Mucilaginibacter polysacchareus sp. nov., an exopolysaccharide-producing bacterial species isolated from the rhizoplane of the herb *Angelica sinensis*

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Three exopolysaccharide-producing bacteria, designated strains DRP28^T, DRP29 and DRP31, were isolated from the rhizoplane of *Angelica sinensis* from the Geumsan, Republic of Korea. Cells were straight rods, Gram reaction-negative, aerobic, non-motile, and catalase- and oxidase-positive. Flexirubin-type pigments were absent. Phylogenetic analysis of the 16S rRNA gene indicated that these bacteria belong to the genus *Mucilaginibacter* in the phylum *Bacteroidetes*. 16S rRNA gene sequence similarities to strains of recognized species of the genus *Mucilaginibacter* were 93.8–97.4 %. The major fatty acids were iso-C_{15:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The strains contained MK-7 as the major isoprenoid quinone. Strains DRP28^T, DRP29 and DRP31 formed a single, distinct genomospecies with DNA G + C contents of 41.9–42.7 mol% and DNA hybridization values of 82.6–86.8 %; the strains exhibited DNA–DNA hybridization values of only 20.4–41.3 % with related species of the genus *Mucilaginibacter*. On the basis of evidence presented in this study, strains DRP28^T, DRP29 and DRP31 were considered to represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter polysacchareus* sp. nov. is proposed. The type strain is DRP28^T (=KACC 15075^T =NBRC 107757^T).

The genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae* (Steyn *et al.*, 1998; Pankratov *et al.*, 2007) and the phylum *Bacteroidetes*, includes Gram-negative, non-motile rods that are facultatively anaerobic and produce large amounts of extracellular polysaccharide (EPS). At the time of writing, the genus *Mucilaginibacter* comprises at least 16 species with validly published names: *Mucilaginibacter paludis* 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), *M. angelicae* (Kim *et al.*, 2012), *M. lappiensis* and *M. mallensis* (Männistö *et al.*, 2010), *M. frigoritolerans* (Männistö *et al.*, 2010), *M.*

boryungensis (Kang *et al.*, 2011), *M. myungsuensis* (Joung & Joh, 2011) and *M. dorajii* (Kim *et al.*, 2010). In this study, we characterized the EPS-producing bacterial strains DRP28^T, DRP29 and DRP31 that were isolated from the rhizoplane of *Angelica sinensis*. Based on phenotypic, phylogenetic and genotypic characteristics, we suggest that these strains represent a novel species of the genus *Mucilaginibacter*.

Strains DRP28^T, DRP29 and DRP31 were isolated from the rhizoplane of *Angelica sinensis* collected from the Geumsan, Republic of Korea, using the standard dilution plating technique. All isolates produced slime indicative of EPS around the colonies when grown on potato dextrose agar (PDA; Difco) medium at 25 °C for 3 days. The isolates were routinely cultured on R2A (Difco) agar medium and preserved at –80 °C as a suspension in distilled water containing 20 % glycerol (w/v).

Cells of strains DRP28^T, DRP29 and DRP31 incubated on R2A medium for 3 days at 25 °C were used for physiological and biochemical tests. Cell morphology and motility was observed by phase-contrast microscopy (Nikon Eclipse 80i).

Abbreviation: EPS, extracellular polysaccharide.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strains DRP28^T, DRP29 and DRP31 are HM748604, HM748605 and HQ396159, respectively.

Three supplementary figures are available with the online version of this paper.

The pH range for growth was determined on R2A agar. Medium sterilized by autoclaving was adjusted to pH 4–11 (in increments of 1 pH unit) by addition of HCl or NaOH. Growth temperature (4, 10, 15, 20, 25, 30, 37, 40 and 42 °C) and NaCl tolerance (0, 1, 2, 3, 5 and 10 %, w/v) were tested on R2A agar. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. The presence of flexirubin-type pigments was investigated as described by Reichenbach (1989). Other physiological and biochemical tests were performed using API 20E, API 20NE and API 50CH kits (bioMérieux). Enzymic activities were tested using the API ZYM kit (bioMérieux) following the manufacturer's instructions.

