Mucilaginibacter myungsuensis sp. nov., isolated from a mesotrophic artificial lake

Yochan Joung and Kiseong Joh

Correspondence

Kiseong Joh
ksjoh@hufs.ac.kr

Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Wangsan, Yongin, Gyeonggi 449-791, Republic of Korea

A non-motile, pale-yellow bacterium, designated strain HMD1056^T, was isolated from an artificial lake located within the campus of Hankuk University of Foreign Studies, Yongin, Korea. The major fatty acids were summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 49.1 %) and iso-C_{15:0} (22.4 %). The major respiratory quinone was MK-7. The DNA G+C content was 46.9 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain HMD1056^T formed a lineage within the genus *Mucilaginibacter* and was closely related to the type strains of *Mucilaginibacter ximonensis* (95.4 % sequence similarity), *Mucilaginibacter kameinonensis* (94.5 %) and *Mucilaginibacter paludis* (93.4 %). On the basis of the evidence presented in this study, strain HMD1056^T represents a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter myungsuensis* sp. nov. is proposed. The type strain is HMD1056^T (=KCTC 22746^T =CECT 7550^T).

The genus *Mucilaginibacter* was proposed by Pankratov *et al.* (2007) and members of the genus are characterized by the ability to produce large amounts of extracellular polymeric substances and to hydrolyse pectin, xylan, pullulan and starch. Strains of *Mucilaginibacter* may also contain iso-C_{15:0} as the major fatty acid and other hydroxy fatty acids as major components. At the time of writing, the genus *Mucilaginibacter* includes 12 species with validly published names: *Mucilaginibacter paludis* (the type species) and *M. gracilis* (Pankratov *et al.*, 2007), *M. kameinonensis* (Urai *et al.*, 2008), *M. daejeonensis* (An *et al.*, 2009), *M. ximonensis* (Luo *et al.*, 2009), *M. oryzae* (Jeon *et al.*, 2009), *M. rigui* (Baik *et al.*, 2010), *M. gossypii* and *M. gossypicola* (Madhaiyan *et al.*, 2010) and *M. frigoritolerans*, *M. lappiensis* and *M. mallensis* (Männistö *et al.*, 2010). *Mucilaginibacter* species have been isolated from rice straw, various soils, acidic *Sphagnum* peat bog, freshwater and lichen.

In the course of a study on the microbial diversity of a mesotrophic artificial lake within the campus of Hankuk University of Foreign Studies in Yongin, Republic of Korea, pale-yellow colonies were isolated on R2A agar at 30 °C using the standard dilution plating technique. Isolate HMD1056^T was maintained on R2A agar at 30 °C and the culture was suspended in water containing 20 % glycerol (w/v) for storage at –80 °C.

Almost-complete sequences of the 16S rRNA gene were obtained for strain HMD1056^T, as described previously (Cho & Giovannoni, 2003). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were performed by using the EzTaxon

server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA version 4 (Tamura *et al.*, 2007). Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms. The robustness of the topologies for the maximum-likelihood and neighbour-joining trees was evaluated by means of bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the sequences. All phylogenetic trees generated in this study indicated that strain HMD1056^T belonged to the genus *Mucilaginibacter* (Fig. 1). Strain HMD1056^T formed a coherent clade with *M. ximonensis* XM-003^T (95.4 %) within the phylogenetically well-resolved *Mucilaginibacter* clade. This phylogenetic inference, coupled with 16S rRNA gene sequence similarities of <97 % (Wayne *et al.*, 1987) between strain HMD1056^T and strains of other *Mucilaginibacter* species, suggested that the strain should be assigned to the genus *Mucilaginibacter* as a representative of a novel species.

M. paludis KACC 13450^T, *M. gracilis* KACC 13451^T, *M. kameinonensis* KCTC 22227^T and *M. ximonensis* KCTC 22437^T were used as reference strains for phenotypic analysis and determination of cellular fatty acid profiles. Cell morphology was examined by light microscopy. Gram staining was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Anaerobic growth was tested on R2A agar at 25 °C by using a GasPak EZ anaerobic container system (BD) according to the manufacturer's instructions. Cellular pigments were extracted with acetone/methanol (1:1, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (UV 6101A; Shimadzu). The

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain HMD1056^T is GQ144415.

