Lysobacter panaciterrae sp. nov., isolated from soil of a ginseng field

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A Gram-negative, aerobic, rod-shaped, non-spore-forming bacterial strain, designated Gsoil 068^T, was isolated from soil of a ginseng field in Pocheon Province (South Korea), and was characterized to determine its taxonomic position by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 068^T belonged to the family *Xanthomonadaceae*, class *Gammaproteobacteria*, and was related most closely to *Lysobacter brunescens* ATCC 29482^T and *Lysobacter gummosus* ATCC 29489^T (96.1 % sequence similarity). The G + C content of the genomic DNA of strain Gsoil 068^T was 67.0 mol%. The detection of a quinone system with ubiquinone Q-8 as the predominant component and a fatty acid profile with iso-C_{15:0}, iso-C_{17:1ω9c}, iso-C_{17:0} and iso-C_{11:0} 3-OH as the major components supported the affiliation of strain Gsoil 068^T to the genus *Lysobacter*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 068^T is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter panaciterrae* sp. nov. is proposed. The type strain is Gsoil 068^T (=KCTC 12601^T =DSM 17927^T).

The genus *Lysobacter*, belonging to the family *Xanthomonadaceae*, class *Gammaproteobacteria*, was established by Christensen & Cook (1978) with the description of four species: *Lysobacter enzymogenes*, *L. antibioticus*, *L. brunescens* and *L. gummosus*. They are Gram-negative, aerobic, gliding organisms with a high DNA G + C content and develop colonies that are highly mucoid and cream, pink or yellow–brown in colour. Members of the genus are strongly proteolytic and characteristically lyse a variety of micro-organisms such as Gram-positive bacteria (including actinomycetes), filamentous fungi, yeasts and green algae, as well as nematodes. *L. enzymogenes* was shown to be a potential biocontrol agent for plant fungal pathogens (Folman *et al.*, 2004; Kilic-Ekici & Yuen, 2003). In addition to the four species mentioned above, at the time of writing the genus *Lysobacter* includes nine other recognized species, namely *Lysobacter capsici* (Park *et al.*, 2008), *L. concretionis* (Bae *et al.*, 2005), *L. daejeonensis* (Weon *et al.*, 2006), *L. defluvii* (Yassin *et al.*, 2007), *L. koreensis* (Lee *et al.*, 2006), *L. niabensis* and *L. niastensis* (Weon *et al.*, 2007), *L. spongiicola* (Romanenko *et al.*, 2008) and *L. yangpyeon-*

ensis (Weon *et al.*, 2006). Except *L. spongiicola*, all members of the genus *Lysobacter* have been isolated from terrestrial environments, mainly from soil.

During the course of a study on the culturable aerobic and facultatively anaerobic bacterial community living in the soil of a ginseng field in Pocheon Province (South Korea), a large number of bacterial strains were isolated. In the present study, we have characterized one of these isolates, namely strain Gsoil 068^T. Phenotypic, chemotaxonomic and phylogenetic analyses established that this isolate was affiliated with the genus *Lysobacter*. The data obtained also suggest that the isolate represents a novel species of the genus.

A soil sample was taken from the rhizosphere of 5-year-old ginseng plants. This was a slightly acidic (pH 6.0), rich, sandy soil with a friable and well-aerated texture. The soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0), serially diluted in the same buffer and suitable 10-fold dilutions were spread onto modified R2A solid medium containing the following (per litre distilled water): 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble

The GenBank/EMBL/DBJ accession number for 16S rRNA gene sequence of strain Gsoil 068^T is AB245359.

starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂ and 15 g agar. The plates were incubated at 30 °C for 2 weeks. Single colonies appearing on the plates were purified by transferring them onto fresh plates of the modified R2A agar and incubating again. One isolate, Gsoil 068^T, was cultured routinely on R2A agar (Difco) at 30 °C and was preserved in a glycerol solution (20 %, w/v) at -70 °C.

