

NOTE

Mucilaginibacter ginsenosidivorax sp. nov., with Ginsenoside Converting Activity Isolated from Sediment

Jin-Kwang Kim^{1,4†}, Tae-Eun Choi^{2†},
Qing-Mei Liu^{1,5}, Hye-Yoon Park³, Tae-Hoo Yi²,
Min-Ho Yoon⁴, Sun-Chang Kim^{1,5},
and Wan-Taek Im^{1*}

¹KI for the BioCentury, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

²Department of Oriental Medicinal Material and Processing College of Life Science, Kyung Hee University, Kyunggi-do 449-701, Republic of Korea

³Microorganism Resources Division, National Institute of Biological Resources, Incheon 404-708, Republic of Korea

⁴Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Republic of Korea

⁵Intelligent Synthetic Biology Center, Daejeon 305-701, Republic of Korea

(Received November 23, 2012 / Accepted February 4, 2013)

A Gram-reaction-negative, strictly aerobic, non-motile, non-spore-forming, and rod-shaped bacterial strain designated KHI28^T was isolated from sediment in Gapcheon (river) and its taxonomic position was investigated using a polyphasic approach. Strain KHI28^T grew at 10–42°C and at pH 5.5–8.5 on R2A and nutrient agar without additional NaCl as a supplement. Strain KHI28^T possessed β -glucosidase activity, which was responsible for its ability to transform ginsenosides Rb₁ and Re (ones of the dominant active components of ginseng) to C-K and Rg₂, respectively. On the basis of 16S rRNA gene sequence similarity, strain KHI28^T was shown to belong to the family *Sphingobacteriaceae* and to be related to *Mucilaginibacter dorajii* DR-f4^T (97.9% sequence similarity), *M. polysacchareus* DRP28^T (97.3%), and *M. lappiensis* ANJLI2^T (97.2%). The G+C content of the genomic DNA was 45.8%. The predominant respiratory quinone was MK-7 and the major fatty acids were summed feature 3 (comprising C_{16:1} ω 6c and/or C_{16:1} ω 7c), iso-C_{15:0} and C_{16:0}. DNA and chemotaxonomic data supported the affiliation of strain KHI28^T to the genus *Mucilaginibacter*. Strain KHI28^T could be differentiated genotypically and phenotypically from the recognized species of the genus *Mucilaginibacter*. The isolate therefore represents a novel species, for which the name *Mucilaginibacter ginsenosidivorax* sp. nov. is proposed, with the type strain KHI28^T (=KACC 14955^T =LMG 25804^T).

[†]These authors contributed equally to this work.

*For correspondence. E-mail: wandra@kaist.ac.kr; Tel.: +82-42-350-4451; Fax: +82-42-350-4450

Keywords: 16S rRNA gene, polyphasic taxonomy, *Mucilaginibacter ginsenosidivorax*, ginsenoside

During the course of a study to screen ginsenoside-converting aerobic bacterial strains in soil and sediment samples, several novel bacterial strains showing ginsenoside converting activity were screened. Among them, a strain designated as KHI28^T isolated from a sediment sample from Gapcheon (river) which could convert ginsenosides Rb₁ and Re became the subject of a taxonomic investigation.

Biotransformation of ginsenoside (deglycosylation) can be achieved by hydrolyzing and removing a sugar moiety from the major ginsenosides using bacterial and fungal strains (Zhao *et al.*, 2009; Park *et al.*, 2010; Choi *et al.*, 2011; Cui *et al.*, 2011; Quan *et al.*, 2011; Wang *et al.*, 2012) because the efficacy of ginsenoside increases with the extent of deglycosylation, which enhances its hydrophobicity and cell wall permeability.

