

Mariniflexile gromovii gen. nov., sp. nov., a gliding bacterium isolated from the sea urchin *Strongylocentrotus intermedius*

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A marine bacterium, designated strain KMM 6038^T, was subjected to taxonomic analysis via a polyphasic approach. Cells of the strain were heterotrophic, orange-pigmented, Gram-negative and motile by means of gliding. 16S rRNA gene sequence analysis indicated that strain KMM 6038^T was closely related to the type species of the genera *Algibacter* and *Yeosuana*, members of the family *Flavobacteriaceae*, with sequence similarities of 93.8 and 93.6% to the respective type strains. However, several chemotaxonomic and phenotypic characteristics, such as the cellular fatty acid profile (iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{15:1}, C_{15:0}, C_{15:1}ω6c, iso-C_{15:0} 3-OH and iso-C_{17:0} 3-OH) and the low G + C content of the DNA (35.7 mol%), indicated that the strain should be separated from these two genera. From the results of phenotypic, genotypic, chemotaxonomic and phylogenetic analyses, the bacterium should be classified as representing a novel genus and species, for which the name *Mariniflexile gromovii* gen. nov., sp. nov. is proposed. The type strain of *Mariniflexile gromovii* is KMM 6038^T (=KCTC 12570^T =LMG 22578^T).

In the course of a study of a microbial population of the sea urchin *Strongylocentrotus intermedius*, a common echinoderm from the north-western Pacific Ocean, a heterotrophic, Gram-negative, gliding and orange-pigmented marine bacterium was recovered. Based on a polyphasic study of the isolate, designated KMM 6038^T, which included phylogenetic, genotypic, chemotaxonomic and phenotypic approaches, the erection of a new genus within the family *Flavobacteriaceae* is proposed.

Strain KMM 6038^T was isolated from the sea urchin *S. intermedius* inhabiting Troitsa Bay, Gulf of Peter the Great, East Sea (also known as the Sea of Japan), in September 2002. For strain isolation, 0.1 ml aliquots of homogenates of sea urchin tissues were transferred onto plates of marine agar 2216 (Difco). After primary isolation and purification, strains were cultivated at 28 °C on the same medium and stored at –80 °C in marine broth (Difco) supplemented with 20% (v/v) glycerol.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed the procedures given by Han *et al.*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Mariniflexile gromovii* KMM 6038^T is DQ312294.

(2003). The sequences obtained were aligned with those of representative members of selected genera belonging to the family *Flavobacteriaceae* by using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated using the Kimura two-parameter model (Kimura, 1980) and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of strain KMM 6038^T (1378 nt) revealed that the strain formed a distinct lineage within the family *Flavobacteriaceae* (Fig. 1). The strain was clustered with *Yuosuana aromatorans* GW1-1^T in the neighbour-joining tree shown, a relationship that was also supported by the maximum-likelihood tree. However, the level of bootstrap support was only 52%, indicating that the topology of tree was not stable. Strain KMM 6038^T showed 16S rRNA gene sequence similarities of 93.6 and 93.8% to *Y. aromatorans* GW1-1^T

Table 1. Fatty acid composition of *Mariniflexile gromovii* gen. nov., sp. nov. KMM 6038^T and its close relatives

Taxa: 1, *M. gromovii* KMM 6038^T; 2, *Yeosuana aromativorans*; 3, *Algibacter lectus*; 4, *Formosa*; 5, *Gelidibacter*; 6, *Psychroserpens burtonensis*; 7, *Winogradskyella*. Those fatty acids accounting for <1% of the total for all taxa are not given. Data for reference taxa are taken from Bowman *et al.* (1997), Macián *et al.* (2002), Nedashkovskaya *et al.* (2004, 2005, 2006), Bowman & Nichols (2005) and Kwon *et al.* (2006).