The Gram reaction was determined by using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000 magnification, with cells grown on R2A agar for 2 days at 30 °C. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Tests for anaerobic growth, nitrate and nitrite reduction and assimilation of 16 amino acids and 31 other compounds (except for substrates included in API 20NE and API ID 32GN kits) were performed as described by Ten *et al.* (2006). In addition, biochemical tests were carried out by using API 20E, API 20NE and API ID 32GN test kits according to the manufacturer's instructions (bioMérieux). Tests for degradation of DNA (by using DNase agar from Scharlau, with DNase activity by flooding plates with 1 M HCl), casein, chitin, starch (Atlas, 1993), lipids (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth at different temperatures (4, 10, 15, 25, 30, 37, 42, 45 and 50 °C) and pH (4.5–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated (at 30 °C).

Cells of strain Gsoil 068^T were aerobic, Gram-negative, rod-shaped, non-spore-forming and non-motile but having gliding activity. The isolate grew on nutrient agar, TSA and MacConkey agar. It did not require NaCl for growth but was able to tolerate up to 3 % (w/v) NaCl. Strain Gsoil 068^T was able to grow at 15–45 °C. The isolate hydrolysed gelatin, indicating proteolytic activity; this was also observed for the type strains of all recognized *Lysobacter* species. The ability to degrade chitin and starch, which is present in some *Lysobacter* species (Christensen & Cook, 1978; Weon *et al.*, 2006, 2007), was not observed in the novel strain. Strain Gsoil 068^T was negative for arginine dihydrolase, urease, indole production and the assimilation of D-ribose, inositol, gluconate, adipate, phenylacetate, itaconate, suberate, malonate, lactate, 4-hydroxybenzoate, salicin and L-alanine, consistent with data for most recognized *Lysobacter* species (Romanenko *et al.*, 2008). Phenotypic and chemotaxonomic characteristics that differentiate strain Gsoil 068^T from recognized *Lysobacter* species are listed in Table 1. In particular, strain Gsoil 068^T could be differentiated readily from most *Lysobacter* species based on its ability to grow at 45 °C, to utilize caprate and to produce catalase.

For phylogenetic analysis, genomic DNA was extracted by using a commercial genomic DNA extraction kit (Solgent).

PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The nearly complete 16S rRNA gene sequence of strain Gsoil 068^T was compiled by using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the CLUSTAL X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated by using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA4 program (Tamura *et al.*, 2007), with bootstrap values based on 1000 replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain Gsoil 068^T was a continuous stretch of 1489 bp. Comparative 16S rRNA gene sequence analyses showed that the novel strain was phylogenetically affiliated with species of the genus *Lysobacter*. In the phylogenetic tree (Fig. 1) based on the neighbour-joining algorithm, strain Gsoil 068^T appeared within the genus *Lysobacter* and formed a branch with *L. brunescens* ATCC 29482^T. On the basis of 16S rRNA gene sequence similarity data, strain Gsoil 068^T was related most closely to *L. brunescens* ATCC 29482^T and *L. gummosus* ATCC 29489^T (96.1 %). Levels of 16S rRNA gene sequence similarity to the type strains of other recognized species within the class *Gammaproteobacteria* were below 96.0 %. The generally accepted criteria for delineating bacterial species state that strains showing 16S rRNA gene sequence dissimilarity above 3 % or showing a level of DNA–DNA relatedness below 70 % (as measured by hybridization) are considered as belonging to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). The data presented here thus indicated that strain Gsoil 068^T was not related to any previously described member of the genus *Lysobacter* at the species level.

For measurement of the G + C content of the chromosomal DNA, the genomic DNA of strain Gsoil 068^T was extracted and purified as described by Moore & Dowhan (1995). The G + C content of the DNA was determined as described by Mesbah *et al.* (1989) after degradation of the DNA to nucleosides by P1 nuclease and alkaline phosphatase and subsequent separation of the nucleosides by reversed-phase HPLC. 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 n-hexane/quinone solution was purified by using Sep-Pak Vac Cartridges Silica (Waters) and was analysed subsequently by HPLC, as described by Hiraishi *et al.* (1996). The cellular fatty acid profile of strain Gsoil 068^T was determined following growth on R2A agar (Difco) for 48 h at 28 °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 analysed by GC (model 6890; Hewlett Packard) by using the Microbial

Table 1. Differential phenotypic characteristics between strain Gsoil 068^T and the type strains of recognized *Lysobacter* species

