Mucilaginibacter angelicae sp. nov., isolated from the rhizosphere of *Angelica polymorpha* Maxim

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A Gram-negative-staining, non-motile rod, designated GG-w14^T, was isolated from the rhizosphere of *Angelica polymorpha* Maxim. Phylogenetic analysis of 16S rRNA gene sequences revealed that the isolate belonged to the genus *Mucilaginibacter* and exhibited 93.9–97.4 % 16S rRNA gene sequence similarity with recognized members of the genus *Mucilaginibacter* (closest relative *Mucilaginibacter gossypii* Gh-67^T). DNA–DNA relatedness between strain GG-w14^T and *M. gossypii* KCTC 22380^T was <41 %. Strain GG-w14^T grew at 4–35 °C, at pH 5.0–8.0 and with 0–1 % (w/v) NaCl. The isolate hydrolysed casein, CM-cellulose and starch and contained menaquinone 7 as the major menaquinone. The major cellular fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 39.9 %), iso-C_{15:0} (24.2 %) and iso-C_{17:0} 3-OH (12.4 %). The DNA G + C content was 42.5 mol%. These data suggest that strain GG-w14^T should be considered as a representative of a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter angelicae* sp. nov. is proposed. The type strain is GG-w14^T (=KCTC 23250^T=NCAIM B 02415^T).

The genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae*, was originally proposed by Pankratov *et al.* (2007) for two isolates from an acidic *Sphagnum* peat bog. Currently, the genus *Mucilaginibacter* comprises 15 species, including *Mucilaginibacter paludis*, the type species of the genus, and the recently proposed species *Mucilaginibacter rigui*, *M. frigiditolerans*, *M. lappiensis*, *M. mallensis*, *M. boryungensis*, *M. myungsuensis*, *M. dorajii*, *M. gossypii* and *M. gossypicola* (Baik *et al.*, 2010; Männistö *et al.*, 2010; Kang *et al.*, 2011; Joung & Joh, 2011; Kim *et al.*, 2010; Madhaiyan *et al.*, 2010).

A novel bacterial strain, designated GG-w14^T, was isolated from the rhizosphere of *Angelica polymorpha* Maxim, which is used as a medicinal herb in Korean traditional medicine (Heo, 1610). Samples of *A. polymorpha* Maxim. were collected at Chungcheongnam-Do, Republic of Korea. Rhizosphere soil collected from the plant roots was serially diluted with sterile 0.85 % (w/v) NaCl and dilutions were spread onto R2A agar (BD). Colonies were subcultivated to obtain pure cultures. Strain GG-w14^T was one of the

isolates obtained. The isolate was routinely cultured on R2A agar at 25 °C under aerobic conditions and stored at –70 °C in 20 % (v/v) glycerol. *M. gossypii* KCTC 22380^T, *M. gossypicola* KCTC 22379^T, *M. oryzae* KACC 12816^T, *M. kameinonensis* KCTC 22227^T, *M. ximonensis* KCTC 22437^T and *Escherichia coli* KCTC 2441^T were obtained from the KCTC and KACC and used as reference strains.

Cell morphology and size were observed using light microscopy (Eclipse 80i; Nikon) and transmission electron microscopy (model H-7600; Hitachi) from cells cultured for 2 days at 25 °C on R2A agar. Colony morphology was observed after culture on R2A agar for 3 days at 25 °C. Growth with 0, 1, 2 and 3 % (w/v) NaCl was determined in NB (BD). Growth at 4, 10, 15, 25, 30, 35, 40 and 45 °C was determined on NA (BD). Growth at pH 4.0–9.0 (in increments of 0.5 pH unit) was tested in pH-adjusted NB. Growth was measured spectrophotometrically at OD₆₀₀ using a DU 730 UV/Vis scanning spectrophotometer (Beckman Coulter). Unless otherwise stated, all physiological characteristics were tested with cells cultured on R2A agar under optimal growth conditions (25–30 °C and pH 6.0–7.0). A Gram-stain kit (BD) was used for Gram-staining and oxidase reagent (bioMérieux) was used for the measurement of oxidase activity. Catalase activity was determined by observing the production of oxygen bubbles with 3 % (v/v) hydrogen peroxide. Anaerobic growth was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GG-w14^T is HM627214.

