Algibacter mikhailovii sp. nov., a novel marine bacterium of the family *Flavobacteriaceae*, and emended description of the genus *Algibacter*

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A novel marine bacterium, designated strain KMM 6171^T, was subjected to taxonomic analysis by using a polyphasic approach. Colonies were yellow-pigmented and cells were Gram-negative, heterotrophic rods displaying slow gliding motility. 16S rRNA gene sequence analysis indicated that strain KMM 6171^T was closely related to the genus *Algibacter*, a member of the family *Flavobacteriaceae*, with sequence similarity of 96.7–96.8 %. The predominant cellular fatty acids were iso-C15 : 1, iso-C15 : 0, anteiso-C15 : 0, C15 : 0, iso-C15 : 0 3-OH, iso-C17 : 0 3-OH and summed feature 3, comprising C16 : 1ω7*c* and/or iso-C15 : 0 2-OH. The DNA G+C content was 35.1 mol%. On the basis of the phenotypic, genotypic, chemotaxonomic and phylogenetic data, strain KMM 6171^T represents a novel species of the genus *Algibacter*, for which the name *Algibacter mikhailovii* sp. nov. is proposed. The type strain is KMM 6171^T (=KCTC 12710^T=LMG 23988^T). An emended description of the genus *Algibacter* based on the new data is also given.

The genus *Algibacter*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), was erected to accommodate Gram-negative, facultatively anaerobic, gliding, orange-pigmented and agarolytic marine bacteria isolated from the surfaces of green algae (Nedashkovskaya *et al.* 2004).

In the present work, we report the isolation and identification of a novel Gram-negative, gliding, yellow-pigmented marine bacterium, designated strain KMM 6171^T. As a result of a polyphasic study, including phylogenetic, genotypic, chemotaxonomic and phenotypic methods, the isolate was identified as a novel member of the genus *Algibacter*.

The agarolytic strain KMM 6171^T was isolated from a sea urchin, *Strongylocentrotus intermedius*, collected in Troitsa Bay, Gulf of Peter the Great, the East Sea (also known as the Sea of Japan). For strain isolation, 0.1 ml homogenates of the 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.

DNA extraction, PCR and 16S rRNA gene sequencing were carried out as described previously (Vancanneyt *et al.*, 2006). Sequence data obtained were aligned with those of representative members of the family *Flavobacteriaceae* retrieved from GenBank, and construction of a neighbour-joining (Saitou & Nei, 1987) phylogenetic tree and bootstrap analysis were carried out as described previously (Cho *et al.*, 2006). In addition, trees were constructed on the basis of maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1993) algorithms.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of strain KMM 6171^T (1473 nt) revealed that the strain was affiliated with the family *Flavobacteriaceae* and formed a distinct lineage within the genus *Algibacter*, which was supported by a high bootstrap level and by the different tree-making algorithms (Fig. 1). The closest relatives of the strain studied were strains of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Algibacter mikhailovii* KMM 6171^T is AM491809.