Strains: 1, Gsoil 068^T (data from the present study); 2, *L. brunescens* ATCC 29482^T (data in columns 2, 3, 5 and 6 from Swings & Christensen, 1989); 3, *L. gummosus* ATCC 29489^T; 4, *L. capsici* KCTC 22007^T (Park *et al.*, 2008); 5, *L. antibioticus* DSM 2044^T; 6, *L. enzymogenes* DSM 2043^T; 7, *L. niastensis* DSM 18481^T (Weon *et al.*, 2007); 8, *L. concretionis* DSM 16239^T (Bae *et al.*, 2005); 9, *L. yangpyeongensis* KACC 11407^T (Weon *et al.*, 2006); 10, *L. daejeonensis* KACC 11406^T (Weon *et al.*, 2006); 11, *L. niabensis* DSM 18244^T (Weon *et al.*, 2007); 12, *L. spongiicola* JCM 14760^T (Romanenko *et al.*, 2008); 13, *L. koreensis* KCTC 12204^T (Lee *et al.*, 2006); 14, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007). All strains are positive for gelatin hydrolysis and negative for urease, indole production and the assimilation of inositol. +, Positive; (+), weakly positive; ±, variable; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Growth at/on:														
5 °C	–	+	–	–	+	+	–	+	–	–	+	+	–	–
45 °C	+	+	–	–	–	–	–	–	–	–	–	–	–	–
MacConkey agar	+	–	–	–	–	+	–	ND	–	–	–	–	+	–
Gliding motility	+	+	+	+	+	+	+	+	–	–	–	–	ND	ND
Nitrate reduction	–	–	–	ND	+	–	+	+	–	+	–	–	–	–
Aesculin hydrolysis	+	+	+	+	+	+	+	–	–	+	–	–	–	–
Starch hydrolysis	–	–	–	–	–	–	+	–	+	–	+	–	–	–
Catalase	–	+	+	+	+	+	+	+	–	(+)	+	+	+	+
Oxidase	+	+	ND	+	+	+	+	+	+	+	+	+	–	+
β-Galactosidase	–	–	±	–	+	±	±	–	–	–	–	–	–	–
NaCl tolerance (range, %)	0–3	0–1	0–2	0–2	0–2	0–2	0–1	ND	0–0.5	0–3	0–1	0–6	0–1	0–6
Assimilation of:*														
Acetate	+	–	(+)	ND	+	(+)	–	+	–	+	–	–	–	ND
N-Acetyl-D-glucosamine	+	–	+	–	+	+	+	–	–	–	–	–	–	–
Glycogen	+	–	+	ND	+	+	–	(+)	+	+	–	–	–	ND
Maltose	+	–	+	–	+	+	+	–	–	+	–	–	–	–
Melibiose	–	–	+	ND	–	+	–	–	–	–	–	–	–	ND
L-Rhamnose	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Sucrose	–	–	+	–	–	+	–	–	–	–	–	–	–	ND
L-Arabinose	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Caprate	+	–	–	ND	–	–	–	–	–	–	–	–	–	–
D-Sorbitol	–	–	–	–	–	–	–	–	–	–	–	–	+	–
D-Mannose	+	–	+	ND	+	+	–	–	–	–	–	–	–	–
D-Mannitol	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Valerate	+	–	(+)	ND	+	+	–	+	–	+	–	–	+	ND
D-Glucose	+	–	+	–	+	+	(+)	–	–	+	–	–	–	–
L-Histidine	–	–	–	ND	+	(+)	+	–	–	–	–	–	–	ND
Citrate	+	–	–	+	–	+	–	–	–	–	–	–	+	+
3-Hydroxybutyrate	+	–	+	ND	+	+	–	+	+	+	–	–	–	ND
5-Ketogluconate	–	–	–	–	–	–	–	–	–	–	–	+	–	ND
Malate	+	–	+	ND	+	+	–	–	–	–	–	–	–	–
L-Proline	–	–	+	ND	+	+	–	+	–	–	–	–	–	ND
DNA G + C content (mol%)	67.0	67.7	65.7	65.4	69.2	69.0	66.6	63.8	67.3	61.7	62.5	69.0	68.9	67.1

*Using API 20NE and API ID 32GN tests.

