

Maribacter polysiphoniae sp. nov., isolated from a red alga

Olga I. Nedashkovskaya,¹ Marc Vancanneyt,² Paul De Vos,^{2,3}
Seung Bum Kim,⁴ Myung Sook Lee⁵ and Valery V. Mikhailov¹

Correspondence

Olga I. Nedashkovskaya
olganedashkovska@piboc.dvo.ru
or
olganedashkovska@yahoo.com

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

²BCCM/LMG Bacteria Collection, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

³Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

⁴Department of Microbiology, Chungnam National University, 220 Gung-dong, Yusong, Daejeon 305-764, Republic of Korea

⁵Food Analysis and Research Institute, Suwon Women's College, 336-27 Sanggi-ri, Bongdam-sup, Hwasung-si, Kyonggi-Do 445-895, Republic of Korea

A novel gliding, heterotrophic, Gram-negative, yellow–orange-pigmented, aerobic, oxidase- and catalase-positive bacterium, designated strain KMM 6151^T, was isolated from the Pacific red alga *Polysiphonia japonica*. Analysis of the 16S rRNA gene sequence of the strain revealed that it formed a distinct lineage within the genus *Maribacter*, family *Flavobacteriaceae*, with sequence similarities in the range 94.6–96.9%. On the basis of phenotypic, genotypic and phylogenetic data, strain KMM 6151^T represents a novel species of the genus *Maribacter*, for which the name *Maribacter polysiphoniae* sp. nov. is proposed. The type strain is KMM 6151^T (=KCTC 22021^T=LMG 23671^T).

The genus *Maribacter*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), accommodates heterotrophic, aerobic, Gram-negative, gliding, yellow-pigmented bacteria isolated from different marine environments. At the time of writing, the genus comprises five recognized species (Nedashkovskaya *et al.*, 2004; Yoon *et al.*, 2005). Two of them, *Maribacter aquivivus* and *Maribacter orientalis*, were isolated from seawater samples (collected in Amursky Bay in the Sea of Japan), *Maribacter ulvicola* was associated with the green alga *Ulva fenestrata*, and the remaining two species, *Maribacter dokdonensis* and *Maribacter sedimenticola*, were isolated from coastal sediments of the Sea of Japan.

In this study, we report the phylogenetic and phenotypic characteristics of a novel isolate, strain KMM 6151^T, which forms a distinct lineage within the genus *Maribacter*.

Strain KMM 6151^T was isolated from a red alga (*Polysiphonia japonica*) collected in the Gulf of Peter the Great in the Sea of Japan during June 2000. For strain isolation, 0.1 ml homogenate of algal tissues was transferred onto plates of marine agar 2216 (Difco). After primary isolation, the strain was cultivated at 28 °C on

marine agar (Difco) and stored at –80 °C in marine broth (Difco) supplemented with 20% (v/v) glycerol.

DNA extraction, PCR amplification and 16S rRNA gene sequencing were carried out as described previously (Vancanneyt *et al.*, 2006). The almost-complete 16S rRNA gene sequence of strain KMM 6151^T (a continuous stretch of 1481 bp) was aligned with sequences retrieved from EMBL and a phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) using the BIONUMERICS software package, version 4.0 (Applied Maths). For the analyses, unknown bases were disregarded. Bootstrapping analysis was undertaken to test the statistical reliability of the topology of the neighbour-joining tree: 500 bootstrap resamplings of the data were performed (Fig. 1). The tree topology obtained with the neighbour-joining method was evaluated and confirmed by means of a maximum-parsimony analysis performed using BIONUMERICS (data not shown).

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of KMM 6151^T revealed that the strain formed a distinct evolutionary lineage within the genus *Maribacter*, family *Flavobacteriaceae*. The nearest neighbours of strain KMM 6151^T were *M. aquivivus* KMM 3949^T and *M. ulvicola* KMM 3951^T, showing similarities of 96.9 and 96.2%, respectively. The sequence similarities between

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 6151^T is AM497875.