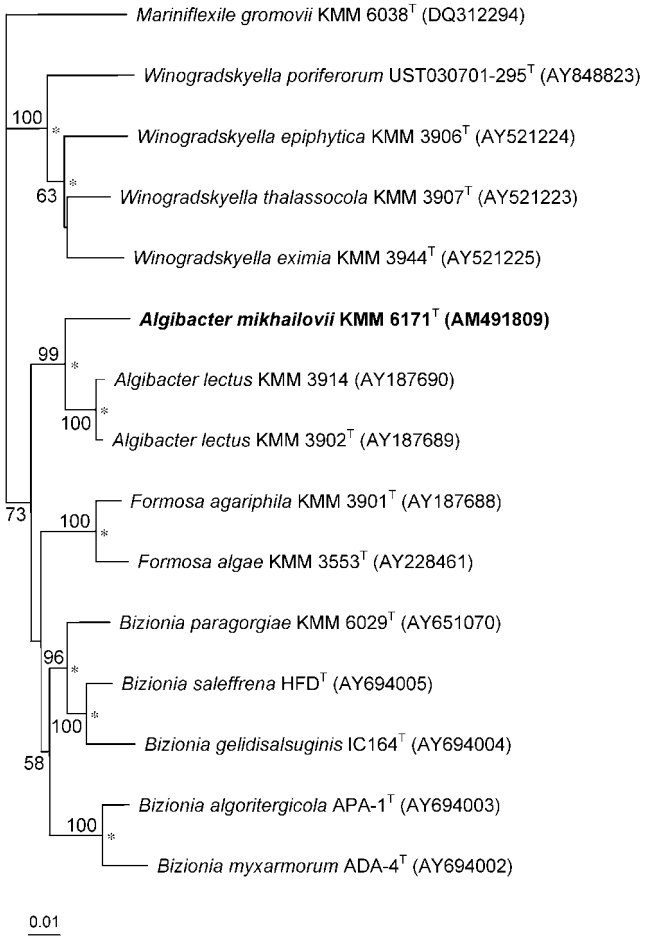

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain KMM 6171^T and representative members of the family *Flavobacteriaceae*. Bootstrap percentages (based on 1000 replications) greater than 50 % are shown at branch points and asterisks indicate branches that were also recovered using the maximum-parsimony and maximum-likelihood algorithms. *Mariniflexile gromovii* KMM 6038^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Algibacter lectus with 16S rRNA gene sequence similarity of 96.7–96.8 %, suggesting that strain KMM 6171^T may represent a novel species in the genus *Algibacter* according to the recommendations of Stackebrandt & Goebel (1994).

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 DNA G+C content of KMM 6171^T was 35.1 mol%.

Analysis of fatty acid methyl esters of strain KMM 6171^T was carried out according to the standard protocol of the Microbial Identification System (Microbial ID), except that the biomass was obtained from culture grown on marine agar 2216 at 25 °C for 48 h.

The fatty acid composition of strain KMM 6171^T was characterized by the predominance of branched-chain saturated and unsaturated fatty acids, namely iso-C15 : 1, iso-C15 : 0, anteiso-C15 : 0, C15 : 0, iso-C15 : 0 3-OH, iso-C17 : 0 3-OH and summed feature 3, comprising C16 : 1ω7*c* and/or iso-C15 : 0 2-OH. A similar fatty acid composition was reported for the type strain of *A. lectus* (Nedashkovskaya *et al.*, 2004).

Phenotypic analysis was performed by using methods described previously (Nedashkovskaya *et al.*, 2003, 2004). API 20E, API 20NE and API ZYM galleries (bioMérieux) were also used for studying the phenotypic features of the strain according to the manufacturer's instructions, except that the galleries were incubated at 28 °C.

Cells of strain KMM 6171^T were heterotrophic, Gram-negative, motile by gliding, agarolytic and formed pale-yellow colonies. Other physiological and biochemical characteristics are listed in the species description and Table 1. Similar to *A. lectus*, the novel bacterium was oxidase-, catalase-, β-galatosidase- and agarase-positive, and was able to grow in media containing 1–6 % NaCl. However, strain KMM 6171^T could be readily distinguished from *A. lectus* by the presence of nitrate reductase and DNase activities, by the absence of amylase and Tween esterase activities, and by its inability to form acid from carbohydrates.

Consequently, significant molecular distinctiveness and clear phenotypic differences support the description of strain KMM 6171^T as a novel species of the genus *Algibacter*, for which the name *Algibacter mikhailovii* sp. nov. is proposed.

The representatives of the single species of the genus *Algibacter*, *A. lectus*, can ferment D-glucose. Consequently, *A. lectus* was characterized as a facultatively anaerobic organism in the genus description (Nedashkovskaya *et al.*, 2004). Conversely, the novel isolate is strictly aerobic and unable to ferment D-glucose. In addition, fatty acid C15 : 1ω6*c*, one of the major components of *A. lectus*, only amounts to 1.7 % in strain KMM 6171^T. These facts justify an emendation of the description of the genus *Algibacter*.

Description of *Algibacter mikhailovii* sp. nov.

Algibacter mikhailovii (mik.ha′i.lo.vi.i. N.L. masc. gen. n. *mikhailovii* of Mikhailov, in honour of Valery V. Mikhailov, a Russian microbiologist, for his contributions to the development of marine microbiology).