Colonies of strains DRP28^T, DRP29 and DRP31 were pale pink (Supplementary Fig. S1, available in IJSEM Online). Cells of these strains were Gram-negative, non-spore-forming, non-motile rods that occurred singly, in pairs or in short chains. Strains DRP28^T, DRP29 and DRP31 grew in the range pH 4–11, with an optimum at pH 6.0–7.0. The temperature range for growth was 4–28 °C, with an optimum at 25 °C. Growth was inhibited completely at NaCl concentrations above 1.0 % (w/v). Detailed results of colony colour, morphological, physiological and biochemical analyses are given in Table 1.

The 16S rRNA gene of each novel strain was amplified by using universal primers 27F [5'-AGAGTTTGATC(AC)T-GGCTCAG-3'] and 1492R [5'-ACGG(CT)TACCTTGTT-

ACGACTT-3'] (Weisburg *et al.*, 1991), and their nucleotide sequences were determined with an ABI prism BigDye terminator cycle sequencing ready reaction kit v.3.1 and ABI 3730XL capillary DNA sequencer (Applied Biosystems). The 16S rRNA gene sequences were compared with available 16S rRNA gene sequences from the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) and the GenBank database using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). 16S rRNA gene sequences of strains DRP28^T, DRP29, DRP31 and representative members of the family *Sphingobacteriaceae* were aligned using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were generated by three algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods, using the PHYLIP program package (Felsenstein, 1984, 2005). Bootstrap analysis to evaluate the stability of phylogenetic tree was achieved using a consensus tree from the neighbour-joining, maximum-parsimony and maximum-likelihood methods, based on 1000 resamplings. Probe labelling for DNA–DNA hybridization was conducted by using the non-radioactive DIG-High Prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using Bio-1D image analysis software (Vilber Lourmat).

The sequences of strains DRP28^T, DRP29 and DRP31 were closely related to those of members of the genus

Table 1. Differentiating phenotypic characteristics of strains DRP28^T, DRP29, DRP31 and related species of the genus *Mucilaginibacter*

Strains: 1, *M. polysacchareus* sp. nov. DRP28^T; 2, *M. polysacchareus* sp. nov. DRP29; 3, *M. polysacchareus* sp. nov. DRP31; 4, *M. gossypii* Gh-67^T (data from Madhaiyan *et al.*, 2010); 5, *M. lappiensis* ANJLI2^T (Männistö *et al.*, 2010); 6, *M. gossypicola* Gh-48^T (Madhaiyan *et al.*, 2010); 7, *M. rigui* WPCB133^T (Baik *et al.*, 2010); 8, *M. oryzae* B9^T (Jeon *et al.*, 2009). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8
Growth temperature range (°C)	4–28	4–28	4–28	20–30	0–31	20–30	4–37	15–35
pH range	4–11	4–11	4–11	4–7	4.5–8	2–8	5–10	5–8
Pigment	Pale pink	Pale pink	Pale pink	Pale pink	Pale red	Pale pink	Pale pink	Pale pink
Flexirubin type pigments	–	–	–	+	–	+	–	–
Nitrate reduction	–	–	–	+	–	+	–	+
Growth with >1 % NaCl	–	–	–	–	+	–	–	+
Hydrolysis of aesculin	–	–	–	+	+	+	+	+
Hydrolysis of gelatin	+	+	+	–	+	–	–	+
Assimilation of:								
Glucose	+	+	+	+	+	+	–	–
Fructose	+	+	+	+	–	+	–	+
Methyl α D-glucoside	+	–	–	+	–	+	–	–
N-Acetylglucosamine	+	–	–	+	–	+	+	–
Starch	+	+	+	–	–	–	–	–
Glycogen	–	–	–	+	–	+	–	+
Acid production from:								
D-Xylose	+	+	+	w	–	+	–	–
D-Fucose	–	–	–	+	–	+	–	+
Quinone(s)	MK-7	MK-7	MK-7	MK-7	MK-6, MK-7	MK-7	MK-7	MK-7
DNA G + C content (mol%) (T _m)	42.7	42.6	41.9	46.7	43.5	44.2	47	44.4

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing relationships between strains DRP28^T, DRP29 and DRP31, members of the genus *Mucilaginibacter* and representative members of the family *Sphingobacteriaceae* and the phylum *Bacteroidetes*. Numbers at nodes are levels of percentage bootstrap support (>50%) based on neighbour-joining analyses of 1000 resampled datasets. Bar, 0.01 nt substitutions per position.