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

evaluated on R2A agar and on R2A agar supplemented with 0.1 % KNO₃ incubated for 14 days under an anaerobic atmosphere (GasPak EZ Anaerobe Pouch System; BD). Motility was tested by culturing the organism in soft R2A medium (0.4 %, w/v, agar). The isolate was tested for growth on MacConkey agar (BD). Starch hydrolysis was determined with Lugol's iodine solution after culture on R2A agar containing 0.2 % (w/v) starch. Pectin hydrolysis was determined with 1 % n-hexadecyltrimethylammonium bromide after culture on R2A agar containing 0.3 % (w/v) citric pectin. Hydrolysis of CM-cellulose and xylan was determined after culture on R2A agar containing 0.5 % (w/v) CM-cellulose and 0.5 % (w/v) xylan, respectively, and the plates were stained with 0.2 % aqueous Congo red and washed with 1 M NaCl to observe the zone of clearing. Hydrolysis of casein, chitin and L-tyrosine was determined after culture on R2A agar containing 3 % (w/v) casein, 1 % (w/v) colloidal chitin and 0.5 % (w/v) L-tyrosine, respectively. Hydrolysis of alginate was determined after culture on R2A agar containing 0.5 % (w/v) sodium alginate and stained with 10 % (w/v) cetylpyridinium chloride (Kawamoto *et al.*, 2006). A zone of clearing around the bacterial colonies indicated positive activity. Hydrolysis of Tweens 20, 40, 60 and 80 was determined by the formation of an opaque halo of precipitation around the colony (Barrow & Feltham, 1993). The presence of flexirubin-type pigments was detected by the bathochromic shift test with 20 % (w/v) KOH (Reichenbach, 1989). The API ZYM system (bioMérieux) was used for observing other enzyme activities. The API 20 NE and API 50 CH systems (bioMérieux) were used to determine other physiological characteristics, with incubation for 7 days at 25 °C.

Susceptibility to antibiotics was determined by the disc diffusion assay using antimicrobial discs (Chang *et al.*, 2009). A bacterial suspension (0.5 McFarland units) was spread on R2A agar, on which were then laid discs (6 mm in diameter; Liofilchem) impregnated with the following antibiotics (µg per disc): ampicillin (10), carbenicillin (100), chloramphenicol (30), erythromycin (15), kanamycin (30), lincomycin (15), nalidixic acid (30), neomycin (30), tetracycline (30), trimethoprim (5) and vancomycin (30). Zones of clearing were measured after 3 days at 25 °C. The strain was considered susceptible if the diameter of the zone of clearing was >12 mm, moderately susceptible if the zone was 8–12 mm and resistant if the zone was <8 mm.

The quinone content of strain GG-w14^T was determined according to the method of Collins & Jones (1981) from cells grown on R2A agar. Quinones were purified by preparative TLC (silica gel F254; Merck) and analysed by HPLC (L-5000; Hitachi). The fatty acid methyl esters of strain GG-w14^T and the reference strains were extracted from cells of the same physiological age, after 1 day on R2A agar (pH 6.0) at 30 °C (Sasser, 2001). The fatty acids were extracted according to the standard protocol of the Sherlock Microbial Identification System (MIDI; Sasser, 2001), separated using GC (HP 6890N; Agilent) and identified using the MIDI software package and database TSBA 40

version 4.0. The genomic DNA of strain GG-w14^T and *E. coli* KCTC 2441^T was extracted according to the method described by Sambrook & Russell (2001). Genomic DNA was hydrolysed with nuclease P1 and dephosphorylated with alkaline phosphatase. The mixture of nucleosides was then analysed by HPLC to determine the G + C content of the DNA (Mesbah *et al.*, 1989).