Fatty acid	1	2	3	4	5	6	7
iso-C _{14:0}							1.4–4.5
iso-C _{14:1}							0–1.4
iso-C _{15:0}	15.0	21.7	12.5	12.7–17.2	3.4–8.8	10.0	6.7–25.6
anteiso-C _{15:0}	5.4	14.9	7.2	1.6–4.7	10.5–17.7	10.4	4.9–15.9
iso-C _{15:1}	16.9	14.8	13.4	6.5–11.4	5.3–11.4	14.1	8.1–11.4
anteiso-C _{15:1}	1.6			0–1.0	11.8–16.6	8.4	1.4–6.3
C _{15:0}	13.8	5.3	13.4	8.7–15.5	2.4–5.3	10.1	1.2–7.9
C _{15:1}	3.1		10.9	6–11.8	2.7–4.2	17.5	0–6.5
iso-C _{16:0}	1.0			1.1–2.1	1.4–4.4		0.8–5.7
iso-C _{16:1}	1.5			0–2.5	1.4–10.3	9.1	2.7–4.7
C _{16:0} 10-methyl		4.7		1.1–1.6			0–6.3
C _{16:0}	1.4						
C _{16:1} ω5c						6.9	
C _{16:1} ω7/iso-C _{15:0} 2-OH	8.4	10.9/–		5.9–15.8	7.4/–		4.2–6.1
iso-C _{17:1}				0–1.4		1.5	0.6–1.1
anteiso-C _{17:1}					1.9–3.4		0–2.3
anteiso-C _{17:0}		4.2					
C _{17:0} cyclo							0–2.4
C _{17:1}	1.1			0–3.5	3.7	2.7	0.9–1.9
iso-C _{14:0} 3-OH							0.9–1.6
C _{15:0} 2-OH	1.4			1.5–1.8			1.0–3.3
iso-C _{15:0} 3-OH	4.3		9.4	6.7–10.5	2.2–6.2		2.6–11.9
C _{15:0} 3-OH	1.6			2.3–4.0			0–2.5
iso-C _{16:0} 3-OH	2.5			3.1–8.9	4.1–12.2		3.2–18.1
C _{16:0} 3-OH	1.1			0–2.1			0–1.0
iso-C _{17:0} 3-OH	8.9		9.1	8.5–10.7	0–3.1		5.4–7.3
C _{17:0} 2-OH				0–1.5			0.8–5.2

In addition to characteristics given for the genus, cells range from 0.4 to 0.5 µm in width and from 2 to 3 µm in length. Colonies are circular, 2–3 mm in diameter, convex, shiny, orange and translucent on marine agar. Does not require Na⁺ ions for growth. Growth occurs at 4–37 °C. Optimal temperature for growth is 23–25 °C. Growth occurs with 0–6 % NaCl. Decomposes gelatin. Does not hydrolyse agar, casein, starch, Tweens 20, 40 or 80, urea, cellulose (CM-cellulose and filter paper), chitin or DNA. Forms acid from L-fucose and DL-xylose, but not from L-arabinose, D-cellobiose, D-galactose, D-glucose, D-maltose, sucrose, D-lactose, D-melibiose, L-raffinose, glycerol, inositol or mannitol. Oxidizes L-rhamnose and *N*-acetylglucosamine. Utilizes D-lactose, D-mannose and sucrose, but not L-arabinose, adonitol, dulcitol, mannitol, inositol, sorbitol, malonate or citrate. Nitrate is not reduced. Indole, H₂S and acetoin (Voges–Proskauer reaction) production are negative.

Susceptible to ampicillin, carbenicillin, lincomycin and tetracycline. Resistant to benzylpenicillin, gentamicin, kanamycin, neomycin, oleandomycin, polymyxin B and streptomycin. Cellular fatty acids accounting for more than 1.0% of the total are iso-C_{15:1} (16.9%), anteiso-C_{15:1} (1.6%), iso-C_{15:0} (15.0%), anteiso-C_{15:0} (5.4%), C_{15:0} (13.8%), C_{15:1}ω6c (3.1%), iso-C_{15:0} 3-OH (4.3%), C_{15:0} 2-OH (1.4%), C_{15:0} 3-OH (1.6%), iso-C_{16:1} (1.5%), iso-C_{16:0} (1.0%), C_{16:0} (1.4%), iso-C_{16:0} 3-OH (2.5%), C_{16:0} 3-OH (1.1%), C_{17:1}ω6c (1.1%), iso-C_{17:0} 3-OH (8.9%) and summed feature 3 (8.4%; comprising C_{16:1}ω7 and/or iso-C_{15:0} 2-OH). The G + C content of the DNA is 35.7 mol%.

