains KMM 6270^T and KMM 6320 were determined by following the procedure described previously (Vancanneyt *et al.*, 2004). The sequence data were aligned with those of representative members of the family *Flavobacteriaceae* retrieved from GenBank and the construction of a neighbour-joining (Saitou & Nei, 1987) phylogenetic tree and bootstrap analysis were performed as described by Cho *et al.* (2006).

Phylogenetic analysis revealed that strains KMM 6270^T and KMM 6320 formed a distinct lineage within the genus *Salinimicrobium* and exhibited 95.0–96.6 % 16S rRNA gene sequence similarity with the type strains of species of the genus *Salinimicrobium* (Fig. 1). *S. xinjiangense* BH206^T was the closest neighbour (96.6 %). Based on the results of the phylogenetic analysis, strains KMM 6270^T and KMM 6320 may be considered as representatives of a novel species of the genus *Salinimicrobium*. The 16S rRNA gene sequence similarity between strains KMM 6270^T and KMM 6320 was 100 %.

DNA was isolated following the method of Marmur (1961) and the DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The G+C contents of the genomic DNA of strains KMM 6270^T and KMM 6320 were 40.9 and 40.8 mol%, respectively.

DNA–DNA hybridization was measured spectrophotometrically and initial renaturation rates were recorded as described by De Ley *et al.* (1970). DNA–DNA relatedness between strains KMM 6270^T and KMM 6320 was 99 %. According to the recommendations of Wayne *et al.* (1987), this value permits the classification of the two isolates as members of the same species.

For the determination of the whole-cell fatty acid profile, strain KMM 6270^T was grown at 25 °C for 48 h on MA and the fatty acid methyl esters were extracted and analysed as described previously (Nedashkovskaya *et al.*, 2006). The cellular fatty acids of strain KMM 6270^T (>1 % of the total) were iso-$C_{15:0}$ (26.0 %), anteiso-$C_{15:0}$ (24.6 %), $C_{15:0}$ (9.3 %), iso-$C_{16:0}$ (7.6 %), $C_{16:1}\omega7c$ (6.2 %), iso-$C_{17:1}$ (5.7 %), iso-$C_{17:0}$ 3-OH (3.1 %), $C_{15:1}\omega6c$ (2.9 %), anteiso-$C_{16:1}$ (2.7 %), $C_{14:0}$ 3-OH (2.2 %), iso-$C_{14:0}$ (1.7 %), iso-$C_{16:0}$ 3-OH (1.3 %), $C_{17:0}$ (1.2 %) and $C_{17:0}$ 3-OH (1.2 %). Isoprenoid quinones were extracted and analysed using a standard procedure (Minnikin *et al.*, 1984). The major respiratory quinone of strain KMM 6270^T was MK-6. These chemotaxonomic data were consistent with data obtained for other members of the genus *Salinimicrobium* (Chen *et al.*, 2008; Lim *et al.*, 2008; Ying *et al.*, 2007).

Phenotypic analysis was performed by using previously described methods (Nedashkovskaya *et al.*, 2004) and the API 20E, API 20NE and API ZYM galleries (bioMérieux), according to the manufacturer's instructions except that the galleries were incubated at 28 °C. Strains KMM 6270^T and KMM 6320 were aerobic, yellow-pigmented, motile by gliding and Gram-negative. Their main physiological and biochemical characteristics are given in Table 1 and in the species and emended genus descriptions. The two isolates differed from each other in several features: in contrast to strain KMM 6270^T, strain KMM 6320 produced acid from arabinose, glycerol and mannitol, hydrolysed starch and was susceptible to doxycycline and tetracycline. Similarly to the recognized species of the genus *Salinimicrobium*, strains KMM 6270^T and KMM 6320 produced catalase, fermented D-glucose and hydrolysed aesculin. However, strains KMM 6270^T and KMM 6320 could be clearly distinguished from the recognized species of the genus *Salinimicrobium* by their ability to move by gliding and produce acid from raffinose and sucrose and by the presence of β-galactosidase activity. The phenotypic traits that differentiate strains KMM 6270^T and KMM 6320 from the recognized species of the genus *Salinimicrobium* are given in Table 1.