The 16S rRNA gene of strain GG-w14^T was amplified by PCR with the universal primers 27F and 1492R (Lane, 1991) and PCR products were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit v3.1 and an ABI 3730XL capillary DNA sequencer (Applied Biosystems). The resultant sequence was assembled using Vector NTI software (Invitrogen) and compared with available 16S rRNA gene sequences in the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) and GenBank using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). 16S rRNA gene sequences of strain GG-w14^T and representative members of the family *Sphingobacteriaceae* were aligned using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were generated with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods implemented in the PHYLIP package (Felsenstein, 2005). Kimura's two-parameter rule (Kimura, 1980) was used for the calculation of phylogenetic distances. Tree topologies were evaluated using a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. DNA–DNA hybridization was performed using the fluorometric hybridization method in microplate wells according to Ezaki *et al.* (1989), in triplicate at 43 °C with 50 % formamide.

A nearly complete 16S rRNA gene sequence (1443 bp) of strain GG-w14^T was determined. Strain GG-w14^T was closely related to, but clearly distinguished from, members of the genus *Mucilaginibacter* (93.9–97.4 % 16S rRNA gene sequence similarity). Strain GG-w14^T showed the highest 16S rRNA gene sequence similarities with *M. gossypii* Gh-67^T (97.4 %) and *M. gossypicola* Gh-48^T (96.5 %). The phylogenetic relationships of strain GG-w14^T with members of the genus *Mucilaginibacter* are shown in the neighbour-joining tree (Fig. 1). Strain GG-w14^T also clustered with the genus *Mucilaginibacter* in the maximum-parsimony and maximum-likelihood trees (Supplementary Figs S1 and S2, available in IJSEM Online). Strain GG-w14^T showed <70 % DNA–DNA relatedness with *M. gossypii* KCTC 22380^T (38.0 ± 2.6 %, reciprocal 40.1 ± 1.7 %). Strains with >70 % DNA–DNA relatedness are considered to represent the same species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). According to this criterion, strain GG-w14^T represented a novel species within the genus *Mucilaginibacter*.

Strain GG-w14^T was a Gram-negative-staining, strictly aerobic, catalase- and oxidase-positive, non-motile rod. Cells were 1.1–1.5 µm long and 0.6–0.8 µm wide and did not have flagella (Supplementary Fig. S3). Colonies were circular, smooth, mucoid, convex, entire and translucent white on R2A agar. Strain GG-w14^T did not grow on MacConkey agar. Strain GG-w14^T grew at 4–35 °C (optimum

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain GG-w14^T in the genus *Mucilaginibacter*. Bootstrap values (>500) based on 1000 resamplings are shown at branch nodes. *Flexibacter flexilis* IFO 15060^T was used as the outgroup. Bar, 0.1 substitutions per nucleotide position.

25–30 °C), at pH 5.0–8.0 (optimum pH 6.0–7.0) and with 0–1 % NaCl (optimum 0 % NaCl). Characteristics that differentiate strain GG-w14^T from closely related type strains of species of the genus *Mucilaginibacter* are given in Table 1 and other physiological and biochemical properties are given in the species description.

The only major quinone of strain GG-w14^T was menaquinone-7 (MK-7), which is the principal quinone of the genus *Mucilaginibacter* (Pankratov *et al.*, 2007) and a characteristic of the family *Sphingobacteriaceae*. The major fatty acids of strain GG-w14^T were similar to those of its closest phylogenetic neighbours (Table 2). After growth on R2A agar for 1 day at 25 °C, the major cellular fatty acids of strain GG-w14^T were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 39.9 %), iso-C_{15:0} (24.2 %) and iso-C_{17:0} 3-OH (12.4 %). Summed feature 3 and iso-C_{15:0} have been previously reported as the predominant fatty acids of genus *Mucilaginibacter* (Pankratov *et al.*, 2007; Baik *et al.*, 2010). The DNA G+C content of strain GG-w14^T was 42.5 mol%, which is within the range for the genus *Mucilaginibacter* (42.4–47 mol%; Baik *et al.*, 2010).

Phylogenetic, phenotypic and chemotaxonomic data show that strain GG-w14^T is a representative of a novel species in the genus *Mucilaginibacter*. The name *Mucilaginibacter angelicae* sp. nov. is proposed.

