

Mucilaginibacter frigoritolerans sp. nov., *Mucilaginibacter lappiensis* sp. nov. and *Mucilaginibacter mallensis* sp. nov., isolated from soil and lichen samples

Minna K. Männistö,¹ Marja Tirola,² Jennifer McConnell³
and Max M. Häggblom³

Correspondence

Minna K. Männistö
minna.mannisto@metla.fi

¹Finnish Forest Research Institute, Eteläranta 55, FI-96300 Rovaniemi, Finland

²Department of Biological and Environmental Science, University of Jyväskylä, FI-40014 Jyväskylä, Finland

³Department of Biochemistry and Microbiology, Rutgers University, 76 Lipman Drive, New Brunswick, NJ 08901, USA

Five cold-adapted bacteria belonging to the genus *Mucilaginibacter* were isolated from lichen and soil samples collected from Finnish Lapland and investigated in detail by phenotypic and phylogenetic analyses. Based on 16S rRNA gene phylogeny, the novel strains represent three new branches within the genus *Mucilaginibacter*. The strains were aerobic, chemo-organotrophic, non-motile rods and formed pigmented, smooth, mucoid colonies on solid media. The strains grew between 0 and 33 °C (optimum growth at 25 °C) and at pH 4.5–8.0 (optimum growth at pH 6.0). The main cellular fatty acids were iso-C_{15:0}, summed feature 3 (C_{16:1ω7c}/iso-C_{15:0} 2-OH) and iso-C_{17:0} 3-OH and the major respiratory quinone was MK-7. The DNA G + C contents were 44.0–46.5 mol%. Based on phylogenetic, phenotypic and chemotaxonomic data, the strains represent three novel species of the genus *Mucilaginibacter* for which the names *Mucilaginibacter frigoritolerans* sp. nov. (type strain FT22^T = ATCC BAA-1854^T = LMG 25359^T), *Mucilaginibacter lappiensis* sp. nov. (type strain ANJLI2^T = ATCC BAA-1855^T = LMG 25358^T) and *Mucilaginibacter mallensis* sp. nov. (type strain MP1X4^T = ATCC BAA-1856^T = LMG 25360^T) are proposed.

The genus *Mucilaginibacter* of the family *Sphingobacteriaceae* was described by Pankratov *et al.* (2007) and at present comprises seven species: *Mucilaginibacter paludis* and *Mucilaginibacter gracilis* (Pankratov *et al.*, 2007), *Mucilaginibacter kameinonensis* (Urai *et al.*, 2008), *Mucilaginibacter daejeonensis* (An *et al.*, 2009), *Mucilaginibacter ximonensis* (Luo *et al.*, 2009), *Mucilaginibacter oryzae* (Jeon *et al.*, 2009) and *Mucilaginibacter rigui* (Baik *et al.*, 2010). Members of the family *Sphingobacteriaceae* are commonly isolated from cold environments such as alpine (Margesin *et al.*, 2003; Shivaji *et al.*, 2005) and tundra soil (Männistö & Häggblom, 2006). Terminal restriction fragment analysis coupled to clone

libraries of soil DNA and rRNA has indicated that bacteria of the family *Sphingobacteriaceae* are dominant members of the Arctic tundra of Finland and retain high activity at low temperatures and after multiple freeze–thaw cycles (Männistö *et al.*, 2009). Through cultivation of soil and lichen samples from northern Finland on various media, we have amassed a collection of more than 50 mucoid Gram-negative strains that are non-pigmented, yellow-pigmented or red-pigmented when grown on R2A agar and contain high amounts of iso/anteiso and branched-chain hydroxy fatty acids (Männistö & Häggblom, 2006; M. K. Männistö, unpublished results). Analysis of 16S rRNA gene sequences indicated that these strains represent a wide diversity of species within the family *Sphingobacteriaceae* and are related to various species of the genera *Pedobacter* and *Mucilaginibacter*. These isolates appear well-adapted to the low temperature environment where they are exposed not only to sub-zero temperatures but also to desiccation and a wide regime of annual temperature variation. In this paper, five strains isolated from samples collected in Finnish Lapland are described. Based on phenotypic and

Abbreviation: TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MP601, ANJLI2^T, RA1BR4, FT22^T and MP1X4^T are FN400858, DQ234446, DQ234507, FN400860 and FN400859, respectively.

Electron micrographs (Supplementary Fig. S1) and differential features (Supplementary Table S1) of the novel isolates are available with the online version of this paper.

phylogenetic analysis, these isolates represent novel members of the genus *Mucilaginibacter*.