The genus *Mucilaginibacter* was recently proposed by Pankratov *et al.* (2007) and emended by Urai *et al.* (2008) and Baik *et al.* (2010) in order to accommodate Gram-negative, strictly aerobic or facultatively anaerobic, chemoheterotrophic, and rod-shaped bacteria. It contains menaquinone-7 (MK-7) as the predominant respiratory quinone and unsaturated and branched saturated fatty acids as the major fatty acids. The G+C content of the genomic DNA of this genus ranges from 42.4 to 47.0 mol% (Pankratov *et al.*, 2007; Urai *et al.*, 2008; Baik *et al.*, 2010). At the time of writing, the genus consisted of 20 validly named species, including the recently described species *Mucilaginibacter rigui* (Baik *et al.*, 2010), *Mucilaginibacter mallensis* (Männistö *et al.*, 2010), *Mucilaginibacter frigoritolerans* (Männistö *et al.*, 2010), *Mucilaginibacter lappiensis* (Männistö *et al.*, 2010), *Mucilaginibacter dorajii* (Kim *et al.*, 2010), *Mucilaginibacter boryungensis* (Kang *et al.*, 2011), *Mucilaginibacter composti* (Cui *et al.*, 2011), *Mucilaginibacter myungsuensis* (Joung and Joh, 2011), *Mucilaginibacter polysacchareus* (Han *et al.*, 2012), and *Mucilaginibacter angelicae* (Kim *et al.*, 2012).

The aim of this study is to determine the taxonomic position of strain KHI28^T by performing a phylogenetic analysis based on the 16S rRNA gene sequence, chemotaxonomic characteristics, and phenotypic characteristics. The results of this study provide evidence that strain KHI28^T represents a new bacterial species.

Strain KHI28^T was originally isolated from the sediment of Gapcheon in Daejeon, South Korea. This sediment sample was thoroughly suspended in 50 mM phosphate buffer (pH

7.0) and spread on nutrient agar (NA, Difco, USA) plates. The plates were incubated at 30°C for 2 weeks. KHI28^T was one of single colonies on the plates, and it was purified by transferring it onto a new nutrient agar plate. It was cultured routinely on R2A agar (Difco) or NA at 30°C and preserved as a suspension in nutrient broth with (20%, w/v) glycerol at -70°C. Strain KHI28^T was deposited to the Korean Agricultural Culture Collection (=KACC 14955^T) and the Belgian Co-ordinated Collections of Micro-organisms/Laboratorium voor Microbiologie (=LMG 25804^T).

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology and motility was observed under a Nikon light microscope at ×1,000 using the hanging drop technique (Perry, 1973), with cells grown on R2A agar for 2 days at 30°C. Catalase and oxidase tests, and spore observation were performed as outlined by Cappuccino and Sherman (2002). Cells grown on R2A agar for 1 day, they were used as an inoculum for the physiological and biochemical tests. Anaerobic growth was determined in serum bottles containing R2A broth supplemented with thioglycolate (1 g/L), in which the upper air layer had been replaced with nitrogen. In addition, biochemical phenotypic test were carried out using API 20NE, API ID 32GN, and API ZYM test kits according to the instructions of the manufacturer (bioMérieux, France). Tests for hydrolysis of DNA (using DNase agar from Scharlau, with DNase activity by flooding plates with 1 M HCl), casein, starch, Tween 80, pullulan, and laminarin (Atlas, 1993), xylan and carboxymethyl-cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth at different temperatures (4, 10, 18, 30, 37, 42, and 45°C) and various pH values (pH 4.5–10.0 at intervals of 0.5 pH units) was assessed after 7 days of incubation. Three different buffers (final concentration, 50 mM) were used to adjust the pH of R2A broth. Acetate buffer was used for pH 5.0–5.5, phosphate buffer was used for pH 6.0–8.0 and Tris buffer was used for pH 8.5–10.0. Salt tolerance was tested on R2A medium supplemented with 1–10% (w/v at intervals of 1% unit) NaCl after 7 days of incubation. Growth on trypticase soy agar (TSA, Difco), and MacConkey agar (Difco) was also evaluated at 30°C.

Ginsenosides Rb₁, Rc, Rd, Re, Rg₁, Rg₂(S), F₂, and compound K (C-K) were purchased from Nanjing Zelang Medical Technology Co., Ltd. (China). Gypenoside XVII was obtained as described by An *et al.* (2010). The reaction mixture, containing 300 µl of 1 mM ginsenosides (Rb₁ and Re, respectively) and 300 µl of a bacterial suspension inoculated in a nutrient broth, was incubated for 5 days, at 200 rpm and 30°C. During the reaction, a 50 µl aliquot was taken daily, extracted with an equal volume of water-saturated *n*-butanol, and subjected to TLC analysis. TLC was performed using 60F₂₅₄ silica gel plates (Merck, Germany) with CHCl₃-CH₃OH-H₂O (65:35:10, v/v, lower phase) as the solvent. The spots on the TLC plates were detected by spraying with 10% (v/v) H₂SO₄ followed by heating at 110°C for 5 min.