Description of *Mucilaginibacter angelicae* sp. nov.

Mucilaginibacter angelicae (an.ge.li'ca.e. N.L. gen. n. *angelicae* of *Angelica*, a herb plant genus).

Gram-negative-staining, catalase- and oxidase-positive, strictly aerobic, non-motile rod (1.1–1.5 × 0.6–0.8 μm). Grows at 4–35 °C (optimum 25–30 °C), but not at 40 °C, at pH 5.0–8.0 (optimum pH 6.0–7.0) and with 0–1 % (w/v) NaCl (optimum 0 %). Colonies are circular, smooth, mucoid, convex, entire and translucent white. Flexirubin-type pigments are absent. Hydrolyses starch, casein, CM-cellulose and Tweens 20, 40 and 60, weakly hydrolyses Tween 80 and L-tyrosine, but does not hydrolyse alginate, chitin, pectin or xylan. With API ZYM, gives a positive result for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, α- and β-glucosidases, N-acetyl-β-glucosaminidase and α-mannosidase, weakly positive for esterase (C4), esterase lipase (C8), cystine arylamidase, trypsin, β-glucuronidase and α-fucosidase, but negative for lipase (C14) and α-chymotrypsin. With API 20 NE, gives a positive reaction for β-galactosidase, hydrolysis of aesculin and gelatin and assimilation of L-arabinose, N-acetylglucosamine, maltose, D-glucose and D-mannose, but a negative result for nitrate reduction, indole production,

Table 1. Differentiating phenotypic characteristics of strain GG-w14^T and its closest phylogenetic neighbours

Strains: 1, GG-w14^T; 2, *M. gossypii* KCTC 22380^T (data from Madhaiyan *et al.*, 2010); 3, *M. gossypicola* KCTC 22379^T (Madhaiyan *et al.*, 2010); 4, *M. oryzae* KACC 12816^T (Jeon *et al.*, 2009); 5, *M. kameinonensis* KCTC 22227^T (Urai *et al.*, 2008); 6, *M. ximonensis* KCTC 22437^T (Luo *et al.*, 2009). All strains are positive for catalase, alkaline phosphatase, leucine arylamidase, acid phosphatase, α - and β -galactosidases, α - and β -glucosidases, *N*-acetyl- β -glucosaminidase, α -mannosidase, hydrolysis of aesculin, assimilation of D-glucose and D-mannose, production of acid from D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-cellobiose, maltose, lactose, melibiose, sucrose, trehalose and D-raffinose and susceptibility to tetracycline (30 μ g). All strains are negative for indole production, glucose fermentation, arginine dihydrolase, urease, lipase (C14), α -chymotrypsin, hydrolysis of alginate and chitin, assimilation of D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid, production of acid from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, methyl- β -D-xylopyranoside, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate and susceptibility to ampicillin (10 μ g), kanamycin (30 μ g), neomycin (30 μ g) and trimethoprim (5 μ g). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Cell dimensions (μ m)						
Width	0.6–0.8	0.2–0.2	0.2–0.3	0.5–0.8	0.3–0.7	0.3–0.5
Length	1.1–1.5	0.8–1.3	0.5–0.8	1.5–2.8	0.8–3.5	0.8–2.1
Colony colour†	TW	P	P	P	P	Y
Range for growth						
pH	5–8	4–7	2–8	5–8	4–9	5–8
Temperature (°C)	4–35	20–30	20–30	15–35	5–30	10–33
Oxidase	+	+	+	+	–	+
Nitrate reduction*	–	+	+	–	–	–
Trypsin*	w	+	+	w	w	–
Hydrolysis of:*						
CM-cellulose	+	–	–	+	+	–
Casein	+	+	–	+	–	–
L-Tyrosine	w	–	–	w	+	–
Tween 60	+	+	+	–	+	–
Tween 80	w	+	+	–	w	–
Assimilation of:*						
L-Arabinose	+	+	+	+	+	–
N-Acetylglucosamine	+	+	+	+	+	–
Maltose	+	+	+	+	+	–
Acid production from:*						
D-Arabinose	+	+	+	+	+	–
Amygdalin	w	+	+	+	+	–
Glycogen	+	–	–	+	+	–
Susceptibility to (μ g per disc):*‡						
Carbenicillin (100)	R	R	R	R	R	S
Chloramphenicol (30)	R	R	R	S	R	S
Erythromycin (15)	R	MS	MS	R	R	R
Lincomycin (15)	R	MS	MS	S	R	S
Nalidixic acid (30)	R	R	R	R	R	S
DNA G + C content (mol%)	42.5	46.7	44.2	44.4	43.7	43.4

*Data from this study.