Strains ANJLI2^T and RA1BR4 originated from oligotrophic lichen-dominated Scots pine forests located in Angeli (68° 57' N 25° 53' E) and Rajajoosseppi (68° 28' N 28° 28' E), respectively. Strain ANJLI2^T was isolated from a decaying lichen using lichenin as the growth substrate as described previously (Männistö & Häggblom, 2006). Strain RA1BR4 was isolated from a soil sample collected under a thick lichen layer after incubation at 15 °C on R2A (Difco) plates as described previously (Männistö & Häggblom, 2006). Strains MP601 and MP1X4^T were isolated from Arctic-alpine tundra soil (elevation approx. 700 m above sea-level) collected from Malla nature reserve located in Kilpisjärvi, in the far north-western part of Finland (69° 01' N 20° 50' E), using R2A agar (Difco) and xylan agar, respectively. The xylan agar contained birchwood xylan (0.6 g l⁻¹), yeast extract (0.3 g l⁻¹) and agar (15 g l⁻¹) in VL55 buffer, pH 5.5 (Sait *et al.*, 2002). Strain FT22^T was isolated from tundra soil (Malla nature reserve) microcosm after two freeze-thaw cycles at -3 °C (Männistö *et al.*, 2009). All strains were maintained on R2A agar (pH 5.5–7.0).

Genomic DNA was extracted using an UltraClean microbial isolation DNA kit (MoBio Laboratories). Nearly complete 16S rRNA genes were amplified using universal primer set 27F and 1387r as described previously (Männistö & Häggblom, 2006). The sequencing of both strands was performed using five internal 16S rRNA gene specific primers, the BigDye terminator cycle sequencing kit version 2.1 (PE Applied Biosystems) and an Applied Biosystems model 3100 sequencer. Sequencing reactions were compiled using the program package Vector NTI (Invitrogen) and sequences were compared with those available in GenBank using the program BLAST (Altschul *et al.*, 1997). Multiple alignments and phylogenetic trees were reconstructed using the program package MEGA4 (Tamura *et al.*, 2007) with the maximum composite likelihood model for nucleotide substitutions (Tamura *et al.*, 2004).

Comparative 16S rRNA gene sequence analysis showed that the novel cold-adapted strains represent three new branches within the genus *Mucilaginibacter*. Strains ANJLI2^T, MP601 and RA1BR4 had identical 16S rRNA gene sequences, whereas strains FT22^T and MP1X4^T differed from the other strains. The sequences of strains FT22^T and MP1X4^T had similarities of 94.8% and 96.4%, respectively, with that of ANJLI2^T, whereas the sequences of strains FT22^T and MP1X4^T shared 95.7% similarity. The closest relatives of strains ANJLI2^T, MP601 and RA1BR4 were the type strains of *M. rigui* (96.3% similarity), *M. ximonensis* (95.1%) and *M. paludis* (94.9%). Strain MP1X4^T was most closely related to the type strains of *M. paludis* (95.3% similarity), *M. rigui* (95.2%), *M. ximonensis* (95.2%), *M. gracilis* (95.0%) and *M. daejeonensis* (94.7%), whereas strain FT22^T was most closely related to the type strains of *M. oryzae* (94.9% similarity), *M. paludis* (94.9%), *M. rigui* (94.6%) and *M. gracilis* (94.6%). In the phylogenetic tree (Fig. 1), all of the studied strains clearly belonged to the lineage defined by the genus *Mucilaginibacter* of the family *Sphingobacteriaceae*. Strains ANJLI2^T, MP601 and RA1BR4 clustered most closely with *M. rigui*, which was supported by high bootstrap values in both the neighbour-joining and maximum-parsimony analyses. Strains FT22^T and MP1X4^T also clustered with *M. rigui* in the neighbour-joining analysis, but this was not supported by high bootstrap values or the maximum-parsimony analysis.

Cellular morphology of the strains was studied by phase-contrast microscopy after 2–14 days cultivation on R2A at 20 °C using a Leitz Laborlux S light microscope. In addition, cells of strains ANJLI2^T, FT22^T and MP1X4^T were examined using transmission electron microscopy (TEM). For TEM analysis, strains were grown in R2A broth at 4 °C and 21 °C for 7 and 2 days, respectively, and cells were fixed with 3% glutaraldehyde and negatively stained. Gram-staining was performed using the Hucker staining method (Murray *et al.*, 1994). On R2A agar (pH 6), strains ANJLI2^T, MP601 and RA1BR4 formed small, circular, convex colonies with diameters of 1–3 mm. The colony

