Gillisia mitskevichiae sp. nov., a novel bacterium of the family *Flavobacteriaceae*, isolated from sea water

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The taxonomic position of a novel marine, heterotrophic, aerobic, pigmented bacterium, non-motile by gliding, that was isolated from a sea-water sample collected in the Sea of Japan, was determined. 16S rRNA gene sequence analysis revealed that strain KMM 6034^T is a member of the genus *Gillisia*. The phenotypic and chemotaxonomic data showed that the isolate represents a novel species of the genus *Gillisia*, for which the name *Gillisia mitskevichiae* sp. nov. is proposed. The type strain is KMM 6034^T (=KCTC 12261^T=NBRC 100590^T=LMG 22575^T).

The genus *Gillisia* accommodates Gram-negative, strictly aerobic, heterotrophic, yellow-pigmented bacteria that are non-motile by gliding and belong to the family *Flavobacteriaceae* (Van Trappen *et al.*, 2004). The strains of the single species of this genus, *Gillisia limnaea*, were isolated from microbial mats from Lake Fryxell in the McMurdo Dry Valleys, Antarctica. The genus *Gillisia* forms a phylogenetic cluster with the genera *Mesonina*, *Salegentibacter* and *Psychroflexus*.

During June 2000, we isolated an unknown bacterium from a sea-water sample collected in Amursky Bay, Gulf of Peter the Great, Sea of Japan. A polyphasic taxonomic study of strain KMM 6034^T, cultured on marine agar 2216 at 25 °C for 48 h, indicates that the isolate represents a novel species of the genus *Gillisia*, for which the name *Gillisia mitskevichiae* sp. nov. is proposed.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed previous procedures (Kim *et al.*, 1998). The sequence data obtained were aligned with those of members of the family *Flavobacteriaceae* using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred by using suitable programs of

the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the model of Kimura (1980) and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets by using SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence (1430 nucleotide positions) revealed that strain KMM 6034^T was a member of the family *Flavobacteriaceae* and formed a distinct lineage within the genus *Gillisia* (Fig. 1). The level of 16S rRNA gene sequence similarity between strains KMM 6034^T and *G. limnaea* LMG 21470^T was 96.7%. 16S rRNA gene sequence similarity values of the strain studied with the next closest relatives *Mesonina algae* KMM 3909^T, [*Cytophaga*] *latercula* ATCC 23177^T, *Salegentibacter salegens* DSM 5424^T, *Psychroflexus torquis* ACAM 623^T and *Kordia algicida* OT-1^T were 91.4, 92.2, 93.1, 91.4 and 90.5%, respectively.

DNA was isolated following the method of Marmur (1961) and the G + C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C content of KMM 6034^T was 36.4 mol%.

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID). The cellular fatty acids comprising more than 1% for strain KMM 6034^T were straight- and branched-chain unsaturated and saturated fatty acids, namely i-14:0 (1.2%), i-15:1 (11.8%), a-15:1 (2.4%),

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Gillisia mitskevichiae* KMM 6034^T is AY576655.

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of KMM 6034^T and related members of the family Flavobacteriaceae. Asterisks indicate branches that were also recovered with the maximum-likelihood algorithm and numbers at nodes are levels of bootstrap support (%) from 1000 resampled datasets. Bar, 0.01 substitutions per nucleotide position.

i-15:0 (7.5%), a-15:0 (5.1%), 15:0 (4.4%), 15:1 ω 6c (1.9%), i-16:1 (6%), i-16:0 (9.3%), i-15:0 3-OH (1.1%), 15:0 2-OH (2.4%), i-17:1 ω 9c (4%), a-17:1 ω 9c (2.1%), 17:1 ω 6c (4.7%), i-16:0 3-OH (7.2%), 16:0 3-OH (1.2%), i-17:0 3-OH (6.7%), 17:0 2-OH (3.8%), and summed feature 3 (11.1%), comprising i-15:0 2-OH and/or 16:1 ω 7c, which corresponds to the fatty acid composition of *G. limnaea* (Van Trappen *et al.*, 2004). Both the strain studied and *G. limnaea* strains contained similar amounts of branched fatty acids (>65% of total).

Phenotypic analysis was performed by using previously described methods (Nedashkovskaya *et al.*, 2003a, b). Gliding motility was determined as described by Bowman (2000).

The physiological, morphological and biochemical characteristics of strain KMM 6034^T are listed in the species description and Table 1. The similarities in phenotypic characteristics support the inclusion of the strain studied in the genus *Gillisia*. However, strain KMM 6034^T clearly differed from strains of *G. limnaea* by the NaCl requirement for growth, growth at 12% NaCl, maximum growth temperature (31 °C), acid production from carbohydrates, utilization of mannitol and the ability to degrade casein, urea, DNA and Tweens 20 and 40 (Table 1).