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain HMD1056^T and strains of the genus *Mucilaginibacter*. Percentages at nodes are levels of bootstrap support (>50 %) based on neighbour-joining analyses of 1000 resampled datasets. Filled and open circles respectively indicate nodes recovered by all three treeing methods or by two treeing methods. *Sphingobacterium spiritivorum* ATCC 33861^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

presence of flexirubin-type pigments was investigated by using the bathochromatic shift test with a KOH solution (Bernardet *et al.*, 2005; Fautz & Reichenbach, 1980). Catalase and oxidase tests were performed according to standard methods (MacFaddin, 1980). The temperature range and optimum for growth were assessed on MA at 4, 10–30 °C (at 5 °C intervals), 37 and 42 °C over 72 h. The pH range for growth was determined in R2A broth at 20 °C. The medium was adjusted to pH 5.0–10.0 (at intervals of 1.0 pH unit) using 0.1 mM sodium acetate buffer (pH 5.0–7.0), 0.1 mM phosphate buffer (pH 7.0–8.0) and 0.1 mM sodium carbonate buffer (pH 9.0–10.0). Growth in the presence of 0.5 % and 1.0–5.0 % (at intervals of 1.0 %) NaCl (w/v) was also tested in R2A broth. Hydrolysis of casein [3 % skimmed milk (Difco), v/v], CM-cellulose [1.0 % CM-cellulose (Sigma), w/v], crystalline cellulose (Whatman No. 1 filter paper, 1 % w/v) and starch (1.0 %, w/v) was tested using R2A agar as the basal medium. MacConkey agar (Difco) and DNase test agar (Difco) were used to test for growth and production of DNase, respectively. Other biochemical tests and carbon source oxidation tests were performed using API 20NE, API 50CH and API ZYM strips (bioMérieux) and Biolog GN2 MicroPlates, according to the manufacturers' instructions. The DNA G+C content was determined by using HPLC (Mesbah & Whitman, 1989). For fatty acid methyl ester analysis, strain HMD1056^T and the four reference strains were grown on MA at 25 °C for 3 days and fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Isoprenoid quinones were isolated according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985).

Morphological, cultural, physiological and biochemical characteristics of strain HMD1056^T are listed in Table 1 and in the species description. Strain HMD1056^T exhibited a number of phenotypic similarities to species of the genus *Mucilaginibacter*, including growth temperature range, pale-yellow pigmentation and obligately aerobic growth. However, several characteristics of HMD1056^T clearly differentiated this strain from the type strains of *Mucilaginibacter* species (Table 1).

The fatty acid profile of strain HMD1056^T contained summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 49.1 %) and iso-C_{15:0} (22.4 %) as major components. The complete fatty acid profile of strain HMD1056^T is given in Table 2. The major fatty acid profile was similar to those of other *Mucilaginibacter* species, although there were differences in the relative proportions of some fatty acids (Table 2). The respiratory quinones were MK-7 (major component) and MK-6 (minor), as reported for other members of genus *Mucilaginibacter*. The DNA G+C content of strain HMD1056^T was 46.9 mol%, a value within the range reported for members of genus *Mucilaginibacter*. The phenotypic data confirm the phylogenetic affiliation of strain HMD1056^T to the genus *Mucilaginibacter*. Therefore, strain HMD1056^T should be classified in the genus *Mucilaginibacter* as a member of novel species, for which the name *Mucilaginibacter myungsuensis* sp. nov. is proposed.

Description of *Mucilaginibacter myungsuensis* sp. nov.

Mucilaginibacter myungsuensis (myung.su.en'sis. N.L. masc. adj. *myungsuensis* pertaining to Myungsu, the name of the mesotrophic freshwater lake from which the type strain was isolated).

Cells are Gram-negative, non-motile, aerobic rods, 0.7–0.8 µm in diameter and 1.4–1.5 µm long. Colonies on R2A are pale yellow, mucoid, convex and round and approximately 5 mm in diameter after 2 days at 25 °C. Good growth occurs on blood agar, TSA and R2A agar. No growth occurs on DNA test agar or MacConkey agar. Growth occurs in the presence of 0–1.0 % (w/v) NaCl (optimum 0.5 %), at pH 5–9 (optimum pH 7) and at 10–30 °C (optimum 30 °C). Oxidase and catalase activities and aesculin hydrolysis activity are present, but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and L-phenylalanine deaminase activities are absent. Casein and starch are hydrolysed, but gelatin and tyrosine are not. Citrate is not utilized. Flexirubin-type pigments are produced. In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine

Table 1. Differential characteristics of strain HMD1056^T and type strains of closely related *Mucilaginibacter* species

Strains: 1, HMD1056^T; 2, *M. paludis* KACC 13450^T; 3, *M. gracilis* KACC 13451^T; 4, *M. kameinonensis* KCTC 22227^T; 5, *M. ximonensis* KCTC 22437^T (data in columns 1–5 from this study unless indicated); 6, *M. gossypii* Gh-67^T; 7, *M. gossypicola* Gh-48^T (data in columns 6 and 7 from Madhaiyan *et al.*, 2010); 8, *M. frigorigerans* FT22^T; 9, *M. lappiensis* ANJL12^T; 10, *M. mallensis* MP1X4^T (data in columns 8–10 from Männistö *et al.*, 2010). +, Positive; w, weakly positive; –, negative; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Isolation source	Water	<i>Sphagnum</i> peat bog ^{ax}	<i>Sphagnum</i> peat bog ^a	Soil ^b	Soil ^c	Soil	Soil	Tundra soil	Lichen	Tundra soil
DNA G + C content (mol%)	46.9	46.1 ^a	42.4 ^a	43.7 ^b	43.4 ^c	46.7	44.2	44.0	43.5	46.0
Major menaquinone(s)	7, 6	7, 6 ^a	7, 6 ^a	7 ^b	7 ^c	7	7	7, 6	7, 6	7, 6
Growth temperature (°C)	10–30	10–30	10–30	10–30	10–30	20–30	20–30	0–33	0–31	–3 to 33
Flexirubin-type pigments	+	–	–	+	–	NA	NA	+	–	+
API 20NE results										
Glucose fermentation	–	–	–	+	+	–	–	–	–	–
Nitrate reduction	–	–	–	–	–	+	+	–	–	+
Urease	–	–	–	–	–	+	–	–	–	–
Gelatinase	–	–	–	–	–	–	–	–	+	–
API ZYM results										
Esterase (C4)	+	+	+	+	–	NA	NA	–	–	–
Cystine arylamidase	+	–	–	+	–	NA	NA	–	+	–
Trypsin	+	–	–	+	–	NA	NA	–	+	–
β-Glucuronidase	–	–	+	+	+	NA	NA	–	–	–
β-Galactosidase	–	+	+	+	+	NA	NA	+	+	+
α-Glucosidase	+	+	+	+	+	NA	NA	+	+	+
β-Glucosidase	–	–	+	+	+	NA	NA	+	+	–
Acid production from:										
D-Arabinose	+	+	–	+	–	–	–	–	+	–
L-Arabinose	–	+	+	–	–	–	–	+	–	+
D-Xylose	+	+	+	–	–	+	+	–	–	–
D-Glucose	+	+	+	+	–	+	+	+	+	+
D-Fructose	+	+	+	+	–	–	–	w	–	+
D-Mannose	+	+	+	+	+	+	+	+	w	+
L-Rhamnose	–	–	–	–	–	+	–	–	–	–
Melibiose	+	+	+	+	+	+	w	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Melezitose	+	–	–	+	–	+	+	+	–	+
Utilization of (Biolog GN2):										
α-Cyclodextrin	–	–	+	–	–	+	+	+	–	+
Dextrin	–	+	+	+	–	+	+	+	+	–
Glycogen	–	+	+	–	–	+	+	–	–	–
N-Acetyl-D-galactosamine	–	–	–	–	–	+	+	–	+	–
N-Acetyl-D-glucosamine	–	–	–	–	–	+	+	+	+	+
Adonitol	–	–	–	–	–	+	+	–	–	–
Cellobiose	+	+	–	–	+	+	+	+	+	+
D-Fructose	+	+	+	–	–	+	–	+	w	+
L-Fucose	–	+	+	–	–	+	+	–	+	w
D-Galactose	–	+	+	–	–	+	+	w	+	+
Gentiobiose	+	+	–	+	+	+	+	+	+	+
Lactose	–	+	–	–	–	+	+	+	+	+
Lactulose	–	–	–	–	+	+	+	+	+	–
Maltose	+	+	+	+	–	+	+	+	+	+
D-Mannose	–	+	+	+	+	+	+	+	+	+
Melibiose	–	+	+	+	+	+	+	+	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10
Methyl β -D-glucoside	—	—	+	+	+	+	+	+	+	+
Raffinose	—	+	+	+	+	+	+	—	+	—
Trehalose	—	+	+	—	+	+	+	+	+	+
Turanose	—	+	+	—	+	+	+	+	+	+
L-Alanine	—	—	—	+	+	+	+	—	+	—
L-Alanyl glycine	—	+	—	+	—	+	+	+	+	+
L-Asparagine	—	—	—	—	—	+	—	—	+	—
L-Glutamic acid	+	—	+	+	+	—	+	+	+	+
L-Proline	+	+	—	+	+	—	+	+	+	w
L-Serine	—	+	+	—	—	—	+	+	—	—
L-Threonine	—	+	—	+	+	—	+	+	—	w
2,3-Butanediol	—	—	—	—	—	+	+	—	—	—
Glycerol	—	+	—	+	—	+	+	+	+	+
DL- α -Glycerol phosphate	—	+	—	—	—	+	+	+	—	—
α -D-Glucose 1-phosphate	—	+	+	—	+	—	—	+	w	—