The genomic DNA of strain KHI28^T was extracted using a commercial genomic DNA-extraction kit (Solgent, Korea). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer pair 9F and 1512R and the purified PCR products were sequenced by Solgent

Co. Ltd (Korea) (Im *et al.*, 2010). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR, USA). The 16S rRNA gene sequences of related taxa were obtained from GenBank and EzTaxon server (Chun *et al.*, 2007). Multiple alignments were performed by ClustalX program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbor-joining (Saitou and Nei, 1987), the maximum-parsimony (Fitch, 1971), and maximum likelihood methods with the MEGA5 Program (Tamura *et al.*, 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Cell biomass of strain KHI28^T for isoprenoid quinones was obtained from cultures grown on R2A agar for 2 days at 30°C. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and re-extracted in *n*-hexane-water (1:1, v/v). The crude quinone in *n*-hexane solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analyzed by reverse-phase HPLC system (Younglin, Korea), as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains grown on R2A agar for 48 h at 30°C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analyzed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990).

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain KHI28^T was extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides. The G+C content was then determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC system (Younglin). DNA-DNA hybridization experiments were performed between strain KHI28^T, and 3 reference strains (*M. dorajii* KACC 14556^T, *M. polysacchareus* KACC 15075^T, and *M. lappiensis* KACC 14978^T) with the method described by Ezaki *et al.* (1989) using photobiotin-labeled DNA probes and micro-dilution wells. Hybridization was performed at 43°C with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were converted to percentage DNA-DNA relatedness values.

Cells of strain KHI28^T were Gram-reaction-negative, strictly aerobic, non-spore-forming, non-motile, rod shaped, oxidase-positive, and catalase-positive. The colonies grown on R2A agar plates for 2 days were smooth, circular, pale pink in color, convex, and 2–3 mm in diameter. On R2A agar, KHI28^T was able to grow at 10–42°C, but not at 45°C. The isolate grew well on nutrient agar and TSA, but not on MacConkey agar. The phenotypic and chemotaxonomic characteristics that differentiate the strain KHI28^T from other closely related *Mucilaginicibacter* species are listed in Table 1. A time course study of the biotransformation of the ginsenosides Rb₁ and Re was conducted. As shown in the TLC result in Fig. 1, ginsenoside Rb₁ was transformed into compound K (C-K) via two types of metabolites, F₂ and gypenoside XVII, of which the R_f value was slightly lower than that of ginsenoside Rd. Ginsenoside Rb₁ was al-

most completely hydrolyzed after 3 days, where the levels of C-K were maximized after 5 days. Ginsenoside Re was transformed into Rg₂(S), of which the characteristics showed

that it was closely related to *Mucilagibacter composti* TR6-03^T (Cui et al., 2011).

The 16S rRNA gene sequences of the strain KHI28^T de-

Table 1. Differentiating characteristics of *Mucilagibacter ginsenosidivorax* KHI28^T and the type strains of related *Mucilagibacter* species

Strains: 1, *Mucilagibacter ginsenosidivorax* KHI28^T; 2, *Mucilagibacter dorajii* KACC 14556^T; 3, *Mucilagibacter polysacchareus* KACC 15075^T; 4, *Mucilagibacter lappiensis* KACC 14978^T; 5, *Mucilagibacter composti* TR6-03^T; 6, *Mucilagibacter rigui* KACC 14990^T. Data were from this study, Männistö et al. (2010), Baik et al. (2010), and Kim et al. (2010). All strains were Gram-reaction-negative, non-motile, rod shaped, positive for oxidase activities. In API 20 NE and API ID 32 GN kits, all strains were positive for assimilation of D-glucose and L-arabinose. All strains were negative for nitrate reduction, indole production, acid production from glucose, hydrolysis of xylan, and assimilation of the following substrates: D-mannitol, caprate, citrate, phenyl-acetate, 4-hydroxybenzoate, inositol, itaconate, suberate, acetate, L-alanine, 5-ketogluconate, 3-hydroxybenzoate and L-serine. In API ZYM kits, all the strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, and N-acetyl- β -glucosaminidase activities. All strains were negative for lipase (C14) and α -chymotrypsin activities. +, Positive; -, negative; w, weakly positive.