Identification software package (Sasser, 1990). Duplicate experiments were performed to give a range of values. Polar lipids were extracted and examined by two-dimensional TLC according to Minnikin *et al.* (1984).

The fatty acid profile of strain Gsoil 068^T (Table 2) was compared with those of the type strains of recognized *Lysobacter* species. The major components were branched fatty acids, namely iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{17:0} and iso-C_{11:0} 3-OH, a profile that is typical of members of the

genus *Lysobacter* (Bae *et al.*, 2005; Weon *et al.*, 2006, 2007; Romanenko *et al.*, 2008). However, some minor qualitative and quantitative differences in the fatty acids were observed between strain Gsoil 068^T and its phylogenetically closest relatives. In particular, strain Gsoil 068^T differed from recognized *Lysobacter* species based on its higher content of iso-C_{17:0} and iso-C_{17:1}ω9c and lower content of iso-C_{16:0}. Strain Gsoil 068^T contained ubiquinone Q-8 as the major respiratory quinone. The polar lipids detected in strain Gsoil 068^T were phosphatidylethanolamine, diphosphati-

Fig. 1. Phylogenetic relationship between strain Gsoil 068^T and the type strains of recognized *Lysobacter* species and other related species within the class *Gammaproteobacteria*. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) of more than 50 % are shown at branch points. The sequence of *Pseudomonas aeruginosa* LMG 1242^T (GenBank accession no. Z76651) was used as an outgroup (not shown). Filled circles indicate that the corresponding notes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position.

dylglycerol, phosphatidylglycerol, one unknown aminolipid and one unknown lipid. These data are in good agreement with those for other members of the genus *Lysobacter* (Bae *et al.*, 2005; Weon *et al.*, 2006, 2007; Park *et al.*, 2008; Romanenko *et al.*, 2008; Yassin *et al.*, 2007). The genomic DNA G + C content of strain Gsoil 068^T was 67.0 mol%, which lies within the range observed for members of the genus *Lysobacter* (61.7–69.2 mol%).

The phenotypic and phylogenetic data presented here indicate that strain Gsoil 068^T belongs to the genus *Lysobacter*. Phylogenetic distinctiveness confirmed that this isolate represent a species that is distinct from recognized *Lysobacter* species. Strain Gsoil 068^T can be differentiated from phylogenetically related *Lysobacter* species based on several phenotypic characteristics (Table 1). Therefore, on the basis of the data presented, strain Gsoil 068^T is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter panaciterrae* sp. nov. is proposed.

Description of *Lysobacter panaciterrae* sp. nov.

Lysobacter panaciterrae (pa.na.ci.ter'rae. N.L. n. *Panax* -*acis* scientific name for ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil of a ginseng field).

Colonies grown on R2A agar plates for 3 days are 1.5–3.0 mm in diameter, smooth, circular, non-glossy and cream-coloured, but take on a weak brownish yellow swarming form after 1 month. The optimum temperature for growth is 30 °C. The pH range for growth is pH 5.0–8.5, with an optimum between pH 6.5 and 7.0. Able to hydrolyse gelatin and casein, but not chitin, starch,

cellulose, xylan, lipids or DNA. Nitrate is not reduced to nitrite and nitrite is not reduced to nitrogen gas. The following substrates are utilized for growth: D-glucose, D-mannose, D-lyxose, L-xylose, *N*-acetyl-D-glucosamine, cellobiose, maltose, trehalose, aesculin, acetate, caprate, citrate, formate, DL-3-hydroxybutyrate, malate, pyruvate, succinate, valerate, glycogen, L-aspartate, L-glutamine and L-tyrosine. The following substrates are not utilized for growth: L-fructose, D- and L-arabinose, L-fucose, D-galactose, L-rhamnose, L-sorbose, D-ribose, D-xylose, salicin, lactose, melibiose, sucrose, raffinose, amygdalin, inulin, dextran, propionate, maleate, fumarate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, lactate, malonate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, 2-ketogluconate, 5-ketogluconate, gluconate, dulcitol, inositol, D-adonitol, D-mannitol, D-sorbitol, xylitol, methanol, ethanol, glycerol, urea, L-alanine, L-arginine, L-asparagine, L-cysteine, L-glutamate, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan and L-valine. Positive for tryptophan deaminase and the Voges–Proskauer test. Production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, β -galactosidase, hydrogen sulfide and indole is negative. Acid is produced from D-glucose, but not from melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose or sucrose. Ubiquinone Q-8 is the predominant quinone. Phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol are the main polar lipids. The major fatty acids are iso-C_{15:0}, iso-C_{17:1} ω 9c, iso-C_{17:0} and iso-C_{11:0} 3-OH. The G + C content of the genomic DNA of the type strain is 67.0 mol%.