Mucilaginibacter but could be clearly distinguished from the type species of the genus. 16S rRNA gene sequence similarity values between strain DRP28^T and the strains representing the species *Mucilaginibacter* were 93.9–97.4%. The 16S rRNA gene sequence of strain DRP28^T had 97.4, 97.2, 97 and 96% pairwise similarity, respectively, to those from *M. gossypii* Gh-67^T, *M. lappiensis* ANJLI2^T, *M. gossypicola* Gh-48^T and *M. oryzae* B9^T. The neighbour-joining tree (Fig. 1) showed that strains DRP28^T, DRP29 and DRP31 formed a monophyletic clade with the members of the genus *Mucilaginibacter* with 100% bootstrap support. DNA–DNA hybridization showed less than 41.3% DNA–DNA relatedness between strains DRP28^T, DRP29, DRP31 and the reference strains (summarized in Table 2). The DNA G+C contents of strains DRP28^T, DRP29 and DRP31 were 42.7, 42.6 and 41.9 mol%, respectively (Table 2).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by TLC on Merck silica gel 60 F254 plates (20×20 cm, 0.5 cm thick) using a mixture of petroleum benzene and diethyl ether (85:15, v/v) as the solvent. Spots were scraped off and then analysed by HPLC as described by Collins & Jones (1981). The predominant respiratory quinone is MK-7 (Table 1).

Cellular fatty acid profiles were determined for strains grown on TSA for one day at 28 °C. Fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification System (MIDI, 1999). Extracted fatty acid methyl esters were separated by using a gas chromatograph (HP6890N GC; Agilent) and identified with the Microbial Identification Sherlock software package (MIDI; database TSBA 40, version 4.0). The major fatty acids of strain DRP28^T were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 45.45%) and iso-C_{15:0} (21.32%) (Table 3). Summed feature 3 and iso-C_{15:0} have been previously reported as the predominant,

characteristic fatty acids of members of the genus *Mucilaginibacter* (Pankratov *et al.*, 2007; Baik *et al.*, 2010).

Polar lipids were analysed according to Minnikin *et al.* (1984). The polar lipid profile consisted of phosphatidylethanolamine, an unidentified aminophospholipid, two unidentified phospholipids, sphingoglycolipid, an unidentified aminolipid and unidentified lipids. Phosphatidylethanolamine and the unidentified aminophospholipid are major polar lipids (Supplementary Fig. S2).

Sphingoglycolipids were analysed by the method of Hirai *et al.* (1995). For detection of alkaline-stable glycolipids, the total extractable lipids were incubated in 1 M KCl and methanol (2:1, v/v) for 2 h at 37 °C and analysed by TLC with a solvent system composed of chloroform, methanol and water (70:30:5, by volume). Strains DRP28^T, DRP29 and DRP31 contained sphingoglycolipid (Supplementary Fig. S3).

Table 2. DNA–DNA relatedness of strains DRP28^T, DRP29, DRP31 and related type strains of the genus *Mucilaginibacter*

Strains: 1, *M. polysacchareus* sp. nov. DRP28^T; 2, *M. polysacchareus* sp. nov. DRP29; 3, *M. polysacchareus* sp. nov. DRP31; 4, *M. gossypii* KCTC 22380^T; 5, *M. lappiensis* LMG 25358^T; 6, *M. gossypicola* KCTC 22379^T; 7, *M. oryzae* KACC 12816^T.

Strain	Hybridization (%) with DNA from:					
	1	2	3	4	5	6
2	86.77					
3	82.60	83.94				
4	41.30	40.80	40.6			
5	36.43	29.41	28.73			
6	20.88	32.58	39.27	38.20	37.56	
7	20.40	28.40	31.20	30.50	32.30	29.10

Table 3. Cellular fatty acid compositions (%) of strains representing the genus *Mucilaginibacter*

Strains: 1, *M. polysacchareus* sp. nov. DRP28^T; 2, *M. polysacchareus* sp. nov. DRP29; 3, *M. polysacchareus* sp. nov. DRP31; 4, *M. gossypii* Gh-67^T (data from Madhaiyan *et al.*, 2010); 5, *M. lappiensis* ANJLI2^T (data from Männistö *et al.*, 2010); 6, *M. gossypicola* Gh-48^T (data from Madhaiyan *et al.*, 2010); 7, *M. rigui* WPCB133^T (data from Baik *et al.*, 2010); 8, *M. oryzae* B9^T (data from Jeon *et al.*, 2009). Values are percentages of total fatty acids. Fatty acids representing less than 0.1 % in all strains were omitted. ECL, Equivalent chain-length; tr, trace (<0.1 %); –, not detected/not reported.