Phylogenetic analysis based on 16S rRNA gene sequences placed strains KMM 6270^T and KMM 6320 in the family *Flavobacteriaceae*. Strains KMM 6270^T and KMM 6320 were most closely related to the type strains of the recognized species of the genus *Salinimicrobium* and formed a distinct phylogenetic lineage. The phenotypic properties of strains KMM 6270^T and KMM 6320 confirmed that they belonged

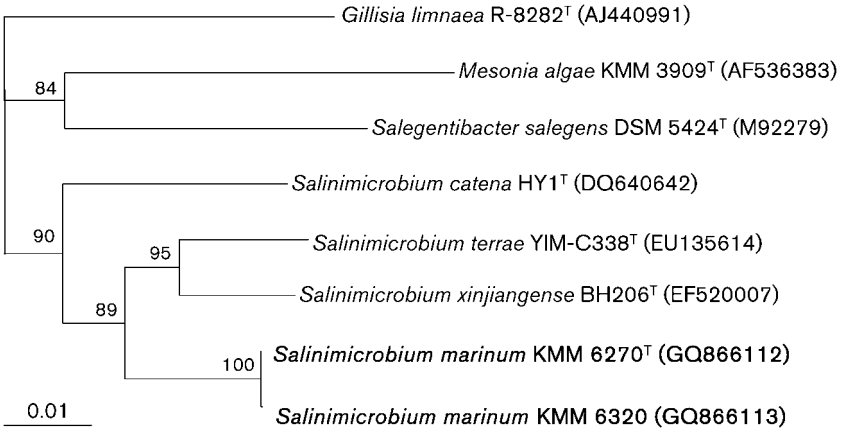

Fig. 1. Neighbour-joining tree of members of the genus *Salinimicrobium* based on Jukes–Cantor distances calculated from 16S rRNA gene sequences. The same tree topology was also obtained with the maximum-likelihood and maximum-parsimony methods. *Gillisia limnaea* R-8282^T, *Mesonia algae* KMM 3909^T and *Salegentibacter salegens* DSM 5424^T were employed as outgroups. Percentages at nodes are bootstrap values calculated from 1000 resamplings. Bar, 0.01 substitutions per nucleotide position.

Downloaded from www.microbiologyresearch.org by
IP: 193.191.134.1
On: Thu, 09 Jun 2016 12:10:24

Table 1. Differential phenotypic characteristics of strains KMM 6270^T and KMM 6320 and the type strains of species of the genus *Salinimicrobium*

Strains: 1, *Salinimicrobium marinum* sp. nov. KMM 6270^T and KMM 6320; 2, *S. catena* HY1^T; 3, *S. terrae* YIM-C338^T; 4, *S. xinjiangense* BH206^T. Data were taken from this study, Chen *et al.* (2008), Lim *et al.* (2008) and Ying *et al.* (2007). All strains were positive for catalase, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), cystine arylamidase, leucine arylamidase, valine arylamidase, α-glucosidase and naphthol-AS-BI-phosphohydrolase activities, hydrolysis of aesculin and utilization of D-glucose. All strains were negative for production of flexirubin-type pigments, hydrolysis of agar, DNA, urea, cellulose and chitin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities and indole production. A, Aerobic; F, facultatively anaerobic; +, positive; −, negative; ND, no data available.