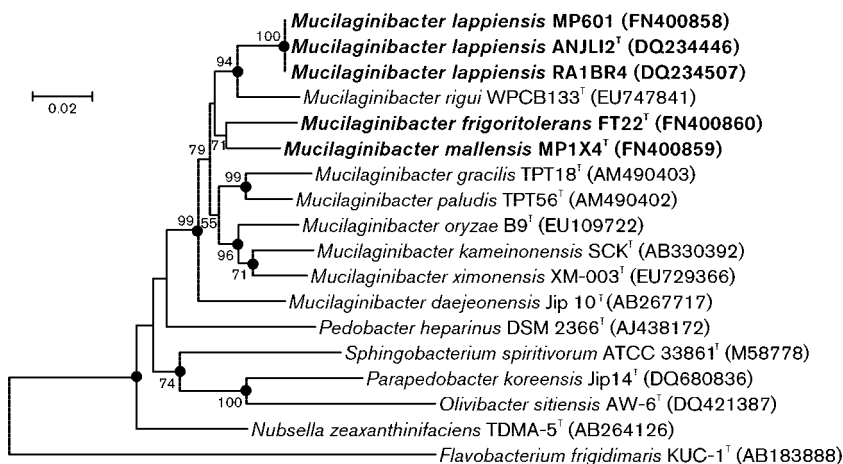


Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1327 nt positions) showing the relationships between *M. frigoritolerans*, *M. lappiensis* and *M. mallensis* strains, other species of the genus *Mucilaginibacter* and related taxa. Bootstrap values (expressed as percentages of 1000 replicates) of >50% are shown at branch points. Solid circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-parsimony tree. Bar, 0.02 substitutions per nucleotide position.

colour varied from light pink to reddish. Strains MP1X4^T and FT22^T formed larger (up to 5 mm diameter) convex, circular and mucoid colonies that were pale yellow to yellow in colour. All strains also grew well on plate count agar (BD) at pH 6.5. Strains ANJLI2^T, MP601 and RA1BR4 grew well on tryptic soy broth agar (BD) at pH 6.5, whereas strain MP1X4^T grew weakly and strain FT22^T did not grow. Cells of all strains were Gram-stain-negative, non-spore-forming, non-motile rods that occurred singly or in pairs. Cells were generally 1–5 µm long, but young cultures (1–2 days) of strains MP1X4^T, ANJLI2^T, MP601 and RA1BR4 occasionally contained cells of 5–15 µm in length or up to 40 µm long chains of cells. Old cultures (2 weeks) of all strains contained shorter (0.5–3.0 µm) cells. Spherical vesicle structures that resembled the L-forms reported in *M. paludis* and *M. gracilis* (Pankratov *et al.*, 2007) were detected in 2-week-old cultures. TEM examination revealed that the cells did not have flagella or fimbria, but the construction of the outer membrane of strain MP1X4^T occasionally resembled pilus-like filaments with a spherical vesicle in the end (see Supplementary Fig. S1 available in IJSEM Online).

Growth of the novel isolates at different temperatures (–3 to +35 °C) and NaCl concentrations (0–3%) was tested on R2A agar (pH 6). The optimum growth temperature and pH were tested on R2A broth at 18, 21, 25 and 28 °C and between pH 4.0 and 9.0. Strains ANJLI2^T, MP601 and RA1BR4 grew at pH 4.5–8.0 and at temperatures of 0–31 °C. Strain MP1X4^T grew at pH 4.5–7.0 and at –3 to 33 °C, whereas strain FT22^T grew at pH 5.0–7.0 and at 0–33 °C. Optimum growth of all strains was observed at 25 °C and pH 6.0. Strains ANJLI2^T, MP601 and RA1BR4 grew well at NaCl concentrations of 1.5%, whereas growth of strains MP1X4^T and FT22^T was inhibited at NaCl concentrations >1%. Flexirubin-type pigments were detected in strains FT22^T and MP1X4^T using the bathochromic shift test with 20% KOH (Bernardet *et al.*, 2002). All five strains were positive for oxidase and catalase activities as determined using Bactident Oxidase strips and Bactident 3% hydrogen peroxide solution (Merck), respectively. The assimilation of various carbon sources was tested after up to 7 days of incubation at 20 °C on API 50 and API 20NE strips (bioMérieux), Biolog GN Microplates and on 96-well plates with DSM 465 mineral medium (pH 6) supplemented with yeast extract (50 mg l⁻¹) and 10 mM of each carbon source. Acid production from carbohydrates was examined using API 50 strips that were inoculated with cultures suspended in MM1 medium (Pankratov *et al.*, 2007) at pH 6.8 and supplemented with 0.5 g yeast extract l⁻¹. After 3 days of incubation at room temperature (22 ± 1 °C), 25 µl methyl red (0.1%) was added to the cupules as a pH indicator. The formation of red colour indicated a positive reaction, whereas an orange colour indicated a weak positive reaction compared to the yellow colour of the control (i.e. no substrate). Enzymic activities in all novel isolates were assayed after 48 h of incubation using the API ZYM

kits (bioMérieux). Hydrolysis of CM-cellulose, birchwood xylan and lichenin (main polysaccharide in lichen) was tested on agar plates following Congo red staining after 3–14 days of incubation at room temperature as described previously (Männistö & Häggblom, 2006).