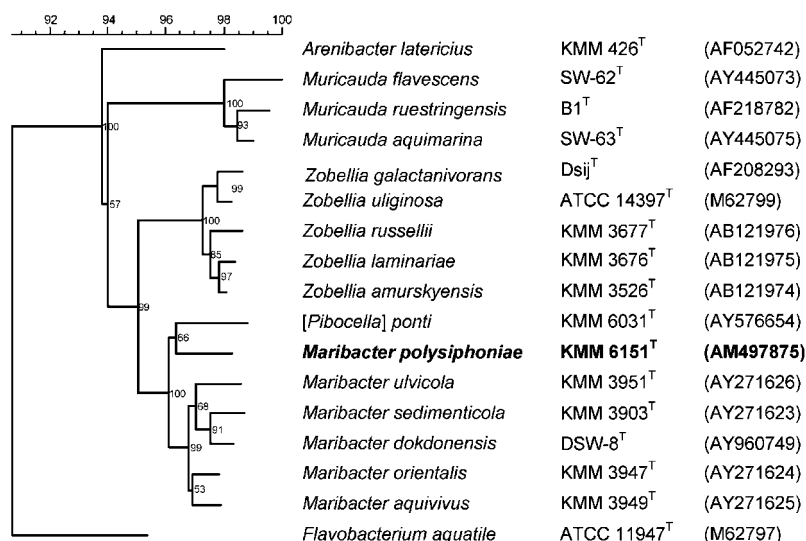


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of strain KMM 6151^T, *Maribacter* species and representative members of related genera in the family *Flavobacteriaceae*, generated using the neighbour-joining method of Saitou & Nei (1987). Numbers at nodes indicate bootstrap percentages (based on 500 bootstrap resamplings of the data) when >50 %. *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup.

strain KMM 6151^T and the other *Maribacter* species were in the range 94.6–96.2 %.

To analyse the DNA base composition, DNA was isolated according to the method of Marmur (1961) and the DNA G + C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C content of KMM 6151^T was 41.1 mol%.

Analysis of fatty acid methyl esters was carried out on cells grown on marine agar at 28 °C for 48 h according to the standard protocol of the Microbial Identification System (Microbial ID). The predominant fatty acids in strain KMM 6151^T were iso-C_{15:1} (9.0 %), iso-C_{15:0} (15.4 %), anteiso-C_{15:0} (7.6 %), C_{15:0} (11.0 %), iso-C_{15:0} 3-OH (5.0 %), iso-C_{17:1}ω_{9c} (5.4 %), iso-C_{16:0} 3-OH (9.1 %), iso-C_{17:0} 3-OH (7.2 %) and summed feature 3 (C_{16:1}ω_{7c} and/or iso-C_{15:0} 2-OH, 10.1 %).

The physiological and biochemical properties of strain KMM 6151^T were examined as described by Nedashkovskaya *et al.* (2004, 2006). The API 20E gallery (bioMérieux) was also used in accordance with the manufacturer's instructions.

The cells of strain KMM 6151^T were Gram-negative and chemo-organotrophic with a respiratory-type metabolism. They were single flexible rods 0.4–0.6 μm in width and 1.2–10 μm in length and showed motility by gliding. Despite having many of the characteristics common in recognized members of the genus *Maribacter*, strain KMM 6151^T had several differentiating features (summarized in Table 1); for example, the maximum growth temperature, oxidation of *N*-acetylglucosamine and acetoin production distinguished this strain from the *Maribacter* species. Strain KMM 6151^T differed from its closest relative, *M. aquivivus* KMM 3949^T, by its ability to hydrolyse starch and to degrade Tween 20, by the absence of agarase and DNase activities, by the utilization of mannitol and by the production of acid from some carbohydrates (Table 1).

The phylogenetic evidence, together with the differentiating phenotypic features, confirms a distinct taxonomic status for strain KMM 6151^T with respect to recognized *Maribacter* species. Therefore, strain KMM 6151^T represents a novel species of the genus *Maribacter* for which the name *Maribacter polysiphoniae* sp. nov. is proposed.