Characteristic	1	2	3	4
Respiration	F	A	A	F
Gliding motility	+	−	−	−
Ranges for growth				
Salinity (%)	0.5–15	0.1–10	0.5–8	0.5–10
Temperature (°C)	4–41	15–42	4–37	10–48
Oxidase	+	−	−	−
Nitrate reduction	+	−	+	−
Production of:				
H_2S	+	+	−	−
Acetoin	+	−	−	ND
Production of acid from:				
Amygdalin	−	ND	+	ND
Fructose	+	−	ND	−
Fucose	+	−	ND	ND
Galactose	+	−	−	+
Glucose	+	+	−	+
Inositol	−	−	+	ND
Lactose	+	−	−	+
Maltose	+	−	+	+
Raffinose	+	−	−	−
Sucrose	+	−	−	−
DL-Xylose	+	−	−	ND
Utilization of:				
Amygdalin	−	ND	+	ND
Arabinose	+	−	+	ND
Inositol	−	−	+	ND
Mannose	+	−	+	+
Sucrose	+	−	+	ND
Hydrolysis of:				
Casein	−	+	−	+
Gelatin	+	+	−	+
Tween 20	−	+	+	ND
Tween 80	−	ND	+	−
Enzyme activity				
α-Chymotrypsin	−	−	−	+
β-Galactosidase	+	−	−	−
N-Acetyl-β-glucosaminidase	+	−	+	+
β-Glucosidase	+	−	+	+
Lipase (C14)	−	+	−	−
Trypsin	−	+	−	−
DNA G+C content (mol%)	40–41	44.4	42.8	42.1

to the genus *Salinimicrobium*. However, because of differences in molecular and phenotypic characteristics, strains KMM 6270^T and KMM 6320 are considered to represent a novel species of the genus *Salinimicrobium*, for which the name *Salinimicrobium marinum* sp. nov. is proposed.

Emended description of the genus *Salinimicrobium* Lim *et al.* 2008 emend. Chen *et al.* 2008

The description of the genus *Salinimicrobium* is as given by Lim *et al.* (2008) and Chen *et al.* (2008) with the following amendments. Some strains can move by gliding and produce cytochrome oxidase.

Description of *Salinimicrobium marinum* sp. nov.

Salinimicrobium marinum (ma.ri′num. L. neut. adj. *marinum* belonging to the sea, marine, isolated from a marine environment).

Cells are Gram-negative, 0.5–1.0 μm in width and 1.2–4.0 μm in length, and move by gliding. On marine agar, colonies are circular, convex, smooth with entire edges, yellow-pigmented and grow to 2–3 mm in diameter after 72 h at 25 °C. Requires Na^+ ions for growth. Grows at 4–41 °C (optimum 28–32 °C) and with 0.5–15 % (w/v) NaCl (optimum 4 %). Ferments D-glucose. Does not produce flexirubin-type pigments. Oxidase- and catalase-positive. Nitrate is reduced to nitrite. Arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase and tryptophan deaminase activities are absent. Hydrolyses aesculin and gelatin, but not agar, casein, DNA, Tweens 20 and 80, cellulose (CM-cellulose and filter paper), chitin or urea. Hydrolysis of starch and Tween 40 is strain-dependent. Produces acid from cellobiose, fructose, L-fucose, galactose, D-glucose, lactose, maltose, raffinose, sucrose and xylose, but not from melibiose, L-rhamnose, trehalose, *N*-acetyl-D-glucosamine, inositol or sorbitol. Acid production from L-arabinose, glycerol and mannitol is strain-dependent. Utilizes arabinose, mannose, sucrose and citrate, but not gluconate, caprate, adipate, malate, phenylacetate, inositol or sorbitol. Utilization of mannitol is strain-dependent. Produces H_2S and acetoin (Voges–Proskauer reaction), but not indole. With API ZYM, positive for esterase (C4), esterase lipase (C8), cystine arylamidase, leucine arylamidase, valine arylamidase, acid phosphatase, alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α- and β-glucosidases and *N*-acetyl-β-glucosidase, but negative for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. Susceptible to ampicillin, benzylpenicillin, carbenicillin, cefalexin, chloramphenicol, erythromycin, lincomycin, ofloxacin, oleandomycin, rifampicin, streptomycin, and vancomycin. Resistant to cefazolin, gentamicin, kanamycin, nalidixic acid, neomycin, oxacillin and polymyxin. Susceptibility to doxycycline and tetracycline is strain-dependent. The predominant fatty acids (>5 %) are iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{15:0}$, iso-$C_{16:0}$, $C_{16:1}\omega7c$ and iso-$C_{17:1}$. The major respiratory quinone is MK-6.