The DNA G+C contents of the strains were determined following the method of Mesbah *et al.* (1989). Genomic DNA was extracted and purified by phenol/chloroform extraction using a method modified from Kerkhof & Ward (1993). RNase A (50 µg ml⁻¹) was added and the mixture was incubated at 37 °C for 30 min, followed by addition of 50 µl 10% SDS and 50–100 µl of 100 µg proteinase K ml⁻¹ and incubation at 37 °C for 1–12 h until loss of turbidity and viscosity was observed. The DNA G+C content (mol%) was determined by HPLC using the method described by Mesbah *et al.* (1989) with the following modifications: a Synergi 4U Fusion-RP 80A C₁₈ reverse phase column (Phenomenex) was used in a Shimadzu HPLC (model LC-10AS) system. The eluent consisted of 7% 20 mM ammonium acetate (pH 4.5) and 93% acetonitrile at a flow rate of 1 ml min⁻¹. Nucleosides were detected at a wavelength of 260 nm. Salmon sperm DNA was used for calibration and *Escherichia coli* DNA was used as a control. The range of DNA G+C contents (44.0–46.5 mol%) was similar to that of other species of the genus *Mucilagibacter* (Table 1).

Cellular fatty acids and respiratory quinones were analysed from cells grown on R2A (pH 6) at 20 °C for 3 days. Analysis of respiratory quinones of strains ANJLI2^T, FT22^T and MP1X4^T was carried out by the Identification Service and Dr B. J. Tindall, DSMZ, Braunschweig, Germany. Total fatty acids were methylated and analysed as described previously (Männistö & Häggblom, 2006) using an HP 5890 series II GC (Hewlett Packard) equipped with Sherlock MIDI Software. The identification of fatty acid methyl esters was also confirmed using an HP GCD Plus GC-MS. The major cellular fatty acids (>4%) of the five strains were: summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH), iso-C_{15:0}, iso-C_{17:0} 3-OH, C_{16:1}ω5c and C_{16:0} (Table 2). Although phylogenetic analysis indicated a close relationship between the novel isolates and *Mucilagibacter rigui*, the fatty acid composition of the isolates was clearly distinguishable from that of *M. rigui*. *M. rigui* contained high amounts of anteiso-C_{15:0}, whereas only traces of summed feature 3 were detected (Table 2). All five strains reported here contained >30% summed feature 3 fatty acids (C_{16:1}ω7c and iso-C_{15:0} 2-OH in approximately equal amounts) and only traces of anteiso-C_{15:0}. The major respiratory quinone of strains ANJLI2^T, FT22^T and MP1X4^T was menaquinone-7 (MK-7); minor quantities (2–3%) of MK-6 were detected in all three strains.

Phylogenetic analysis, DNA G+C content (44.0–46.5 mol%), major cellular fatty acids (iso-C_{15:0}, summed feature 3 and iso-C_{17:0} 3-OH) and MK-7 as the major quinone associated the strains with members of the genus *Mucilagibacter*. However, the degree of 16S rRNA gene

Table 1. Physiological characteristics of the novel isolates and the type strains of species of the genus *Mucilaginibacter*

Strains: 1, ANJLI2^T; 2, RA1BR4; 3, MP601; 4, FT22^T; 5, MP1X4^T; 6, *M. paludis* TPT56^T (data from Pankratov *et al.*, 2007); 7, *M. gracilis* TPT18^T (Pankratov *et al.*, 2007); 8, *M. daejeonensis* Jip 10^T (An *et al.*, 2009); 9, *M. ximonensis* XM-003^T (Luo *et al.*, 2009); 10, *M. oryzae* B9^T (Jeon *et al.*, 2009); 11, *M. rigui* WPCB133^T (Baik *et al.*, 2010); 12, *M. kameinonensis* SCK^T (Urai *et al.*, 2008). All strains are positive for acid production from cellobiose, galactose and maltose and catalase and oxidase activities. All strains are negative for Gram-staining and indole production. +, Positive; -, negative; w, weakly positive reaction; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Isolation source	Lichen	Tundra soil	Forest soil	Freeze-thaw-treated tundra soil	Tundra soil	Peat bog	Peat bog	Dried rice straw	Tibetan soil	Rice paddy soil	Wetland freshwater	Garden soil
Growth temperature range (°C)	0–31	0–31	0–31	0–33	–3 to 33	2–33	2–33	15–37	10–33	15–35	4–37	5–30
pH range	4.5–8.0	4.5–8.0	4.5–8.5	5.0–7.0	4.5–7.0	4.2–8.2	4.2–8.2	4.5–8.5	5.0–8.0	5.0–8.0	5.0–10.0	4.0–9.0
Pigment	Pale red	Pale red	Pale red	Yellow	Pale yellow	Pink	Yellow/orange	Orange	Pale yellow	Pale pink	Pale pink	Pale pink
Flexirubin-type pigments	–	–	–	+	+	–	–	NA	–	–	–	+
Oxidase activity	+	+	+	+	+	+	+	+	+	+	+	–
Nitrate reduction	–	–	–	–	+	–	–	–	–	–	–	–
Growth with >1 % NaCl	+	+	+	–	–	–	–	+	–	+	–	–
Hydrolysis of aesculin	+	+	+	+	+	–	–	+	+	+	+	+
Hydrolysis of gelatin	+	+	+	–	–	NA	NA	NA	NA	+	–	NA
Arginine dihydrolase	+	+	+	–	–	NA	NA	+	–	NA	–	–
H ₂ S production	–	–	–	–	–	–	–	NA	–	–	–	–
Assimilation of:												
Acetic acid	–	–	–	–	–	–	–	–	+	+	–	–
Dextrin	+	+	+	+	–	NA	NA	NA	+	+	+	+
Glycogen	–	–	–	+	–	NA	NA	–	–	+	–	+
D-Glucose	+	+	+	+	+	+	+	+	–	–	+	+
D-Mannose	+	+	+	+	+	+	–	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	–	–	+	+
Gluconic acid	–	–	–	–	–	+	–	NA	NA	+	–	–
Melibiose	+	+	+	+	+	–	–	–	–	+	+	+
Raffinose	+	+	+	–	–	–	+	NA	+	+	+	+
myo-Inositol	–	–	–	–	–	+	–	–	–	–	–	–
N-Acetyl-D-galactosamine	+	+	+	–	–	NA	NA	NA	–	–	–	–
N-Acetyl-D-glucosamine	+	+	+	+	+	+	–	+	+	w	+	w
D-Galacturonic acid	–	–	–	–	–	+	–	NA	–	–	–	–
Acid production from:												
D-Arabinose	+	+	+	–	–	NA	NA	+	NA	NA	–	+
L-Arabinose	–	–	–	+	+	NA	NA	+	NA	+	–	+
D-Glucose	+	+	+	+	+	+	+	+	–	+	–	+
D-Fructose	–	–	–	w	+	+	+	+	+	+	–	+
D-Mannose	w	w	w	+	+	+	–	+	+	+	+	+
Melezitose	–	–	–	+	–	NA	NA	–	–	NA	–	+
Melibiose	+	+	+	+	+	–	–	+	w	+	+	+
L-Rhamnose	–	–	–	–	–	+	–	+	NA	NA	–	–
Sucrose	+	w	w	+	+	+	+	+	–	+	+	+
D-Xylose	–	–	–	–	–	+	+	–	+	NA	–	+
Trehalose	+	w	w	+	+	–	+	+	+	+	+	+
L-Xylose	–	–	–	–	–	NA	NA	–	NA	NA	NA	+
Quinone(s)	MK-7, MK-6	NA	NA	MK-7, MK-6	MK-7, MK-6	MK-7, MK-6	MK-7, MK-6	MK-7	MK-7	MK-7	MK-7	MK7

