Gillisia limnaea gen. nov., sp. nov., a new member of the family Flavobacteriaceae isolated from a microbial mat in Lake Fryxell, Antarctica

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A taxonomic study was performed on three strains isolated from microbial mats in Lake Fryxell, McMurdo Dry Valleys, Antarctica. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these strains belong to the family Flavobacteriaceae, in which they form a distinct lineage. The isolates are Gram-negative, chemoheterotrophic, aerobic, rod-shaped cells. They are psychrophilic and yellow-pigmented, with DNA G + C contents in the range 37.8–38.9 mol%. Whole-cell fatty acid profiles revealed mainly branched fatty acids and 17 : 0 2-OH. On the basis of genotypic, phenotypic, chemotaxonomic and phylogenetic results, it is proposed that the isolates represent a novel species in a new genus, Gillisia limnaea gen. nov., sp. nov. The type strain is LMG 21470T (=DSM 15749T).

Members of the Cytophaga–Flavobacterium cluster constitute one of the dominant bacterial groups in marine and freshwater environments (Bowman et al., 1997b; Pinhassi et al., 1997; Glöckner et al., 1999). In addition, it is now thought that flavobacteria play an important role in the uptake and degradation of complex dissolved and particulate organic matter (Kirchman, 2002). Therefore, this group has an important and central role in remineralization processes in aquatic systems. Recently, several new genera of the Flavobacteriaceae have been described, i.e. Cellulophaga, Zobellia, Muricauda, Arenibacter, Tenacibaculum, Vitellibacter, Mesonia and Ulvibacter (Johansen et al., 1999; Barbeyron et al., 2001; Bruns et al., 2001; Ivanova et al., 2001; Suzuki et al., 2001; Nedashkovskaya et al., 2003a, 2003b, 2004). Members of several of these genera, i.e. Gelidibacter, Psychroserpens, Psychroflexus, Polaribacter and Salegentibacter (Bowman et al., 1997a, 1998; Gosink et al., 1998; McCammon & Bowman, 2000), were originally isolated from Antarctic maritime lakes and the surrounding Southern Ocean, whereas isolates of the genus Aequorivita were found in terrestrial and marine Antarctic habitats (Bowman & Nichols, 2002).

During the MICROMAT project (November 1998–August 2001), 746 bacterial strains were isolated under heterotrophic conditions from microbial mat samples collected from 10 Antarctic lakes in the Vestfold Hills (Lakes Ace, Druzhby, Grace, Highway, Pendant, Organic and Watts), the Larsemann Hills (Lake Reid) and the McMurdo Dry Valleys (Lakes Hoare and Fryxell) (Van Trappen et al., 2002). Numerical analysis of their fatty acid composition revealed 41 clusters and 16S rRNA gene sequence analysis, performed on representative strains, showed that these isolates belong to the α-, β- and γ-Proteobacteria, the high- and low-G+C-containing Gram-positives and the phylum Bacteroidetes (Van Trappen et al., 2002).

In the present work, the taxonomic relationship between the three strains from fatty acid cluster 4 (as delineated by Van Trappen et al., 2002) was studied by a polyphasic taxonomic approach. A novel genus of the family Flavobacteriaceae is described, Gillisia gen. nov., with Gillisia limnaea sp. nov. as the type species.

The strains investigated were LMG 21470T (=DSM 15749T (=R-8282T)), LMG 21966 (=R-7730) and LMG 21965 (=R-7610), isolated as described by Van Trappen et al. (2002) from microbial mat samples (FR1 and FR2) taken from Lake Fryxell, McMurdo Dry Valleys, Antarctica. The strains were routinely cultivated on marine agar 2216 (Difco) at 20 °C for 48 h, except when mentioned otherwise.

DNA extracts were prepared using the method of Pitcher et al. (1989). Genomic relatedness between the novel strains was determined by DNA–DNA hybridizations, carried out with photobiotin-labelled probes in microplate wells as described by Willems et al. (2001), using an HTS7000 BioAssay Reader (Perkin Elmer) for fluorescence measurements. The hybridization temperature was 30 °C and

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The EMBL accession number for the 16S rRNA gene sequence of strain LMG 21470T is AJ440991.
The almost complete 16S rRNA gene sequence (1483 nt) of strain LMG 21470 T was obtained as described earlier (Van Trappen et al., 2002). The closest related sequences were found using the program FASTA. Sequences from reference strains were aligned and editing of the alignment and reformatting was performed with BIOEDIT (Hall, 1999) and FORCON (Raes & De Peer, 1999). Evolutionary distances were calculated using the Jukes & Cantor evolutionary model and a phylogenetic tree (Fig. 1) was constructed using the neighbour-joining method (Saitou & Nei, 1987) with TREECON (Van de Peer & De Wachter, 1994). Dendrograms obtained by maximum-parsimony and maximum-likelihood analyses showed essentially the same topography (data not shown).

Results of the phylogenetic analysis revealed that the novel strains form a distinct lineage within the family Flavobacteriaceae (Bernardet et al., 2002) and belong to a cluster of species: Salegentibacter salegens, Mesonia algae, Psychroflexus torquis, Psychroflexus gondwanensis, Geildibacter algens, Geildibacter mesophilus, Psychroserpens burtonensis and the misclassified strains [Flexibacter] tractuosus IFO 15980 and [Cytophaga] laterula ATCC 23177T (see Fig. 1). The 16S rDNA sequence similarity values between strain LMG 21470 T and its closest relatives, [F.] tractuosus, S. salegens and Psychroflexus gondwanensis, were 93-0, 92-8 and 92-0 %, respectively. The 16S rDNA sequence of the recently described M. algae (Nedashkovskaya et al., 2003b) showed only 91-5 % similarity with that of strain LMG 21470 T. The low level of sequence similarity between the novel strains and other bacteria belonging to the Flavobacteriaceae (87-4–93-0 %) clearly demonstrates that they represent a new genus.