It should be noted that strain KMM 6151^T also clustered with [*Pibocella*] *ponti* KMM 6031^T, the closest relative of the genus *Maribacter*, showing a 16S rRNA gene sequence similarity of 95.7 %. Consequently, the phylogenetic data obtained in this study indirectly allow the assignment of strain KMM 6031^T to the genus *Maribacter*. Moreover, another seawater isolate, KMM 6033, sharing 99 % 16S rRNA gene sequence similarity with the original [*Pibocella*] *ponti* KMM 6031^T (AY576654), shows a fatty acid profile consistent with those of *Maribacter* species (data not shown). However, the transfer of [*Pibocella*] *ponti* to the genus *Maribacter* cannot be formally proposed, as the type (and only) strain, KMM 6031^T, has been lost. The strain labelled KMM 6031^T (=KCTC 12262^T=LMG 22573^T=NBRC 100591^T) currently available in culture collections is actually a member of the genus *Gillisia*, as shown by its 99.7 % 16S rRNA gene sequence similarity to, and its 93 % DNA–DNA relatedness with, *Gillisia mitskevichiae* KMM 6034^T (data not shown).

Description of *Maribacter polysiphoniae* sp. nov.

Maribacter polysiphoniae (po.ly.si.pho'ni.ae. N.L. gen. n. *polysiphoniae* from the generic name of the red alga *Polysiphonia japonica*, from which the type strain was isolated).

The main characteristics are as given for the genus. In addition, cells are 0.4–0.6 μm in width and 1.2–10 μm in length. On marine agar, colonies are circular, yellow–orange in colour, shiny, translucent, have entire edges and are 2–4 mm in diameter. Grows with 1–8 % NaCl, at

Table 1. Phenotypic characteristics of *Maribacter* species

Species: 1, strain KMM 6151^T; 2, *M. aquivivus* KMM 3949^T; 3, *M. dokdonensis* DSW-8^T; 4, *M. orientalis* KMM 3947^T; 5, *M. sedimenticola* KMM 3903^T; 6, *M. ulvicola* KMM 3951^T. All of the strains are positive for the following characteristics: respiratory metabolism; gliding motility; oxidase, catalase and alkaline phosphatase activities; and hydrolysis of aesculin and Tween 40. All of the strains are negative for the following characteristics: production of flexirubin-type pigments; hydrolysis of urea, CM-cellulose, casein and chitin; acid production from L-sorbose; utilization of citrate, adonitol, glycerol, inositol and sorbitol; production of indole and H₂S. Data are from Nedashkovskaya *et al.* (2004, 2005) and Yoon *et al.* (2005). w, Weakly positive reaction; ND, not detected.

Characteristic	1	2	3	4	5	6
Nitrate reduction	—	+	—	—	+	—
β -Galactosidase activity	+	+	—	+	—	+
Acetoin production	w	—	—	—	—	—
Temperature range for growth (°C)	4–41	4–30	4–35	4–32	4–33	4–32
Salinity range for growth (%)	1–8	1–7	1–10	1–5	1–6	1–4
Hydrolysis of:						
Agar	—	+	+	—	+	+
Gelatin	+	+	—	+	—	—
Starch	+	—	—	—	+	—
DNA	—	+	ND	—	—	—
Tween 20	+	—	+	+	+	+
Tween 80	+	+	+	—	+	+
Acid production from:						
L-Arabinose	+	—	+	+	+	—
D-Cellobiose, D-glucose, D-lactose, sucrose	+	—	+	+	—	+
D-Galactose, D-melibiose, DL-xylose	+	—	+	+	—	—
Maltose	+	—	+	+	—	+
D-Raffinose	+	—	+	—	—	—
L-Rhamnose	—	—	—	—	—	+
N-Acetylglucosamine	+	—	—	—	—	—
Mannitol	+	—	+	—	—	—
Utilization of:						
L-Arabinose	+	—	+	+	+	—
D-Glucose, D-mannose, sucrose	+	+	+	+	—	+
D-Lactose	+	+	+	+	—	+
Mannitol	+	—	+	—	—	—
DNA G + C content (mol%)	41.1	35.0	35–37	39.0	37.0	35–37