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
DNA G+C content (mol%)	43.5	46.5	44.0	44.0	46.0	46.1	42.4	40.4	43.4	44.4	47	43.7

sequence divergence (>3%) and a number of phenotypic and chemotaxonomic features (Table 1, Table 2 and Supplementary Table S1) distinguish them from other species of the genus *Mucilaginibacter*. Strains ANJLI2^T, MP601 and RA1BR4 were nearly identical in their physiological, phylogenetic and chemotaxonomic characteristics

and should be placed in the same species. Based on the phylogenetic, phenotypic and chemotaxonomic data, it is concluded that the strains represent three novel species of the genus *Mucilaginibacter* with the proposed names *Mucilaginibacter frigitolerans* sp. nov., *Mucilaginibacter lappiensis* sp. nov. and *Mucilaginibacter mallensis* sp. nov.

Table 2. Cellular fatty acid composition (%) of the novel isolates and the type strains of species of the genus *Mucilaginibacter*

Strains: 1, ANJLI2^T; 2, RA1BR4; 3, MP601; 4, FT22^T; 5, MP1X4^T; 6, *M. paludis* TPT56^T (data from Pankratov *et al.*, 2007); 7, *M. gracilis* TPT18^T (Pankratov *et al.*, 2007); 8, *M. daejeonensis* Jip 10^T (An *et al.*, 2009); 9, *M. ximonensis* XM-003^T (Luo *et al.*, 2009); 10, *M. oryzae* B9^T (Jeon *et al.*, 2009); 11, *M. rigui* WPCB133^T (Baik *et al.*, 2010); 12, *M. kameinonensis* SCK^T (Baik *et al.*, 2010). tr, Trace (<0.3%); –, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
C _{14:0}	0.3	0.4	0.5	0.4	tr	tr	tr	–	1.3	–	tr	2.4
C _{15:0}	tr	0.4	tr	1.2	1.3	3.2	4.5	–	–	–	–	1.7
C _{16:0}	3.7	5.4	4.8	3.1	4.8	3.1	tr	9.3	6.8	8.5	tr	25.2
C _{17:0}	–	–	–	–	–	tr	tr	–	–	–	–	1.3
C _{18:0}	–	–	tr	–	tr	tr	–	1.7	1.4	–	–	19.5
C _{15:1} ω6c	–	–	–	1.1	tr	tr	1.2	–	–	–	–	–
C _{16:1} ω5c	6.8	6.6	7.9	2.7	4.0	5.8	2.6	5.9	3.1	–	–	1.3
C _{17:1} ω8c	–	–	–	–	–	tr	1.5	–	–	–	–	–
C _{17:1} ω6c	–	–	–	–	–	tr	tr	–	–	–	–	–
C _{18:1} ω5c	–	–	–	–	–	–	–	–	–	–	1.4	–
C _{18:1} ω7c	–	–	–	–	–	–	–	–	–	–	–	2.9
C _{18:1} ω9c	tr	tr	0.5	tr	0.5	tr	–	–	–	–	–	2.5
C _{18:3} ω6c	–	–	–	–	–	–	–	1.3	–	–	–	–
iso-C _{14:0}	–	–	–	–	–	–	–	–	–	–	3.3	–
iso-C _{15:0}	18.9	16.9	17.6	21.9	26.0	15.7	32.2	26.5	26.2	23.9	34.1	10.0
iso-C _{16:0}	tr	0.4	1.5	tr	–	1.5	tr	–	–	–	1.3	–
iso-C _{17:0}	tr	0.3	tr	0.6	1.2	tr	tr	–	2.0	–	1.1	–
iso-C _{17:1}	2.7	2.3	2.7	4.1	9.5	tr	2.7	3.0	6.2	–	3.4	–
anteiso-C _{15:0}	–	tr	–	1.6	0.9	21.1	5.8	1.8	–	–	26.7	–
anteiso-C _{17:0}	–	–	–	–	0.4	4.5	–	–	–	–	–	–
C _{15:0} 2-OH	–	–	–	0.3	tr	tr	tr	–	–	–	2.1	–
C _{15:0} 3-OH	0.4	0.5	0.7	0.4	0.3	–	tr	–	–	–	–	–
C _{16:0} 2-OH	tr	–	–	–	0.3	–	–	–	–	–	–	–
C _{16:0} 3-OH	3.2	2.6	2.9	0.6	tr	tr	tr	2.3	1.5	–	–	2.3
C _{17:0} 2-OH	tr	–	–	0.9	1.4	tr	tr	–	–	–	1.1	–
C _{17:0} 3-OH	–	–	–	–	–	tr	tr	–	–	–	–	–
iso-C _{13:0} 3-OH	0.4	1.0	0.3	–	–	tr	tr	–	–	–	–	–
iso-C _{15:0} 3-OH	2.2	3.0	2.0	2.9	2.9	1.8	3.2	2.2	1.9	–	5.9	–
iso-C _{16:0} 3-OH	–	–	–	tr	0.3	tr	tr	–	–	–	2.6	–
iso-C _{17:0} 3-OH	14.3	13.5	13.9	16.4	12.5	8.9	13.1	7.1	10.0	5.4	5.4	6.3
Summed feature 2*	0.7	0.7	0.7	–	–	–	–	–	–	–	–	–
Summed feature 3*	45.0	45.4	43.3	38.7	32.9	24.7	21.5	39.1	26.7	43.7	tr	24.2

*Summed features: 2, iso-C_{16:1} and/or C_{14:0} 3-OH; 3, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