Characteristics	1	2	3	4	5	6
Isolation source	Sediment	Rhizosphere	Rhizoplane	Lichen	Compost	Wet land freshwater
Colony color on R2A	Pale pink	Light yellow	Pale pink	Light pink to reddish	Light yellow	Pale pink
Growth temperature (°C)	10–42	4–30	4–28	0–31	4–42	4–37
pH range	5.5–8.5	5.0–8.0	5.5–9.0	4.5–8.0	6.0–8.0	5.0–10.0
Salt tolerance at 1% (w/v)	-	+	+	+	-	-
Catalase ^a	+	+	+	+	-	+
Arginine dihydrolase ^a	+	-	-	+	+	+
Urease ^a	+	-	-	+	+	+
Hydrolysis of: ^a						
CM-Cellulose	-	+	-	+	-	-
Starch	+	+	-	-	-	-
Tween 80	-	+	-	-	-	-
Gelatin	-	-	-	+	-	-
Carbon utilization of: ^a						
D-Glucose	+	+	+	-	+	+
L-Arabinose	+	+	+	-	+	+
D-Mannose	+	+	-	+	-	+
N-Acetyl-D-glucosamine	+	-	+	+	-	-
D-Maltose	+	+	+	+	-	+
Gluconate	-	-	+	+	-	-
Adipate	-	-	-	+	-	-
Malate	-	-	-	+	-	+
Salicin	+	+	+	-	+	-
D-Melibiose	+	+	+	+	-	+
L-Fucose	-	-	+	+	-	-
D-Sorbitol	-	-	-	+	-	-
Propionate	-	-	-	+	-	-
Valerate	-	-	-	+	-	-
L-Histidine	-	-	-	+	-	-
2-Ketogluconate	-	-	-	+	-	-
3-Hydroxy-butyrate	-	-	-	+	-	-
L-Proline	-	-	-	+	-	+
L-Rhamnose	+	-	+	-	-	-
D-Ribose	-	-	-	+	-	-
D-Sucrose	+	+	+	-	-	+
Malonate	-	-	-	+	-	-
Lactate	-	-	-	+	-	-
Glycogen	-	w	w	-	-	+
API ZYM test results: ^a						
Trypsin	+	-	+	+	-	+
β -Glucuronidase	-	-	+	+	-	-
α -Mannosidase	+	+	+	+	-	+
α -Fucosidase	+	+	+	+	-	+
Quinone	MK-7	MK-7	MK-7	MK7,MK6	MK-7	MK-7
G+C contents (mol%)	45.8	42.6	42.7	43.5	45.6	47

^aAll these data from this study.

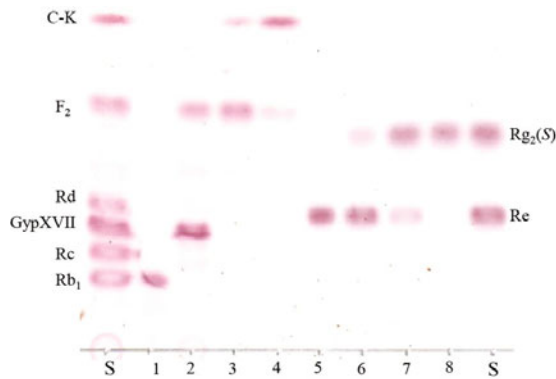


Fig. 1. TLC analyses of time-course transformation of ginsenosides Rb₁ and Re by strain KHI28^T. Developing solvent: CHCl₃/MeOH/H₂O (65:35:10, by vol.). S, saponin standards; 1, ginsenoside Rb₁; 2, reaction mixture of Rb₁ after 1 day; 3, 3 days; 4, 5 days; 5, ginsenoside Re; 6, reaction mixture of Re after 1 day; 7, 3 days; 8, 5 days. Abbreviations: C-K, compound K; GypXVII, gypenoside XVII.