*Data taken from: a, Pankratov *et al.* (2007); b, Urai *et al.* (2008); c, Luo *et al.* (2009).

†Reported as negative by Luo *et al.* (2009).

Table 2. Cellular fatty acid compositions of strain HMD1056^T and type strains of closely related *Mucilaginibacter* species

Strains: 1, HMD1056^T; 2, *M. paludis* KACC 13450^T; 3, *M. gracilis* KACC 13451^T; 4, *M. kameinonensis* KCTC 22227^T; 5, *M. ximonensis* KCTC 22437^T (data in columns 1–5 from this study); 6, *M. gossypii* Gh-67^T; 7, *M. gossypicola* Gh-48^T (data in columns 6 and 7 from Madhaiyan *et al.*, 2010); 8, *M. frigoritolerans* FT22^T; 9, *M. lappiensis* ANJL12^T; 10, *M. mallensis* MP1X4^T (data in columns 8–10 from Männistö *et al.*, 2010). All strains were grown for 2 days at 25 °C on R2A. Values are percentages of total fatty acids. Only fatty acids that account for more than 1% for one or more strains are indicated. tr, Trace; ND, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9	10
C _{14:0}	2.6	1.6	1.1	1.7	tr	1.2	1.7	tr	tr	tr
iso-C _{15:0}	24.4	23.2	20.6	22.9	33.2	25.8	20.0	21.9	18.9	26.0
anteiso-C _{15:0}	tr	15.8	ND	12.5	tr	ND	ND	1.6	ND	tr
C _{15:1} ω 6c	1.1	1.8	tr	1.1	3.1	tr	tr	ND	ND	ND
C _{15:1} ω 5c	ND	tr	ND	ND	ND	2.8	1.3	1.1	ND	tr
C _{15:0}	ND	ND	ND	ND	ND	ND	ND	1.2	tr	1.3
C _{16:1} ω 5c	3.7	8.8	5.0	6.8	3.7	2.8	1.3	2.7	6.8	4.0
C _{16:0}	4.5	3.7	7.9	5.8	2.3	15.0	17.0	3.1	3.7	4.8
iso-C _{15:0} 3-OH	2.9	1.7	2.0	2.6	3.7	1.9	4.5	2.9	2.2	2.9
C _{15:0} 2-OH	ND	1.2	tr	1.4	tr	ND	tr	tr	ND	tr
C _{17:1} ω 8c	ND	1.0	tr	tr	tr	tr	tr	ND	ND	ND
C _{16:0} 3-OH	1.4	tr	3.3	1.4	1.2	5.1	4.6	tr	3.2	tr
C _{18:0}	tr	tr	tr	tr	ND	1.6	ND	ND	ND	tr
iso-C _{17:0} 3-OH	5.5	3.0	4.9	6.7	6.0	6.5	6.1	16.4	14.3	12.5
C _{17:0} 2-OH	ND	tr	ND	ND	ND	ND	ND	0.9	tr	1.4
iso-C _{17:1}	ND	ND	ND	ND	ND	ND	ND	4.1	2.7	9.5
Summed feature 3*	49.1	30.5	49.3	34.2	37.7	33.0	39.6	38.7	45.0	32.9
Summed feature 9*	1.6	tr	1.4	ND	4.6	tr	tr	ND	ND	ND