Description of *Mucilaginibacter frigitolerans* sp. nov.

Mucilaginibacter frigitolerans (fri.go.ri.to'le.rans. L. neut. n. *frigus*, *frigoris* the cold; L. part. adj. *tolerans* tolerating; N.L. part. adj. *frigitolerans* cold tolerating).

Cells are Gram-negative, non-motile rods, 0.3–0.5 µm wide and 1–4 µm long, that occur singly or in pairs. Colonies on R2A agar are yellow, circular, convex and smooth. Grows at 0 to 33 °C and pH 5–7, with optimum growth at 25 °C and pH 6. Growth is inhibited at NaCl concentrations >1%. Aerobic. Catalase- and oxidase-positive. Hydrolyses CM-cellulose, lichenin and aesculin, but not gelatin. Positive for acid production from L-arabinose, D-glucose, D-mannose, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, gentiobiose and turanose. Negative for acid production from ribose, D-arabinose, D- and L-xylose, L-sorbose, L-rhamnose, dulcitol, inositol, mannitol, sorbitol, raffinose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D- and L-fucose, and D- and L-arabitol. Weakly positive for acid production from galactose, D-fructose, *N*-acetylglucosamine and inulin. Assimilates L-arabinose, cellobiose, D-glucosamine, maltose, D-mannose, sucrose, xylose, trehalose, D-galactose, D-arabinose, D-glucose and lactose, but not D-ribose, D-sorbitol, D-glycine, succinate, formate, L-ornithine, glucuronate, maleic acid, pyruvate, acetate, mannitol, D- and L-alanine, or L-leucine. Positive for oxidation of the following Biolog GN2 microplate substrates: α -cyclodextrin, dextrin, glycogen, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, gentiobiose, α -D-glucose, α -lactose, lactulose, maltose, D-mannose, melibiose, methyl β -D-glucoside, sucrose, trehalose, turanose, L-alanyl-glycine, L-glutamic acid, L-proline, L-serine, L-threonine, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and α -D-glucose 6-phosphate. Weakly positive for the oxidation of D-galactose. Negative for the oxidation of Biolog GN2 substrates acetic acid, *N*-acetyl-D-galactosamine, *cis*-aconitic acid, adonitol, L-alaninamide, D- and L-alanine, γ -aminobutyric acid, 2-aminoethanol, L-arabinose, D-arabitol, L-asparagine, L-aspartic acid, bromosuccinic acid, 2,3-butanediol, D- and L-carnitine, citric acid, i-erythritol, formic acid, L-fucose, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, glucuronamide, D-glucuronic acid, glycolyl L-aspartic acid, glycolyl L-glutamic acid, L-histidine, α -, β - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, L-hydroxyproline, inosine, *myo*-inositol, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, D- and L-lactic acid, L-leucine, malonic acid, D-mannitol, L-ornithine, L-phenylalanine, phenylethylamine, propionic acid, D-psicose, putrescine, L-pyroglytamic acid, pyruvic acid methyl ester, quinic acid, raffinose, L-rhamnose, D-saccharic acid, sebacic acid, D-serine, D-sorbitol, succinamic acid, succinic acid, succinic acid monomethyl ester, thymidine, Tweens 40 and 80, uridine, urocanic acid and xylitol. Produces alkaline phosphatase, esterase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase,