terminated in this study were continuous stretches of 1477 bp (base position 16–1492 with respect to the *E. coli* numbering system), which were deposited in a GenBank database (accession numbers HM204914). A sequence similarity values between KHI28^T and other *Mucilaginigibacter* species using the EzTaxon server [http://www.eztaxon.org/; Chun *et al.* (2007)] ranged from 97.9% to 93.9%, and strain KHI28^T showed highest similarity to *Mucilaginigibacter dorajii* DR-f4^T (97.9%) and was followed by *M. polysacchareus* DRP28^T (97.3%) and *M. lappiensis* ANJLI2^T (97.2%). The relationship between strain KHI28^T and other members of the genus *Mucilaginigibacter* was also evident in the neighbor-joining tree, which used over 1350 nt (Fig. 2). In this phylogenetic tree, the isolate formed a distinct lineage. Strain KHI28^T,

M. dorajii FR-f4^T, *M. lappiensis* ANJLI2^T, *M. composti* TR6-03^T, and *M. rigui* WPCB133^T formed a monophyletic group although it didn't have high bootstrap value, which was supported by maximum-parsimony tree making method.

The predominant respiratory quinone of strain KHI28^T was menaquinone 7 (MK-7), in line with all other members of the genus *Mucilaginigibacter*. The cellular fatty acids of strain KHI28^T and related type strains are listed in the Table 2. The major fatty acids of all 6 compared strains were summed feature 3 (comprising C_{16:1} ω6c and/or C_{16:1} ω7c), iso-C_{15:0} and C_{16:0}. According to the Table 2, qualitative and quantitative differences in fatty acid content could be observed between strain KHI28^T and its phylogenetically closest relatives.

The DNA G+C content of strain KHI28^T was 45.8 mol%, similar to those of *M. dorajii* DR-f4^T, *M. polysacchareus* DRP28^T, *M. lappiensis* ANJLI2^T, *M. composti* TR6-03^T, and *M. rigui* WPCB133^T, which were in the range of 42.6–47.0 mol%. DNA-DNA relatedness values between strain KHI28^T and *M. dorajii* DR-f4^T, *M. polysacchareus* DRP28^T, and *M. lappiensis* ANJLI2^T were 35% (SD, 4.5%), 13% (SD, 2.5%), and 8% (SD, 1.7%), respectively. These values are below the threshold, i.e. 70%, for determining bacterial genomic species (Wayne *et al.*, 1987). Thus, it is clear that the DNA-DNA hybridization test confirmed that strain KHI28^T should be classified as a different genomic species based on incorporating the phenotypic data.

The characteristics of strain KHI28^T were consistent with the description of the genus *Mucilaginigibacter* with regard to morphological, biochemical, and chemotaxonomic properties. However, phylogenetic distance between strain KHI28^T and recognized *Mucilaginigibacter* species, also indicated by relatively low 16S rRNA gene sequence similarity (<97.9%) and the combination of unique phenotypic characteristics (Table 1) warrant assignment of strain KHI28^T to the genus *Mucilaginigibacter* as the type strain of a novel species, for which the