*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c and summed feature 9 comprised iso-C_{17:1} ω 9c and/or 10-methyl C_{16:0}.

arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase and α -fucosidase activities are present, but lipase (C14), α -chymotrypsin, β -galactosidase, β -glucuronidase, β -glucosidase and α -mannosidase activities are absent. The following compounds are utilized as sole carbon sources in Biolog GN2 microplates: cellobiose, D-fructose, gentiobiose, α -D-glucose, maltose, sucrose, L-glutamic acid and L-proline. The following carbon sources are not utilized: α -cyclodextrin, dextrin, glycogen, Tweens 40 and 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-erythritol, L-fucose, D-galactose, *myo*-inositol, lactose, lactulose, D-mannitol, D-mannose, melibiose, methyl β -D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, trehalose, turanose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -, β - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D- and L-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. The major fatty acids are summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c) and iso-C_{15:0}. The DNA G+C content of the type strain is 46.9 mol%.

The type strain, HMD1056^T (=KCTC 22746^T =CECT 7550^T), was isolated from a mesotrophic freshwater lake in Wangsan, Yongin, Gyeonggi, Republic of Korea.

Acknowledgements

This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Korea.

References

- An, D. S., Yin, C. R., Lee, S. T. & Cho, C. H. (2009). *Mucilaginibacter daejeonensis* sp. nov., isolated from dried rice straw. *Int J Syst Evol Microbiol* **59**, 1122–1125.
- Baik, K. S., Park, S. C., Kim, E. M., Lim, C. H. & Seong, C. N. (2010). *Mucilaginibacter rigui* sp. nov., isolated from wetland freshwater, and emended description of the genus *Mucilaginibacter*. *Int J Syst Evol Microbiol* **60**, 134–139.
- Bernardet, J. F., Vancanneyt, M., Matte-Tailliez, O., Grisez, L., Tailliez, P., Bizet, C., Nowakowski, M., Kerouault, B. & Swings, J. (2005). Polyphasic study of *Chryseobacterium* strains isolated from diseased aquatic animals. *Syst Appl Microbiol* **28**, 640–660.
- Cho, J. C. & Giovannoni, S. J. (2003). *Parvularcula bermudensis* gen. nov., sp. nov., a marine bacterium that forms a deep branch in the α -Proteobacteria. *Int J Syst Evol Microbiol* **53**, 1031–1036.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Jeon, Y., Lee, S. S., Chung, B. S., Kim, J. M., Bae, J. W., Park, S. K. & Jeon, C. O. (2009). *Mucilaginibacter oryzae* sp. nov., isolated from soil of a rice paddy. *Int J Syst Evol Microbiol* **59**, 1451–1454.
- Luo, X., Zhang, L., Dai, J., Liu, M., Zhang, K., An, H. & Fang, C. (2009). *Mucilaginibacter ximonensis* sp. nov., isolated from Tibetan soil. *Int J Syst Evol Microbiol* **59**, 1447–1450.
- MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Madhaiyan, M., Poonguzhali, S., Lee, J. S., Senthilkumar, M., Lee, K. C. & Sundaram, S. (2010). *Mucilaginibacter gossypii* sp. nov. and *Mucilaginibacter gossypicola* sp. nov., plant-growth-promoting bacteria isolated from cotton rhizosphere soils. *Int J Syst Evol Microbiol* **60**, 2451–2457.
- Männistö, M. K., Tirola, M., McConnell, J. & Häggblom, M. M. (2010). *Mucilaginibacter frigorigerans* sp. nov., *Mucilaginibacter lappiensis* sp. nov. and *Mucilaginibacter mallensis* sp. nov., isolated from soil and lichen samples. *Int J Syst Evol Microbiol* **60**, 2849–2856.
- Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Pankratov, T. A., Tindall, B. J., Liesack, W. & Dedysh, S. N. (2007). *Mucilaginibacter paludis* gen. nov., sp. nov. and *Mucilaginibacter gracilis* sp. nov., pectin-, xylan- and laminarin-degrading members of the family *Sphingobacteriaceae* from acidic *Sphagnum* peat bog. *Int J Syst Evol Microbiol* **57**, 2349–2354.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Urai, M., Aizawa, T., Nakagawa, Y., Nakajima, M. & Sunairi, M. (2008). *Mucilaginibacter kameionensis* sp. nov., isolated from garden soil. *Int J Syst Evol Microbiol* **58**, 2046–2050.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.