The type strain, KMM 6038^T (=KCTC 12570^T =LMG 22578^T), was isolated from the sea urchin *Strongylocentrotus intermedius*, collected in Troitsa Bay, East Sea (also known as the Sea of Japan).

Table 2. Differential characteristics of *Mariniflexile gromovii* gen. nov., sp. nov. KMM 6038^T and its close relatives within the family *Flavobacteriaceae*

Data are taken from Bowman *et al.* (1997), Macián *et al.* (2002), Bowman & Nichols (2005), Kwon *et al.* (2006), Lau *et al.* (2005), Nedashkovskaya *et al.* (2004, 2005, 2006) and this study. v, Variable. Strain KMM 6038^T and all members of the genera shown are positive for catalase activity.

Characteristic	Strain KMM 6038 ^T	<i>Yeosuana</i>	<i>Algibacter</i>	<i>Formosa</i>	<i>Gelidibacter</i>	<i>Psychroserpens</i>	<i>Winogradskyella</i>
Fermentation of D-glucose	+	–	+	+	–	–	–
Gliding motility	+	–	+	+	+	–	+
Oxidase	+	–	+	+	–	–	+
β-Galactosidase	+	+	+	v	v	v	–
Salinity range for growth (% v/v)	0–6	0.5–3.0	1–6	0–8	0–8	2.4–8	1–8
Temperature range for growth (°C)	4–37	23–39	4–35	4–34	–2 to 37	0–19	4–44
Requirement for Ca ²⁺ and Mg ²⁺ for growth	–	+	–	–	–	–	–
Acid formation from carbohydrates	+	–	+	+	+	–	v
Hydrolysis of:							
Agar	–	–	+	v	–	–	v
Casein	–	–	–	–	v	+	v
Starch	–	–	+	v	v	–	v
DNA G+C content (mol%)	35–36	51–52	31–33	34–36	36–42	27–29	32–37

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References

- Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposal minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.
- Bowman, J. P. & Nichols, D. S. (2005). Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. *Int J Syst Evol Microbiol* **55**, 1471–1486.
- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- Felsenstein, J. (1993). PHYLIP – Phylogeny Inference Package, version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.
- Han, S. K., Nedashkovskaya, O. I., Mikhailov, V. V., Kim, S. B. & Bae, K. S. (2003). *Salinibacterium amurskyense* gen. nov., sp. nov., a novel genus of the family *Microbacteriaceae* from the marine environment. *Int J Syst Evol Microbiol* **53**, 2061–2066.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kwon, K. K., Lee, H.-S., Jung, H.-B., Kang, J.-H. & Kim, S.-J. (2006). *Yeosuana aromativorans* gen. nov., sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae*, isolated from estuarine sediment of the South Sea, Korea. *Int J Syst Evol Microbiol* **56**, 727–732.
- Lau, S. C. K., Tsoi, M. M. Y., Li, X. & 7 other authors (2005). *Winogradskyella poriferorum* sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas. *Int J Syst Evol Microbiol* **55**, 1589–1592.
- Macián, M. C., Pujalte, M. J., Márquez, M. C., Ludwig, W., Ventosa, A., Garay, E. & Schleifer, K. H. (2002). *Gelidibacter mesophilus* sp. nov., a novel marine bacterium in the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **52**, 1325–1329.
- 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.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K. & 7 other authors (2004). *Algibacter lectus* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from green algae. *Int J Syst Evol Microbiol* **54**, 1257–1261.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K. & 9 other authors (2005). *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **55**, 49–55.
- Nedashkovskaya, O. I., Kim, S. B., Vancanneyt, M. & 9 other authors (2006). *Formosa agariphila* sp. nov., a budding bacterium of the family *Flavobacteriaceae* isolated from marine environments, and emended description of the genus *Formosa*. *Int J Syst Evol Microbiol* **56**, 161–167.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.