Table 2. Cellular fatty acid profiles of strain Gsoil 068^T and type strains of recognized *Lysobacter* species

Strains: 1, Gsoil 068^T (data from the present study); 2, *L. brunescens* ATCC 29482^T; 3, *L. gummosus* ATCC 29489^T; 4, *L. capsici* KCTC 22007^T (Park *et al.*, 2008); 5, *L. antibioticus* DSM 2044^T; 6, *L. enzymogenes* DSM 2043^T; 7, *L. niastensis* DSM 18481^T; 8, *L. concretionis* DSM 16239^T; 9, *L. yangpyeongensis* KACC 11407^T; 10, *L. daejeonensis* KACC 11406^T; 11, *L. niabensis* DSM 18244^T; 12, *L. spongiicola* JCM 14760^T (Romanenko *et al.*, 2008); 13, *L. koreensis* KCTC 12204^T; 14, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007). Data are from Weon *et al.* (2007) unless indicated otherwise. All strains were grown on R2A agar for 48 h at 28 °C, except *L. capsici* KCTC 22007^T (TSA, 48 h, 28 °C), *L. spongiicola* JCM 14760^T (R2A agar, 72 h, 28 °C) and *L. defluvii* DSM 18482^T (BHI broth, 1 week, 37 °C). Values are percentages of total fatty acids; –, <1% or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14
C _{14:0}	—	—	—	1.9	1.1	1.0	—	—	—	—	—	—	—	—
C _{16:0}	5.2	1.5	6.0	10.8	8.0	8.6	—	1.6	3.1	1.4	1.1	—	—	2.9
C _{17:0} cyclo	—	—	1.0	—	7.2	6.2	—	1.9	—	—	—	—	—	3.2
iso-C _{10:0}	—	—	—	—	—	—	—	—	—	—	1.0	—	1.1	—
iso-C _{11:0}	3.8	5.9	3.8	2.3	3.1	3.4	4.1	5.7	4.3	3.7	6.4	9.5	5.3	1.8
iso-C _{12:0}	—	—	—	—	—	—	—	—	1.1	2.0	1.3	—	1.1	—
iso-C _{14:0}	—	3.7	—	—	1.3	1.4	4.2	2.3	4.5	11.2	8.7	3.3	4.0	—
iso-C _{15:0}	29.5	19.6	25.2	23.3	24.9	20.5	21.9	33.6	14.5	13.1	12.7	23.0	12.5	40.9
iso-C _{16:0}	3.4	23.5	5.7	—	10.3	13.8	23.3	20.4	27.5	33.7	23.7	32.5	26.3	19.3
iso-C _{17:0}	16.0	2.3	7.8	3.7	3.4	2.9	1.3	4.1	1.9	—	1.6	2.8	1.8	11.1
anteiso-C _{15:0}	4.5	2.6	5.5	—	3.8	3.8	3.8	1.2	5.1	3.2	5.9	—	—	—
anteiso-C _{17:0}	1.4	—	1.4	—	—	—	—	—	1.1	—	—	—	—	—
C _{10:0} 3-OH	—	—	—	—	—	1.1	—	—	—	—	—	—	—	—
iso-C _{11:0} 3-OH	6.9	7.2	9.7	3.8	8.0	6.6	8.0	6.9	5.5	6.0	9.3	15.5	9.0	7.2
iso-C _{12:0} 3-OH	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—
iso-C _{15:1} AT 5	—	—	1.7	—	1.0	—	1.6	—	3.1	—	3.4	—	4.4	—
iso-C _{15:1} F	—	1.7	—	—	—	—	—	3.2	—	3.2	—	—	—	—
iso-C _{16:1} H	—	1.5	—	—	—	—	1.3	—	1.1	2.6	1.0	—	2.1	—
C _{16:1} ω11c	—	—	4.5	2.2	4.1	—	—	—	2.2	—	1.0	—	—	—
C _{16:1} ω7c alcohol	—	—	1.7	—	1.6	—	4.5	—	8.8	—	7.8	—	10.8	—
iso-C _{17:1} ω9c	23.1	15.5	12.2	—	6.4	4.7	10.9	15.1	6.7	6.7	10.0	13.2	16.7	5.8
C _{18:1} ω7c	—	—	2.5	6.5	1.7	3.3	—	—	—	—	—	—	—	—
Summed feature 4*	4.8	9.5	6.4	20.4	8.3	15.8	6.5	—	3.3	6.1	2.0	—	1.4	—
ECL 11.799†	1.4	—	1.8	—	2.0	1.5	1.4	—	—	—	—	—	—	—