4–41 °C and at pH 5.5–10.0. Grows optimally with 1.5–2.0 % NaCl, at 30–32 °C and at pH 7.5–8.5. Oxidase, catalase, β -galactosidase and alkaline phosphatase activities are present. Arginine dihydrolase, lysine- and ornithine decarboxylases and tryptophan deaminase activities are absent. Decomposes aesculin, gelatin, starch and Tweens 20, 40 and 80. Does not hydrolyse agar, casein, DNA, CM-cellulose, urea or chitin. Forms acid from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-melibiose, D-raffinose, sucrose, DL-xylose, N-acetylglucosamine and mannitol, but not from L-rhamnose, citrate, adonitol, dulcitol, glycerol or inositol. Utilizes arabinose, glucose, lactose, mannose, sucrose and mannitol but not inositol, sorbitol, malonate or citrate. Nitrate is not reduced. Acetoin (Voges–Proskauer reaction) is weakly produced, but H₂S and indole are not produced. Fatty acids amounting to more than 1 % are as follows: iso-C_{14:0} (1.4 %), iso-C_{15:1} (9 %), iso-C_{15:0} (15.4 %), anteiso-C_{15:0} (7.6 %), C_{15:0} (11 %), C_{15:1} ω 6c (3.9 %), iso-C_{16:1} (1.4 %), iso-C_{16:0} (1.8 %), C_{16:0} (1 %), iso-C_{15:0} 3-OH (5 %), C_{15:0}

3-OH (2.1 %), iso-C_{17:1} ω 9c (5.4 %), C_{17:1} ω 8c (1.2 %), C_{17:1} ω 6c (1.4 %), iso-C_{16:0} 3-OH (9.1 %), iso-C_{17:0} 3-OH (7.2 %), C_{17:0} 2-OH (1.4 %) and summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH, 10.1 %). The DNA G + C content is 41.1 mol%.

The type strain, KMM 6151^T (=KCTC 22021^T=LMG 23671^T), was isolated from the red alga *Polysiphonia japonica*, collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan.

Acknowledgements

We deeply appreciate the assistance of Claudine Vereecke (Public Collection Curator) and Dr Bart Hoste of the BCCM/LMG Bacteria Collection (Ghent, Belgium) with the preparation of this paper. This research was supported by grants from the Russian Foundation for Basic Research no. 05-04-48211, the Presidium of the Far-Eastern Branch of the Russian Academy of Sciences no. 06-04-96067, the Presidium of the Russian Academy of Sciences ‘Molecular and Cell Biology’ and the President of the Russian Federation ‘Scientific Schools’.

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.
- 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., Lysenko, A. M., Rohde, M., Rhee, M.-S., Frolova, G. M., Falsen, E., Mikhailov, V. V. & Bae, K. S. (2004).** *Maribacter* gen. nov., a new member of the family *Flavobacteriaceae*, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov., *Maribacter aquivivus* sp. nov., *Maribacter orientalis* sp. nov. and *Maribacter ulvicola* sp. nov. *Int J Syst Evol Microbiol* **54**, 1017–1023.
- Nedashkovskaya, O. I., Kim, S. B., Lee, K. H., Bae, K. S., Frolova, G. M., Mikhailov, V. V. & Kim, I. S. (2005).** *Pibocella ponti* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi*. *Int J Syst Evol Microbiol* **55**, 177–181.
- Nedashkovskaya, O. I., Kim, S. B., Vancanneyt, M., Lysenko, A. M., Shin, D. S., Park, M. S., Lee, K. H., Jung, W. J., Kalinovskaya, N. I. & other authors (2006).** *Echinicola pacifica* gen. nov., sp. nov., a novel flexibacterium isolated from the sea urchin *Strongylocentrotus intermedius*. *Int J Syst Evol Microbiol* **56**, 953–958.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Vancanneyt, M., Naser, S. M., Engelbeen, K., De Wachter, M., Van der Meulen, R., Cleenwerck, I., Hoste, B., De Vuyst, L. & Swings, J. (2006).** Reclassification of *Lactobacillus brevis* strains LMG 11494 and LMG 11984 as *Lactobacillus parabrevis* sp. nov. *Int J Syst Evol Microbiol* **56**, 1553–1557.
- Yoon, J. H., Kang, S.-J., Lee, S.-Y., Lee, C.-H. & Oh, T.-K. (2005).** *Maribacter dokdonensis* sp. nov., isolated from sea water off a Korean island, Dokdo. *Int J Syst Evol Microbiol* **55**, 2051–2055.