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 aromativorans* 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. aromativorans* GW1-1^T

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of strain KMM 6038^T and representative members of the family *Flavobacteriaceae*, constructed by the neighbour-joining method. Asterisks indicate branches that were also recovered by using the maximum-likelihood algorithm. Numbers at nodes indicate bootstrap values (%) from 1000 resamplings. Bar, 0.01 substitutions per nucleotide position.

and *Algibacter lectus* KMM 3902^T, respectively, and less than 93.1% to other members of the family *Flavobacteriaceae*. *A. lectus* KMM 3902^T shared higher sequence similarity with KMM 6038^T than *Y. aromativorans* GW1-1^T but formed a separate phylogenetic cluster, given that *A. lectus* KMM 3902^T also shared high sequence similarities with other strains such as *Formosa algae* KMM 3553^T (95.1%). *Y. aromativorans* GW1-1^T shared 93.6% sequence similarity with *Gelidibacter algens* ACAM 536^T. Therefore, it is evident from the phylogenetic analysis that KMM 6038^T is not the closest neighbour of either *A. lectus* or *Y. aromativorans*.

DNA was isolated following the method of Marmur (1961) and the G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G+C content of strain KMM 6038^T was 35.7 mol%.

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID), except that marine agar 2216 was used to obtain cell biomass. The dominant fatty acids are detailed in the species description and in Table 1. Isoprenoid quinones were extracted from lyophilized cells and analysed as described previously (Nedashkovskaya *et al.*, 2004); the major respiratory quinone was MK-6. These results are consistent with those obtained previously for members of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002).

Phenotypic analysis was performed by using our previously described methods (Nedashkovskaya *et al.*, 2004).

Cells of strain KMM 6038^T were Gram-negative, chemo-organotrophic and motile by means of gliding. The isolate did not require Na⁺ ions or seawater for growth, which occurred in media that contained 0–6% NaCl, with optimum growth at 1–2%. The maximum growth temperature of strain KMM 6038^T was 37 °C, with optimum growth occurring at 23–25 °C. The pH range for growth was 5.5–10.0, with optimum growth occurring between pH 7.5 and 8.3. Other physiological and biochemical characteristics of the strain are listed in the species description and in Table 2. Strain KMM 6038^T has several characteristics that help to differentiate it from its nearest neighbours, *Y. aromativorans* and *A. lectus* (Table 2). Phenotypic features such as the ability to move by means of gliding, to ferment D-glucose, to produce oxidase and to form acids from carbohydrates, together with a lower DNA G+C content, strongly support the differentiation of strain KMM 6038^T from *Y. aromativorans*. The sea urchin isolate is able to grow without Na⁺ ions or seawater and does not decompose agar or starch, in contrast to *A. lectus* KMM 3902^T. Although KMM 6038^T is related most closely to strains of *A. lectus*, with 16S rRNA gene sequence similarity of 93.8%, the phylogenetic tree topology placed the strain studied more closely to *Y. aromativorans* GW1-1^T. In addition to the above-mentioned phylogenetic and phenotypic distinctiveness between KMM 6038^T and its nearest neighbours, differences in cellular fatty acid composition clearly separate them.

Thus, the polyphasic data presented herein support the conclusion that the sea urchin bacterium studied could not be affiliated to any taxa currently included in the family *Flavobacteriaceae*. Consequently, we propose that KMM 6038^T is the type strain of a novel species in a new genus, *Mariniflexile gromovii* gen. nov., sp. nov.

Description of *Mariniflexile* gen. nov.

Mariniflexile (Ma.ri.ni.fle'xi.le. L. adj. *marinus*, -a, -um marine; L. part. adj. *flexilis* -e pliant, pliable, flexible; N.L. neut. n. *Mariniflexile* a flexible marine bacterium).

Cells are rod-shaped and motile by gliding. Gram-negative. Do not form endospores. Can ferment D-glucose. Produce non-diffusible orange pigments. Chemo-organotrophs. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The predominant cellular fatty acids are straight-chain saturated, branched-chain saturated and unsaturated fatty acids, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{15:1}, C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1} ω7 and/or iso-C_{15:0} 2-OH). The main lipoquinone is MK-6. As determined by 16S rRNA gene sequence analysis, the genus is a member of the family *Flavobacteriaceae*, within the phylum *Bacteroidetes*. The type species is *Mariniflexile gromovii*.

Description of *Mariniflexile gromovii* sp. nov.

Mariniflexile gromovii (gro'mo.vi.i. N.L. gen. n. *gromovii* of Gromov, in honour of B. V. Gromov, the Russian aquatic and marine microbiologist).

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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