The above-mentioned phenotypic features (Table 1) in association with molecular differences presented in this paper allow the differentiation of strain KMM 6034^T from *G. limnaea*. Thus, we propose that strain KMM 6034^T be placed in the genus *Gillisia* as the type strain of *Gillisia mitskevichiae* sp. nov.

Description of *Gillisia mitskevichiae* sp. nov.

Gillisia mitskevichiae (mit.ske'vi.chi.ae. N.L. gen. n. *mitskevichiae* of Mitskevich, in honour of Irina N. Mitskevich, Russian marine microbiologist, for her contributions to the development of marine microbiology).

Table 1. Phenotypic characteristics that distinguish *Gillisia mitskevichiae* sp. nov. from *G. limnaea*

Data from Van Trappen *et al.* (2004) and this study. Strains of both species are Gram-negative and are positive for respiratory type of metabolism, oxidase, catalase and alkaline phosphatase activities, growth at 1–5% NaCl and at 5–30 °C, hydrolysis of gelatin and Tween 80, utilization of glucose and sucrose and susceptibility to ampicillin, carbenicillin, lincomycin and oleandomycin. Strains of both species are negative for gliding motility, requirement for organic growth factors, flexirubin pigments, nitrate reduction, H₂S, indole and acetoin production, degradation of agar, starch, cellulose (CM-cellulose, filter paper) and chitin, acid formation from arabinose, cellobiose, fucose, galactose, lactose, maltose, melibiose, raffinose, rhamnose, sorbose, xylose, inositol, mannitol, sorbitol, glycerol, adonitol, dulcitol and citrate, utilization of arabinose, lactose, mannose, inositol, sorbitol, malonate and citrate and susceptibility to gentamicin, kanamycin, neomycin and polymyxin B.

Characteristic	<i>G. mitskevichiae</i> KMM 6034 ^T	<i>G. limnaea</i> (n=3)
Na ⁺ requirement for growth	+	–
Degradation of:		
Casein	+	–
DNA	+	–
Urea	+	–
Tween 20	–	+
Tween 40	+	–
Growth at:		
12% NaCl	+	–
31 °C	+	–
Acid from:		
Glucose, sucrose	+	–
N-Acetylglucosamine	+	–
Utilization of mannitol	–	+
Susceptibility to streptomycin and tetracycline	+	–
DNA G + C content (mol%)	36.4	37–39

Cells are Gram-negative, strictly aerobic with respiratory metabolism, chemo-organotrophic, asporogenic rods, non-motile by gliding, 0.5–0.7 µm wide and 3–4 µm long. Oxidase-, catalase-, urease- and alkaline phosphatase-positive. Colonies are circular, convex, shiny with entire edges, 1–3 mm in diameter on marine agar 2216. Produces yellow non-diffusible pigments. No growth is observed without Na⁺; grows at 1–12% NaCl. Flexirubin pigments are absent. Growth occurs at 4–31 °C. The pH range of growth is 5.5–10.0, with optimum growth occurring between pH 7.6 and 8.3. Casein, gelatin, DNA, urea and Tweens 40 and 80 are degraded; does not hydrolyse agar, starch, cellulose (CM-cellulose and filter paper), chitin or Tween 20. Forms acid from glucose, sucrose and N-acetylglucosamine, but not from arabinose, cellobiose, fucose, galactose, lactose, maltose, melibiose, raffinose, rhamnose, xylose, adonitol, dulcitol, glycerol, inositol or

mannitol. Does not utilize arabinose, lactose, mannose, inositol, mannitol, sorbitol or citrate as sole sources of carbon and energy. Nitrate reduction is negative. H₂S, indole and acetoin (Voges-Proskauer reaction) are not produced. Susceptible to ampicillin, carbenicillin, oleandomycin, lincomycin, streptomycin and tetracycline; resistant to kanamycin, benzylpenicillin, neomycin, gentamicin and polymyxin B. The predominant cellular fatty acids are straight-chain unsaturated, branched-chain unsaturated and saturated, namely i-15:0 (7.5%), a-15:0 (5.1%), i-15:1 (11.8%), i-16:1 (6%), i-16:0 (9.3%), i-16:0 3-OH (7.2%), i-17:0 3-OH (6.7%) and summed feature 3 (11.1%), consisting of i-15:0 2-OH and/or 16:1 ω 7c (64.7% of total). The G+C content of the DNA is 36.4 mol%.

The type strain is KMM 6034^T (=KCTC 12261^T=NBRC 100590^T=LMG 22575^T), isolated from sea-water collected in Amursky Bay, Gulf of Peter the Great, Sea of Japan.

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