N-acetyl- β -glucosaminidase and α -mannosidase, but not trypsin, α -chymotrypsin, β -glucuronidase or α -fucosidase. Weakly positive for the following activities (API ZYM): esterase (C4), lipase (C14) and cystine arylamidase. The major isoprenoid quinone is MK-7. The major cellular fatty acids are summed feature 3 (C_{16:1} ω 7*c*/iso-C_{15:0} 2-OH), iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{17:1}, C_{16:0}, iso-C_{15:0} 3-OH and C_{16:1} ω 5*c*.

The type strain is FT22^T (=ATCC BAA-1854^T =LMG 25359^T), isolated from freeze-thaw treated tundra soil collected from Malla nature reserve, northern Finland. The DNA G + C content of the type strain is 44.0 mol%.

Description of *Mucilaginibacter lappiensis* sp. nov.

Mucilaginibacter lappiensis [lap.pi.en'sis. N.L. masc. adj. *lappiensis* pertaining to Lapland (Finn. *Lappi*)].

Cells are Gram-negative, non-motile straight rods, 0.3–0.5 µm wide and 1–3 µm long, that occur singly or in pairs. Colonies grown on R2A agar are light pink to reddish, circular, convex and smooth. Growth occurs from 0 to 31 °C and at pH 4.5–8.0, with optimum at 25 °C and pH 6. Growth is inhibited at NaCl concentrations >1.5%. Aerobic. Catalase- and oxidase-positive. Hydrolyses CM-cellulose, lichenin, aesculin and gelatin, but not xylan. Positive for acid production from D-arabinose, D-galactose, D-glucose, amygdalin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, gentiobiose and L-fucose. Negative for acid production from ribose, L-arabinose, D-fructose, D- and L-xylose, L-sorbose, L-rhamnose, dulcitol, inositol, inulin, melezitose, mannitol, sorbitol, raffinose, starch, glycogen, xylitol, D-lyxose, turanose, D-tagatose, D-fucose, and D- and L-arabitol. Weakly positive for acid production from D-mannose, *N*-acetylglucosamine and arbutin. Assimilates L-arabinose, cellobiose, D-glucosamine, maltose, D-mannose, sucrose, xylose, trehalose, D-galactose, D-arabinose, D-glucose and lactose, but not D-ribose, D-sorbitol, D-glycine, succinate, formate, L-ornithine, glucuronate, maleic acid, pyruvate, acetate, mannitol, D-alanine or L-leucine. Positive for oxidation of the following Biolog GN2 microplate substrates: dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, cellobiose, L-fucose, D-galactose, gentiobiose, α -D-glucose, α -lactose, lactulose, maltose, D-mannose, melibiose, methyl β -D-glucoside, raffinose, sucrose, trehalose, turanose, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, L-proline and glycerol. Weakly positive for oxidation of D-fructose, α -D-glucose 1-phosphate, α -D-glucose 6-phosphate, and D- and L- α -glycerol phosphate. Oxidation of D-gluconic acid and L-ornithine varies between strains. Negative for oxidation of the Biolog GN2 substrates acetic acid, *cis*-aconitic acid, adonitol, L-alaninamide, D-alanine, γ -aminobutyric acid, 2-aminoethanol, L-arabinose, D-arabitol, L-aspartic acid, bromosuccinic acid, 2,3-butanediol, D- and L-carnitine, citric acid, α -cyclodextrin, i-erythritol, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic

acid, glucuronamide, glycogen, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, α -, β - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, L-hydroxyproline, inosine, *myo*-inositol, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, D- and L-lactic acid, malonic acid, D-mannitol, L-phenylalanine, phenylethylamine, propionic acid, D-psicose, putrescine, L-pyroglutamic acid, pyruvic acid methyl ester, quinic acid, L-rhamnose, D-saccharic acid, sebacic acid, D-serine, D-sorbitol, succinamic acid, succinic acid, succinic acid monomethyl ester, thymidine, Tweens 40 and 80, uridine, urocanic acid and xylitol. Produces alkaline phosphatase, esterase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, but not α -chymotrypsin or lipase. Weakly positive (API ZYM) for esterase (C4) and β -glucuronidase. The major isoprenoid quinone is MK-7. The major cellular fatty acids are summed feature 3 ($C_{16:1\omega7d}/iso-C_{15:0}$ 2-OH), $iso-C_{15:0}$, $iso-C_{17:0}$ 3-OH, $C_{16:1\omega5c}$, $C_{16:0}$, $C_{16:0}$ 3-OH and $iso-C_{17:1}$. The DNA G + C content is 43.5–46.5 mol%.