Strain	1	2	3	4	5	6	7	8
Saturated acids								
C _{14:0}	1.2	1.55	0.97	1.2	tr	1.7	tr	1.7
C _{15:0}	3.09	0.54	3.51	–	tr	–	–	1.6
C _{16:0}	5.79	10.11	6.01	15	3.7	17	tr	8.5
C _{17:0}	–	–	–	0.2	–	0.3	–	–
C _{18:0}	–	–	–	1.6	–	–	–	–
C _{20:0}	5	1.48	4.03	–	–	–	–	–
Branched chain								
iso-C _{11:0}	0.36	0.49	0.47	0.2	–	–	–	0.1
iso-C _{13:0}	0.22	0.27	0.39	0.2	–	–	–	–
iso-C _{14:0}	–	–	–	–	–	–	3.3	–
iso-C _{15:0}	21.32	18.65	23.01	25.8	18.9	20.2	34.1	23.9
anteiso-C _{15:0}	–	–	–	–	tr	–	26.7	0.2
iso-C _{15:1} F	–	–	–	–	–	–	1.9	–
iso-C _{16:0}	–	–	–	–	tr	–	1.3	–
iso-C _{17:0}	–	–	–	0.8	tr	–	1.1	0.3
anteiso-C _{17:0}	–	–	–	–	–	0.5	–	–
iso-C _{17:1}	–	–	–	–	2.7	–	–	–
iso-C _{17:1} ω9c	1.14	0.99	1.16	1.1	–	0.5	3.4	1.9
Unsaturated acids								
C _{15:1} ω6c	0.94	–	0.88	0.7	–	0.6	–	0.4
C _{16:1} ω5c	4.02	0.82	4.32	2.8	6.8	1.3	–	1.4
C _{17:1} ω5c	–	–	–	1.5	–	–	–	–
C _{17:1} ω8c	0.49	–	–	0.3	–	0.5	–	0.3
C _{18:1} ω5c	–	–	–	–	–	–	1.4	–
C _{18:1} ω7c	–	–	–	0.2	–	–	–	–
C _{18:1} ω9c	–	–	–	0.5	–	–	–	0.1
Hydroxylated								
C _{14:0} 2-OH	0.45	1.04	–	0.3	–	0.6	–	0.6
C _{15:0} 2-OH	0.56	–	0.56	–	–	0.6	2.1	0.3
C _{16:0} 2-OH	–	–	–	–	–	–	–	0.2
C _{16:0} 3-OH	1.43	1.62	1.02	5.1	3.2	4.6	–	4.6
C _{17:0} 2-OH	–	–	–	–	tr	–	1.1	–
iso-C _{11:0} 3-OH	–	–	–	0.3	–	–	–	–
iso-C _{14:0} 3-OH	–	–	–	–	–	–	–	0.2
iso-C _{15:0} 3-OH	2.12	3.33	2.19	1.9	2.2	4.5	5.9	2.2
iso-C _{16:0} 3-OH	–	–	–	0.3	–	0.5	2.6	0.2
iso-C _{17:0} 3-OH	3.55	1.69	3.14	6.5	14.3	6.1	5.4	5.4
Summed features*								
1	–	–	–	–	–	–	1.7	–
2	–	–	–	–	–	0.5	–	0.4
3	45.45	55.28	42.96	33	45	39.6	tr	43.7
4	–	–	–	0.5	–	0.6	1.7	–
Unknown								
ECL 13.565	2.87	2.14	4.79	–	–	–	–	0.7
ECL 14.263	–	–	–	–	–	–	2.2	–
ECL 16.582	–	–	–	–	–	–	–	0.3

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 comprised C_{13:0} 3-OH and/or iso-C_{15:1} H; summed feature 2 comprised iso-C_{16:1} I and/or C_{14:0} 3-OH; summed feature 3 comprised C_{16:1} ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 comprised anteiso-C_{17:1} B and/or iso-C_{17:1} I.

Description of *Mucilaginibacter polysacchareus* sp. nov.

Mucilaginibacter polysacchareus (po.ly.sac.cha're.us. Gr. adj. *polu* many; Gr. n. *sakchâr* sugar; L. masc. suff. *-eus* suffix denoting made of or belonging to; N.L. masc. adj. *polysacchareus* with many saccharides).