Cells range from 0.3 to 0.4 μm in width by 2 to 10 μm in length and move slowly by gliding. On marine agar colonies are circular, 1–3 mm in diameter after 72 h of incubation at 25 °C, convex, shiny, sunken into the agar and pale-yellow-pigmented. Requires Na^+ ions for growth. Growth occurs at 4–37 °C and with 1–6 % NaCl. Optimal growth is observed at 23–25 °C and with 2–3 % NaCl. Heterotrophic, strictly aerobic. D-Glucose is not fermented. Flexirubin-type pigments are not produced. Oxidase, catalase, β-galactosidase and alkaline phosphatase activities

Table 1. Differential phenotypic characteristics of *Algibacter* species

All strains were positive for: gliding motility; oxidase, catalase, β-galactosidase and alkaline phosphatase activities; requirement of NaCl for growth; growth at 1–6 % NaCl and at 4–35 °C; hydrolysis of agar and gelatin; utilization of D-glucose, D-lactose and D-mannose; susceptibility to carbenicillin, oleandomycin and lincomycin. All strains were negative for: production of flexirubin pigments; urease activity; H_2S, indole and acetoin production; hydrolysis of casein, cellulose (CM-cellulose and filter paper), chitin and Tween 80; acid formation from L-arabinose, D-lactose, D-melibiose, D-raffinose, L-rhamnose, L-sorbose, glycerol, citrate, fumarate, malate, adonitol, inositol, mannitol and sorbitol; utilization of inositol, citrate and malonate; susceptibility to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. Data are from Nedashkovskaya *et al.* (2004) and this study.

Characteristic	*A. mikhailovii* KMM 6171^T	*A. lectus* (*n*=3)
Fermentation of D-glucose	−	+
Nitrate reduction	+	−
Growth at 37 °C	+	−
Hydrolysis of:		
Starch	−	+
Tween 40	−	+
DNA	+	−
Acid production from carbohydrates	−	+
Utilization of:		
Sucrose	−	+
Arabinose and sorbitol	+	−
Susceptibility to tetracycline	+	−
DNA G+C content (mol%)	35.1	31–33

are present. Decomposes agar, aesculin, gelatin and DNA. Does not hydrolyse casein, starch, Tweens 20, 40 and 80, cellulose (CM-cellulose and filter paper), chitin or urea. Acid is not produced from arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, melibiose, raffinose, rhamnose, sucrose, xylose, *N*-acetyl-D-glucosamine, glycerol, inositol and mannitol. Arabinose, glucose, lactose, mannose and sorbitol are utilized, but sucrose, adonitol, dulcitol, mannitol and inositol are not. According to API galleries, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate are not utilized; arginine dihydrolase activity is absent, and acid is not produced from amygdalin. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidases and *N*-acetyl-β-glucosidase activities are present, but lipase (C14), α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase or α-fucosidase activities are absent. Nitrate is reduced to nitrite. Indole, H_2S and acetoin (Voges–Proskauer reaction) are not produced. Susceptible to carbenicillin, lincomycin, oleandomycin and tetracycline. Resistant to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymixin B and streptomycin. The fatty acids amounting to more than 1 % of total are iso-C15 : 1 (13 %), anteiso-C15 : 1 (2.1 %), iso-C15 : 0 (11.3 %), anteiso-C15 : 0 (4.6 %), C15 : 0 (7 %), C15 : 1ω6*c* (1.7 %), iso-C16 : 1 H (2 %), C16 : 0 10 methyl (2.1 %), C16 : 0 (2.3 %), iso-C15 : 0 3-OH (5.8 %), C15 : 0 2-OH (2.4 %), C15 : 0 3-OH (1.3 %), C16 : 0 3-OH (1.4 %), iso-C17 : 0 3-OH (13 %), C17 : 0 2-OH (2.4 %), C18 : 1ω5*c* (1.8 %) and summed feature 3 (22.2 %), consisting of iso-C15 : 0 2-OH and/or C16 : 1ω7*c*. The DNA G+C content is 35.1 mol%.

The type strain, KMM 6171^T (=KCTC 12710^T=LMG 23988^T), was isolated from a sea urchin, *Strongylocentrotus intermedius*, collected in Troitsa Bay, East Sea.

Emended description of the genus *Algibacter* Nedashkovskaya *et al.* 2004

The description of the genus *Algibacter* is as given by Nedashkovskaya *et al.* (2004) and this study, with the following amendments. Some strains can ferment D-glucose. The main cellular fatty acids are straight-chain unsaturated and branched-chain unsaturated iso-C15 : 0, anteiso-C15 : 0, iso-C15 : 1, C15 : 0, iso-C15 : 0 3-OH, iso-C17 : 0 3-OH and summed feature 3, consisting of iso-C15 : 0 2-OH and/or C16 : 1ω7*c*. As determined by 16S rRNA gene sequence analysis, the genus *Algibacter* is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The type species is *A. lectus*.

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