The type strain is ANJLI2^T (=ATCC BAA-1855^T =LMG 25358^T), isolated from decaying lichen collected in an oligotrophic Scots pine forest at Angeli, Northern Finland. Two reference strains are MP601 and RA1BR4.

Description of *Mucilagibacter mallensis* sp. nov.

Mucilagibacter mallensis (mal.len'sis. N.L. masc. adj. *mallensis* pertaining to Malla Nature Reserve, Finland).

Cells are Gram-negative, non-motile, straight rods, 0.4–0.6 μ m wide and 1–2 μ m long that occur singly or in pairs. Colonies grown on R2A agar are yellow, circular, convex and smooth. Growth occurs between –3 and 33 °C and at pH 4.5–7.0, with optimum at 25 °C and pH 6. Growth is inhibited at NaCl concentrations >1%. Catalase- and oxidase-positive. Hydrolyses CM-cellulose, xylan and aesculin, but not gelatin or lichenin. Reduces nitrate to nitrite. Positive for acid production from L-arabinose, galactose, D-glucose, D-fructose, D-mannose, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, raffinose and gentiobiose. Negative for acid production from ribose, D-arabinose, D- and L-xylose, L-sorbose, L-rhamnose, dulcitol, inositol, mannitol, melezitose, sorbitol, glycogen, xylitol, D-lyxose, D-tagatose, turanose, D- and L-fucose, and D- and L-arabitol. Weakly positive for acid production from *N*-acetylglucosamine and starch. Assimilates D- and L-arabinose, cellobiose, D-glucosamine, maltose, D-mannose, sucrose, xylose, trehalose, D-galactose, D-glucose and lactose, but not D-ribose, D-sorbitol, D-glycine, succinate, formate, L-ornithine, glucuronate, maleic acid, pyruvate, acetate, mannitol, D- and L-alanine, or L-leucine. Positive for oxidation of the following Biolog GN2 microplate substrates: α -cyclodextrin, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α -D-glucose, α -lactose, maltose, D-mannose, melibiose,

methyl α -D-glucoside, sucrose, trehalose, turanose, L-alanyl-glycine, L-glutamic acid and glycerol. Weakly positive for the oxidation of L-fucose, L-proline and L-threonine. Negative for oxidation of the Biolog GN2 substrates acetic acid, *N*-acetyl-D-galactosamine, *cis*-aconitic acid, adonitol, L-alaninamide, D- and L-alanine, γ -aminobutyric acid, 2-aminoethanol, L-arabinose, D-arabitol, L-asparagine, L-aspartic acid, bromosuccinic acid, 2,3-butanediol, D- and L-carnitine, citric acid, dextrin, i-erythritol, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, glucose 1-phosphate, glucose 6-phosphate, glucuronamide, D-glucuronic acid, D- and L- α -glycerol phosphate, glycogen, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, α -, β - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, L-hydroxyproline, inosine, *myo*-inositol, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, D- and L-lactic acid, L-leucine, malonic acid, D-mannitol, L-ornithine, L-phenylalanine, phenylethylamine, propionic acid, D-psicose, putrescine, L-pyroglutamic acid, pyruvic acid methyl ester, quinic acid, raffinose, L-rhamnose, D-saccharic acid, sebacic acid, D- and L-serine, D-sorbitol, succinamic acid, succinic acid, succinic acid monomethyl ester, thymidine, Tweens 40 and 80, uridine, urocanic acid and xylitol. Produces alkaline phosphatase, esterase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase and α -fucosidase, but not lipase, cystine arylamidase, trypsin or α -chymotrypsin. Weakly positive for the following activities (API ZYM): esterase (C4), β -glucosidase, β -glucuronidase and α -mannosidase. The major isoprenoid quinone is MK-7. The major cellular fatty acids are summed feature 3 ($C_{16:1\omega7d}/iso-C_{15:0}$ 2-OH), $iso-C_{15:0}$, $iso-C_{17:0}$ 3-OH, $iso-C_{17:1}$, $C_{16:0}$, $iso-C_{15:0}$ 3-OH and $C_{16:1\omega5c}$.

The type strain is MP1X4^T (=ATCC BAA-1856^T =LMG 25360^T), isolated from tundra soil of a wind-exposed ridge in Malla nature reserve, northern Finland. The DNA G + C content of the type strain is 46.0 mol%.

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