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified; ECL, equivalent chain length.

The type strain, Gsoil 068^T (=KCTC 12601^T=DSM 17927^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

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References

- Atlas, R. M. (1993).** *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Bae, H.-S., Im, W.-T. & Lee, S.-T. (2005).** *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* **55**, 1155–1161.
- Buck, J. D. (1982).** Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin/Cummings.

Christensen, P. & Cook, F. D. (1978). *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* **28**, 367–393.

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.

Folman, L. B., De Klein, M. J. E. M., Postma, J. & van Veen, J. A. (2004). Production of antifungal compounds by *Lysobacter enzymogenes* isolate 3.1T8 under different conditions in relation to its efficacy as a biocontrol agent of *Pythium aphanidermatum* in cucumber. *Biol Control* **31**, 145–154.

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.

Hiraishi, A., Ueda, Y., Ishihara, J. & 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.
- Kilic-Ekici, O. & Yuen, G. Y. (2003). Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3. *Biol Control* **93**, 1103–1110.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β -glucosidase-producing bacterium in the family *Sphingomonadaceae* in α -4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kouker, G. & Jaeger, K.-E. (1987). Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol* **53**, 211–213.
- Lee, J. W., Im, W.-T., Kim, M. K. & Yang, D.-C. (2006). *Lysobacter koreensis* sp. nov., isolated from a ginseng field. *Int J Syst Evol Microbiol* **56**, 231–235.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- 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.
- Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, chapter 2, pp. 2–11. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Park, J. H., Kim, R., Aslam, Z., Jeon, C. O. & Chung, Y. R. (2008). *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Microbiol* **58**, 387–392.
- Romanenko, L. A., Uchino, M., Tanaka, N., Frolova, G. M. & Mikhailov, V. V. (2008). *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* **58**, 370–374.
- 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 through fatty acid analysis. In *Methods in Phytobacteriology*, pp. 199–204. Edited by Z. Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kiado.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Swings, J. & Christensen, P. (1989). Genus *Lysobacter* Christensen and Cook 1978, 372^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2083–2089. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & 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.
- Ten, L. N., Liu, Q.-M., Im, W.-T., Lee, M., Yang, D.-C. & Lee, S.-T. (2006). *Pedobacter ginsengisoli* sp. nov., a DNase-producing bacterium isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **56**, 2565–2570.
- 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.
- 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.
- Weon, H.-Y., Kim, B.-Y., Baek, Y.-K., Yoo, S.-H., Kwon, S.-W., Stackebrandt, E. & Go, S.-J. (2006). Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. *Int J Syst Evol Microbiol* **56**, 947–951.
- Weon, H.-Y., Kim, B.-Y., Kim, M.-K., Yoo, S.-H., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2007). *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. *Int J Syst Evol Microbiol* **57**, 548–551.
- Yassin, A. F., Chen, W.-M., Hupfer, H., Siering, C., Kroppenstedt, R. M., Arun, A. B., Lai, W.-A., Shen, F.-T., Rekha, P. D. & Young, C. C. (2007). *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Microbiol* **57**, 1131–1136.