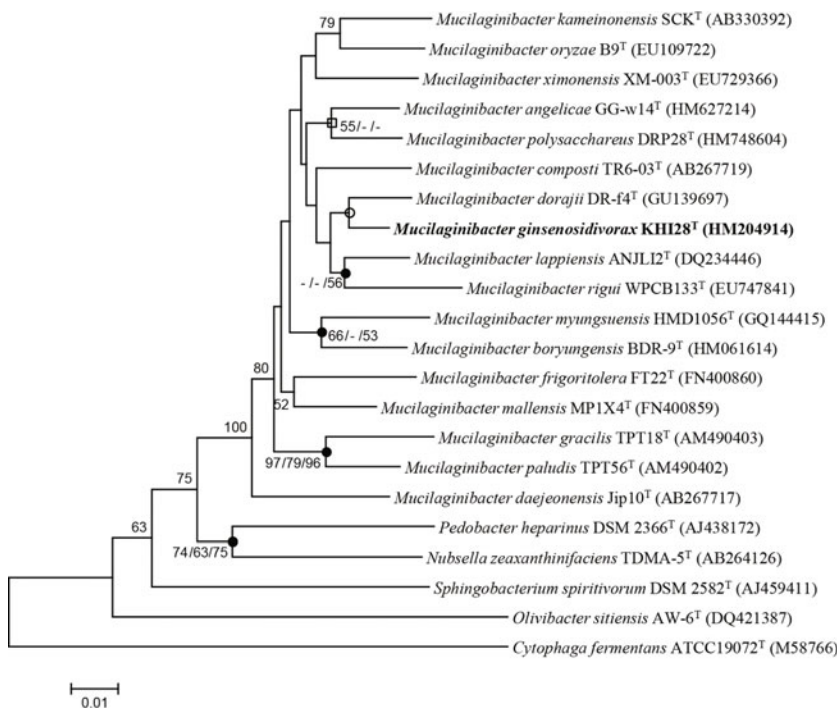


Fig. 2. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of *M. ginsenosidivorax* KHI28^T with other related species. This tree was made using the neighbor-joining method (Saitou and Nei, 1987) with a Kimura (1983) two-parameter distance matrix and pairwise deletion. Filled circles, open circles and a square indicate generic branches that were also recovered using the maximum-parsimony and maximum-likelihood algorithm, using the maximum-parsimony algorithm, and maximum-likelihood algorithm, respectively. Numbers at nodes indicate bootstrap values as calculated by neighbor-joining/maximum-parsimony/maximum-likelihood probabilities in percent. Bootstrap values greater than 50% are shown at the branch points. Bar, 0.01 substitutions per 1 nucleotide position.

Table 2. Cellular fatty acid profiles of strain KHI28^T and recognized *Mucilaginibacter* species

Strains: 1, *Mucilaginibacter ginsenosidivorax* KHI28^T; 2, *Mucilaginibacter dorajii* DR-f4^T (data from Kim et al., 2010); 3, *Mucilaginibacter polysacchareus* KACC 15075^T; 4, *Mucilaginibacter lappiensis* KACC 14978^T; 5, *Mucilaginibacter composti* TR6-03^T; 6, *Mucilaginibacter rigui* KACC 14990^T (column 3-6 data from this study). All cells were cultured on R2A agar for 2 days with MIDI version 6.0. -, not detected. tr, trace amount (<0.5%); ECL, equivalent chain length. Major fatty acids are shown in bold type. Some fatty acids that account for less than 0.5% of the total fatty acids in all strains are excluded. Therefore, the percentages do not add up to 100%.

Fatty acid	1	2	3	4	5	6
Saturated						
C _{14:0}	1.0	0.6	0.8	0.6	0.9	0.6
C _{15:0}	-	2.8	3.0	-	-	-
C _{16:0}	12.2	7.8	14.1	7.3	8.2	7.7
Unsaturated						
C _{14:1} ω5c	2.5	-	-	1.4	3.2	2.4
C _{15:1} ω6c	-	tr	0.9	-	-	0.6
C _{16:1} ω5c	10.8	6.8	2.1	3.4	9.5	4.6
C _{17:1} ω8c	-	0.6	-	-	0.5	0.9
Branched-chain fatty acid						
iso-C _{13:0}	-	-	tr	-	-	0.6
iso-C _{15:0}	19.2	15.0	22.5	23.2	19.1	25.6
iso-C _{15:0} 3OH	2.4	1.8	2.8	2.1	3.1	2.1
iso-C _{16:0}	-	tr	-	0.5	-	1.2
iso-C _{17:0}	0.5	tr	-	0.8	1.4	1.0
iso-C _{17:0} 3OH	11.3	7.0	10.1	14.9	13.0	9.9
anteiso-C _{15:0}	2.3	-	-	0.8	2.2	1.7
anteiso-C _{17:0}	1.2	-	-	0.8	1.7	1.4
iso-C _{17:1} ω9c	2.4	1.8	-	4.2	4.1	4.6
Hydroxy fatty acids						
C _{15:0} 2OH	-	0.5	-	-	-	0.7
C _{15:0} 3OH	0.6	tr	-	-	-	0.5
C _{16:0} 3OH	-	1.6	3.5	2.1	-	0.8
Summed feature						
3; C _{16:1} ω7c/ C _{16:1} ω6c	33.69	45.6	39.4	37.0	32.8	31.8
Unknown						
ECL 13.565	-	1.2	0.9	-	-	-
ECL 16.582	-	0.5	-	-	-	-
ECL 18.81	-	0.6	-	-	-	-

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed features consist of: 3, C_{16:1} ω7c/ C_{16:1} ω6c

name *Mucilaginibacter ginsenosidivorax* sp. nov. is proposed.