†P, Pale pink; TW, translucent white; Y, pale yellow.

‡MS, Moderately susceptible; R, resistant; S, susceptible.

glucose fermentation, arginine dihydrolase, urease and assimilation of potassium gluconate, D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. With API 50 CH, gives a positive result for acid production from D- and L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, gentiobiose, turanose, D-lyxose

and L-fucose, weakly positive for acid production from amygdalin, but negative for acid production from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, methyl- β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, D-tagatose, D-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. Susceptible to (μ g per disc) tetracycline (30), but resistant to ampicillin (10), carbenicillin

Table 2. Cellular fatty acid compositions of strain GG-w14^T and its closest phylogenetic neighbours

Strains: 1, GG-w14^T; 2, *M. gossypii* KCTC 22380^T; 3, *M. gossypicola* KCTC 22379^T; 4, *M. oryzae* KACC 12816^T; 5, *M. kameinonensis* KCTC 22227^T; 6, *M. ximonensis* KCTC 22437^T. All data were taken from this study from cells cultured on R2A agar at 25 °C for 1 day. Fatty acids comprising <0.5 % of the total in all strains are not shown. ECL, Equivalent chain-length; tr, trace (<0.5 %); –, not detected.

Fatty acid (%)	1	2	3	4	5	6
Saturated						
C _{14:0}	0.7	0.8	0.7	0.7	0.8	tr
C _{15:0}	0.9	1.1	3.4	2.7	1.9	1.8
C _{15:0} 3-OH	–	–	–	0.5	–	–
C _{16:0}	10.1	10.0	7.4	9.3	11.0	5.0
C _{16:0} 3-OH	3.5	3.1	2.8	4.5	3.7	1.4
Unsaturated						
C _{15:1} ω6c	–	tr	0.5	0.5	tr	tr
C _{16:1} ω5c	2.3	3.8	5.3	3.7	3.3	3.8
C _{17:1} ω8c	tr	tr	0.7	0.6	0.5	tr
iso-C _{17:1} ω9c	1.8	2.3	2.0	2.3	1.7	6.1
Branched						
iso-C _{15:0}	24.2	23.4	23	19.5	23.4	39.7
iso-C _{15:0} 3-OH	2.1	2.0	1.9	2.0	1.8	2.2
iso-C _{17:0}	tr	0.6	0.5	0.9	0.6	1.6
iso-C _{17:0} 3-OH	12.4	11.9	11.6	11.6	7.1	10.7
anteiso-C _{15:0}	–	–	tr	–	–	0.7
Unknown ECL 16.582*	0.7	tr	0.5	–	tr	0.8
Summed features†						
3	39.9	37.6	37.4	37.9	37.8	23.8
4	–	tr	tr	–	–	0.8

*This compound does not have a listed name in the Peak Library File of the Microbial Identification System and therefore was not identified.

†Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 4 consisted of iso-C_{17:1} 1 and/or anteiso-C_{17:1} B.

(100), chloramphenicol (30), erythromycin (15), kanamycin (30), lincomycin (15), nalidixic acid (30), neomycin (30), trimethoprim (5) and vancomycin (30). The predominant fatty acids are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), iso-C_{15:0} and iso-C_{17:0} 3-OH. The major quinone is menaquinone 7.

The type strain, GG-w14^T (=KCTC 23250^T=NCAIM B 02415^T), was isolated from the rhizosphere of *Angelica polymorpha* Maxim. The genomic DNA G+C content of the type strain is 42.5 mol%.

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