Downloaded from www.microbiologyresearch.org by
IP: 193.191.134.1
On: Thu, 09 Jun 2016 12:10:24

The type strain (=KMM 6270^T=KCTC 12719^T=LMG 25395^T) was isolated from a sediment collected from Rudnaya Bay, the East Sea (also known as the Sea of Japan). The DNA G+C content of the type strain is 40.9 mol%.

Emended description of *Salinimicrobium catena* Lim *et al.* 2008

The description of the species is as given by Ying *et al.* (2007) and Lim *et al.* (2008) with the following amendments. Produces cytochrome oxidase. Does not produce acid from cellobiose, fructose, galactose, ribose, glycerol, mannitol or *N*-acetylglucosamine.

Acknowledgements

This research was supported by grants from Russian Foundation for Basic Research (no. 08-04-00099), Presidium of the Russian Academy of Sciences 'Molecular and Cell Biology' and Federal Agency for Science and Innovations of the Russian Federation (state contract no. 02.518.11.7169).

References

Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

Chen, Y.-G., Cui, X.-L., Zhang, Y.-Q., Li, W.-J., Wang, Y.-X., Kim, C.-J., Lim, J.-M., Xu, L.-H. & Jiang, C.-L. (2008). *Salinimicrobium terrae* sp. nov., isolated from saline soil, and emended description of the genus *Salinimicrobium*. *Int J Syst Evol Microbiol* **58**, 2501–2504.

Cho, S. H., Han, J. H., Seong, C. N. & Kim, S. B. (2006). Phylogenetic diversity of acidophilic sporoactinobacteria isolated from various soils. *J Microbiol* **44**, 600–606.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Lim, J.-M., Jeon, C.O., Lee, S.S., Park, D.-J., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2008). Reclassification of *Salegentibacter catena* Ying *et al.* 2007 as *Salinimicrobium catena* gen. nov., comb. nov. and description of *Salinimicrobium xinjiangense* sp. nov., a halophilic bacterium isolated from Xinjiang province in China. *Int J Syst Evol Microbiol* **58**, 438–442.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

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

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

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Rhee, M. S., Lysenko, A. M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2004). *Ulvibacter litoralis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 119–123.

Nedashkovskaya, O. I., Kim, S. B., Zhukova, N. V., Kwak, J., Mikhailov, V. V. & Bae, K. S. (2006). *Mesonia mobilis* sp. nov., isolated from seawater, and emended description of the genus *Mesonia*. *Int J Syst Evol Microbiol* **56**, 2433–2436.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Vancanneyt, M., Mengaud, J., Cleenwerck, I., Hoste, B., Dawyndt, P., Degivry, M. C., Ringuet, D., Janssens, D. & Swings, J. (2004). Reclassification of *Lactobacillus kefirgranum* Takizawa *et al.* 1994 as *Lactobacillus kefiranofaciens* subsp. *kefirgranum* subsp. nov., and emended description of *L. kefiranofaciens* Fujisawa *et al.* 1988. *Int J Syst Evol Microbiol* **54**, 551–556.

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.

Ying, J.-Y., Liu, Z.-P., Wang, B.-J., Dai, X., Yang, S.-S. & Liu, S.-J. (2007). *Salegentibacter catena* sp. nov., and emended description of the genus *Salegentibacter*. *Int J Syst Evol Microbiol* **57**, 219–222.

Downloaded from www.microbiologyresearch.org by
IP: 193.191.134.1
On: Thu, 09 Jun 2016 12:10:24