Description of *Mucilaginibacter ginsenosidivorax* sp. nov.

Mucilaginibacter ginsenosidivorax (gin.se.no.si.di.vo^orax. N.L. n. *ginsenosidum* ginsenoside; L. adj. vorax, ravenous, voracious, devouring; N.L. masc. adj. *ginsenosidivorax*, consuming ginsenoside).

Cells are Gram-reaction-negative, strictly aerobic, non-motile and non-spore-forming rods (0.4–0.6 μm in diameter and 1.3–2.4 μm in length) after culture on R2A agar for 2 days. Colonies are smooth, transparent, convex, circular with regular margins, pale pink in color, and 2–3 mm in diameter after 2 days on R2A agar. Growth also occurs on nutrient agar and TSA, but not on MacConkey. Grows on R2A at 10–42°C and at pH 5.5–8.5 but not at 45°C. Optimum growth occurs at 30–37°C and at pH 7.0. Growth does not occur with 1% NaCl supplement. Catalase is negative, oxidase is positive. Hydrolyze starch and gelatin. Does not hydrolyze DNA, casein, pullulan, and laminarin, xylan and carboxymethyl-cellulose. Carbon assimilation tests as a sole carbon sources (API ID 32 GN, API 20 NE) and the enzyme

activities (API ZYM) are listed in Table 1. MK-7 is the predominant respiratory quinone, and summed feature 3 (comprising C_{16:1} ω6c and/or C_{16:1} ω7c), iso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3OH, and C_{16:1} ω5c are the major cellular fatty acids (>10%). The G+C content of the genomic DNA is 45.8 mol%.

The type strain, KHI28^T (=KACC 14955^T =LMG 25804^T) was isolated from the sediment of Gapcheon (river) in Daejeon, South Korea.

This work was supported by the Intelligent Synthetic Biology Center of Global Frontier Project funded by the Ministry of Education, Science and Technology (2011-0031967) and 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., Cui, C.H., Lee, H.G., Wang, L., Kim, S.C., Lee, S.T., Jin, F., Yu, H., Chin, Y.W., Lee, H.K., Im, W.T., and Kim, S.G. 2010. Identification and characterization of a novel *Terrabacter gin-*