The G+C content of DNA from the Antarctic strains was determined using an HPLC method, as described by Van Trappen et al. (2003). The G+C contents of strains LMG 21470 T, LMG 21966 and LMG 21965 were respectively 37-8, 38-7 and 38-9 mol%. These values are consistent with G+C contents observed in the family Flavobacteriaceae (27–44 mol%) (Bernardet et al., 2002).

Cellular fatty acid patterns of the novel strains have been published previously (Van Trappen et al., 2002; cluster 4). The strains showed similar profiles and the major constituents were branched fatty acids (<65 % of total), which is typical for members of the Flavobacteriaceae (Bernardet et al., 2002). Significant differences in the fatty acid compositions of the novel strains and related taxa were found, e.g. extracts of Gillisia limnaea strains contained considerable amounts of 17:0 2-OH (13-1 % of total), 17:1 0c iso (7-1 %), 17:1 0c anteiso (7-4 %) and summed feature 3 (8-2 %; comprises 15:0 iso 2-OH and/or 16:1 0c or both), whereas these fatty acids were not detected in S. salegens, Psychroflexus gondwanensis or [C.] laterula (Bowman et al., 1998).

Morphological, physiological and biochemical tests were performed, as described previously (Van Trappen et al., 2002). The strains show similar properties and the major characteristics are given in the species description. Results of polyphasic analysis support the formation of a new genus within the family Flavobacteriaceae, Gillisia gen. nov., with Gillisia limnaea sp. nov. as the type species. The new genus can be clearly differentiated from related members of the Flavobacteriaceae by several phenotypic characteristics (Table 1).

**Description of Gillisia gen. nov.**

Gillisia (Gil.lis.i.a. N.L. fem. n. Gillisia after Monique Gillis, a Belgian bacteriologist who has made major contributions to bacterial taxonomy).

Gram-negative, rod-shaped cells that are strictly aerobic, moderately halotolerant, psychrophilic and chemoheterotrophic. Produces yellow pigments. No flexirubins are

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**Fig. 1.** Neighbour-joining dendrogram showing the estimated phylogenetic relationship of Gillisia limnaea gen. nov., sp. nov. and related members of the family Flavobacteriaceae on the basis of 16S rRNA gene sequences. *Weeksellia zoohelicum* was chosen as the outgroup. Bootstrap values over 50 % are shown (percentages of 500 replicates). Bar, 1 nt substitution per 10 nt. EMBL accession numbers for reference strains are shown in parentheses.
formed. Gliding motility is not detected. Does not form endospores. Positive for cytochrome oxidase, catalase and formed. Gliding motility is not detected. Does not form endospores. Positive for cytochrome oxidase, catalase and β-galactosidase. The main cellular fatty acids are 15:0 iso, 15:0 anteiso, 15:1 iso, 16:0 iso, 17:0 2-OH, 17:0 iso 3-OH, 17:0 9c iso, 17:0 9c anteiso and summed feature 3 (comprising 15:0 iso 2-OH and/or 16:1 ω7c). 16S rRNA gene sequence analysis reveals that the genus Gillisia belongs to the family Flavobacteriaceae of the phylum Bacteroidetes. The type species is Gillisia limnaea.

Description of Gillisia limnaea sp. nov.

Gillisia limnaea (lim.nae’a. Gr. adj. limnaios pertaining to, living in lakes; N.L. fem. adj. limnaea living in the water, referring to the isolation source, microbial mats in Lake Fryxell).

The main characteristics are the same as given for the genus. In addition, cells are 3-0 × 0-7 μm. Grows at 5-25 °C; optimal growth at 20 °C. Weak growth is observed at 30 °C and no growth occurs at 37 °C. Yellow, convex, translucent colonies with diameters of 1-3 mm and entire margins are formed on marine agar plates after 6 days incubation. Colonies on Anacker & Ordal’s agar are flat, round with entire margins and 0-7-0-9 mm in diameter after 14 days incubation. Growth also occurs on nutrient agar and R2A and colonies do not adhere to the agar. No growth on trypticase soy agar. Degrades aesculin and gelatin. Growth is not observed (API 20NE) on glucose, mannose, maltose, L-arabinose, mannitol, N-acetylcysteine, gluconate, caprate, adipate, malate, citrate or phenylacetate. Acids are not produced from carbohydrates (API 20E). Agar, alginate, pectin, chitin, casein, carboxymethylcellulose, DNA, starch, Tween 80, tyrosine and urea are not degraded. Congo red is not absorbed. No brown diffusible pigment is produced on L-tyrosine agar and no precipitate is formed on egg-yolk agar. Tests for indole production, citrate utilization, nitrate reduction, the Voges–Proskauer reaction and hydrogen sulfide production are negative. None of the strains has the following enzyme activities: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase (API 20E), lipase (C14), x-galactosidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-fucosidase (API ZYM). Weak enzymic activity is observed for cystine arylamidase, β-glucuronidase and x-glucosidase, medium activity for esterase (C4), esterase lipase (C8) and trypsin and strong activity for alkaline and acid phosphatases, leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase. Variable results are observed for x-chymotrypsin activity. Growth occurs in up to 5 % NaCl, but not in 10 % NaCl, indicating that strains are moderately halotolerant but not halophilic. DNA G+C content is 37-8-38-9 mol%.

The type strain is LMG 21470T (= DSM 15749T). Isolated from microbial mats from Lake Fryxell in the McMurdo Dry Valleys, Antarctica.

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