Cells are Gram-reaction-negative, non-endospore-forming, non-motile, aerobic rods. Colonies on R2A agar are circular, convex with entire margins, mucoid, translucent, pale pink and approximately 3.0 mm in diameter after 3 days at 25 °C (pH 7). Large amounts of exopolysaccharides are produced. Growth occurs at pH 4–11 (optimum, pH 6–7) and at 4–28 °C (optimum, 25 °C). NaCl inhibits growth at concentrations above 1 % (w/v). Flexirubin-type pigments are absent. Oxidase- and catalase- positive. Positive for hydrolysis of Tween 20 and starch but negative for hydrolysis of alginate, casein, cellulose, chitin, CM-cellulose, pectin, Tween 80, L-tyrosine and xylan. Positive reactions (API 20E and 20NE systems) for β -glucosidase, protease activity, assimilation of mannose, *N*-acetylglucosamine and maltose, and fermentation of glucose, arabinose, rhamnose, sucrose and melibiose. Negative reactions for arginine dihydrolase, nitrate reduction, fermentation of mannitol, and assimilation of gluconate, caprate, adipate, malate, citrate and phenylacetate. In the API 50CH system, acid is produced from D-xylose, galactose, glucose, fructose, mannose, methyl α -D-glucoside, *N*-acetylglucosamine, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, gentiobiose, turanose and L-fucose; acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, ribose, L-xylose, adonitol, methyl β -D-xylopyranoside, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannopyranoside, arbutin, inulin, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. In the API ZYM gallery, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, α -galactosidase, β -galactosidase, α -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and α -fucosidase activities are present; but esterase (C4), esterase lipase (C8), lipase (C14), α -chymotrypsin, naphthyl-AS-BI-phosphohydrolase, β -glucuronidase, α -mannosidase and trypsin activities are absent. The major fatty acids are summed feature 3 and iso-C_{15:0}. The major cellular polar lipids are phosphatidylethanolamine and an unidentified aminophospholipid. Sphingoglycolipids are detected. The DNA G + C content of the type strain is 42.7 mol%. The only respiratory quinone detected is menaquinone 7 (MK-7).

The type strain, DRP28^T (=KACC 15075^T =NBRC 107757^T), was isolated from the rhizoplane of *Angelica sinensis*, the sample being collected from the Geumsan, located in the Republic of Korea.

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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.
- 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. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1984). Distance methods for inferring phylogenies: a justification. *Evolution* **38**, 16–24.
- Felsenstein, J. (2005). PHYLIP (phylogeny inference package), version 3.65. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Hirai, Y., Haque, M., Yoshida, T., Yokota, K., Yasuda, T. & Oguma, K. (1995). Unique cholesteryl glucosides in *Helicobacter pylori*: composition and structural analysis. *J Bacteriol* **177**, 5327–5333.
- 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.
- Joung, Y. & Joh, K. (2011). *Mucilaginibacter myungsuensis* sp. nov., isolated from a mesotrophic artificial lake. *Int J Syst Evol Microbiol* **61**, 1506–1510.
- Kang, S. J., Jung, Y. T., Oh, K. H., Oh, T. K. & Yoon, J. H. (2011). *Mucilaginibacter boryungensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **61**, 1549–1553.
- Kim, B. C., Lee, K. H., Kim, M. N., Lee, J. & Shin, K. S. (2010). *Mucilaginibacter dorajii* sp. nov., isolated from the rhizosphere of *Platycodon grandiflorum*. *FEMS Microbiol Lett* **309**, 130–135.
- Kim, B. C., Poo, H., Lee, K. H., Kim, M. N., Kwon, O. Y. & Shin, K. S. (2012). *Mucilaginibacter angelicae* sp. nov., isolated from the rhizosphere of *Angelica polymorpha* Maxim. *Int J Syst Evol Microbiol* **62**, 55–60.
- Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703–704.
- 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.
- 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 frigoritolerans* 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.
- MIDI (1999).** *Sherlock Microbial Identification System Operating Manual*, version 3.0. Newark, DE: MIDI, Inc.
- 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.
- Reichenbach, H. (1989).** Order I. Cytophagales Leadbetter 1974. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2011–2013. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998).** Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* **48**, 165–177.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Urai, M., Aizawa, T., Nakagawa, Y., Nakajima, M. & Sunairi, M. (2008).** *Mucilaginibacter kameinonensis* sp. nov., isolated from garden soil. *Int J Syst Evol Microbiol* **58**, 2046–2050.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.