- senosidimitans* sp. nov. beta-glucosidase that transforms ginsenoside Rb₁ into the rare gypenosides XVII and LXXV. *Appl. Environ. Microbiol.* **76**, 5827–5836.
- Atlas, R.M.** 1993. Handbook of Microbiological Media. CRC Press, Boca Raton, Florida, USA.
- Baik, K.S., Park, S.C., Kim, E.M., Lim, C.H., and Seong, C.N.** 2010. *Mucilagibacter rigui* sp. nov., isolated from wetland freshwater, and emended description of the genus *Mucilagibacter*. *Int. J. Syst. Evol. Microbiol.* **60**, 134–139.
- Buck, J.D.** 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* **44**, 992–993.
- Cappuccino, J.G. and Sherman, N.** 2002. Microbiology: a laboratory manual, 6th ed. Pearson Education, Inc., California, USA.
- Choi, J.R., Hong, S.W., Kim, Y., Jang, S.E., Kim, N.J., Han, M.J., and Kim, D.H.** 2011. Metabolic activities of ginseng and its constituents, ginsenoside Rb₁ and Rg₁, by human intestinal microflora. *J. Ginseng Res.* **35**, 301–307.
- Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and 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.
- Cui, C.H., Choi, T.E., Yu, H., Jin, F., Lee, S.T., Kim, S.C., and Im, W.T.** 2011. *Mucilagibacter composti* sp. nov., with ginsenoside converting activity, isolated from compost. *J. Microbiol.* **49**, 393–398.
- Ezaki, T., Hashimoto, Y., and Yabuuchi, E.** 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39**, 224–229.
- Felsenstein, J.** 1985. Confidence limit 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.
- Hall, T.A.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.
- Han, S.I., Lee, H.J., Lee, H.R., Kim, K.K., and Whang, K.S.** 2012. *Mucilagibacter polysacchareus* sp. nov., an exopolysaccharide-producing bacterial species isolated from the rhizoplane of the herb *Angelica sinensis*. *Int. J. Syst. Evol. Microbiol.* **62**, 632–637.
- Hiraishi, A., Ueda, Y., Ishihara, J., and Mori, T.** 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* **42**, 457–469.
- Im, W.T., Kim, S.Y., Liu, Q.M., Yang, J.E., Lee, S.T., and Yi, T.H.** 2010. *Nocardioides ginsengisegetis* sp. nov., isolated from soil of a ginseng field. *J. Microbiol.* **48**, 623–628.
- Joung, Y. and Joh, K.** 2011. *Mucilagibacter 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., and Yoon, J.H.** 2011. *Mucilagibacter 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., and Shin, K.S.** 2010. *Mucilagibacter 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., and Shin, K.S.** 2012. *Mucilagibacter angelicae* sp. nov., isolated from the rhizosphere of *Angelica polymorpha* Maxim. *Int. J. Syst. Evol. Microbiol.* **62**, 55–60.
- Kimura, M.** 1983. The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press, Cambridge, New York, N.Y., USA.
- Männistö, M.K., Tirola, M., McConnell, J., and Häggblom, M.M.** 2010. *Mucilagibacter frigitolerans* sp. nov., *Mucilagibacter lappiensis* sp. nov. and *Mucilagibacter mallensis* sp. nov., isolated from soil and lichen samples. *Int. J. Syst. Evol. Microbiol.* **60**, 2849–2856.
- Mesbah, M., Premachandran, U., and Whitman, W.** 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**, 159–167.
- Moore, D.D. and Dowhan, D.** 1995. Preparation and analysis of DNA, pp. 2–11. In Ausubel, F.W., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (eds.), Current Protocols in Molecular Biology. Wiley, New York, N.Y., USA.
- Pankratov, T.A., Tindall, B.J., Liesack, W., and Dedysh, S.N.** 2007. *Mucilagibacter paludis* gen. nov., sp. nov. and *Mucilagibacter 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.
- Park, C.S., Yoo, M.H., Noh, K.H., and Oh, D.K.** 2010. Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. *Appl. Microbiol. Biotechnol.* **87**, 9–19.
- Perry, L.B.** 1973. Gliding motility in some non-spreading flexibacteria. *J. Appl. Bacteriol.* **36**, 227–232.
- Quan, L.H., Piao, J.Y., Min, J.W., Kim, H.B., Kim, S.R., Yang, D.U., and Yang, D.C.** 2011. Biotransformation of ginsenoside Rb₁ to prosapogenins, gypenoside XVII, ginsenoside Rd, ginsenoside F₂, and compound K by *Leuconostoc mesenteroides* DC102. *J. Ginseng Res.* **35**, 344–351.
- Saitou, N. and 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., MIDI Inc., Newark, DE, USA.
- Tamara, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S.** 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.
- Ten, L.N., Im, W.T., Kim, M.K., Kang, M.S., and Lee, S.T.** 2004. Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J. Microbiol. Methods* **56**, 375–382.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G.** 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **24**, 4876–4882.
- Urai, M., Aizawa, T., Nakagawa, Y., Nakajima, M., and Sunairi, M.** 2008. *Mucilagibacter kameionensis* sp. nov., isolated from garden soil. *Int. J. Syst. Evol. Microbiol.* **58**, 2046–2050.
- Wang, L., An, D.S., Kim, S.G., Jin, F.X., Kim, S.C., Lee, S.T., and Im, W.T.** 2012. *Ramlibacter ginsenosidimitans* sp. nov., with ginsenoside-converting activity. *J. Microbiol. Biotechnol.* **22**, 311–315.
- 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., Stackebrandt, E., and et al.** 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.
- Zhao, X., Wang, J., Li, J., Fu, L., Gao, J., Du, X., Bi, H., Zhou, Y., and Tai, G.** 2009. Highly selective biotransformation of ginsenoside Rb₁ to Rd by the phytopathogenic fungus *Cladosporium fulvum* (syn. *Fulvia fulva*). *J. Ind. Microbiol. Biotechnol.* **36**, 721–726.