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Lysobacter ginsengisoli sp. nov., a Novel Species Isolated from Soil in Pocheon Province, South Korea

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A Gram-negative, aerobic, rod-shaped, nonspore-forming bacterial strain, designated Gsoil 357^T, was isolated from soil sample of a ginseng field in Pocheon Province (South Korea). The isolate contained Q-8 as the predominant ubiquinone and iso-$C_{16:0}$, iso-$C_{17:1}$ ω9c, and iso-$C_{15:0}$ as the major fatty acids. The G+C content of the genomic DNA was 69.3 mol%. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Gsoil 357^T was most closely related to *Lysobacter gummosus* (97.6%) and *Lysobacter antibioticus* (97.6%). However, the DNA-DNA relatedness value between strain Gsoil 357^T and its phylogenetically closest neighbors was less than 17%. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 357^T should be classified as representing a novel species in the genus *Lysobacter*, for which the name *Lysobacter ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 357^T (=KCTC 12602^T=DSM 18420^T).

Keywords: *Lysobacter ginsengisoli*, polyphasic taxonomy, ginseng-field soil

The genus *Lysobacter* was established by Christensen and Cook [4] for nonfruiting body gliding bacteria with high G+C content, and at present comprises eleven species with validly published names, namely *Lysobacter antibioticus* [4], *Lysobacter brunescens* [4], *Lysobacter concretionis* [2], *Lysobacter daejeonensis* [16], *Lysobacter defluvii* [18], *Lysobacter enzymogenes* [4], *Lysobacter gummosus* [4], *Lysobacter koreensis* [7], *Lysobacter niabensis* [17], *Lysobacter niastensis* [17], and *Lysobacter yangpyeongensis* [16]. During a course of study on the culturable aerobic bacterial community in soil of a ginseng field in Pocheon Province (South Korea), a large number of bacteria were isolated. Some of the strains were already characterized and classified as novel species of the genera *Bacillus* [13], *Chitinophaga* [1], and *Cohnella* [19]. In this study, we have characterized one of these isolates, strain Gsoil 357^T. The phenotypic, chemotaxonomic, and phylogenetic analyses established the affiliation of the isolate to the genus *Lysobacter* in the *Gammaproteobacteria* lineage. The data obtained suggest that the isolate represents a novel species of the genus *Lysobacter*.

Strain Gsoil 357^T was isolated from a soil sample by using modified R2A medium as described previously [13]. The isolate was routinely cultured on R2A agar at 25–30°C and preserved in a glycerol solution (20%) at −70°C. The Gram reaction was performed by the nonstaining method, as described by Buck [3]. Cell morphology was observed under a light microscope (Nikon) at ×1,000, with cells grown on R2A agar for 2 days at 25°C. The assimilation of single carbon sources was determined at 25°C as described previously [14]. Some physiological characteristics were determined using API 20E and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Catalase and oxidase activities, anaerobic growth, growth at different temperatures, degradation of natural biopolymers, and other characteristics were tested as reported previously [13, 14]. Cells of strain Gsoil 357^T were Gram-negative, aerobic, nonspore-forming, and rod shaped with various sizes (0.7–1.0 by 1.0–4.5 μm). The isolate grew well on R2A, but did not grow on nutrient agar, tryptic soy agar (TSA, Difco), and MacConkey agar. Flagella movement was not observed by microscopy. Colony has the ability to swarm and spread on the surface of R2A agar plates by means of gliding. Strain Gsoil 357^T was able to grow at 20–30°C, but did not grow at 15 or 37°C. Gelatin was liquefied within one day, indicating proteolytic activity; this was also observed for previously reported members of the genus *Lysobacter* [17, 18]. The capability to degrade chitin and starch that is present in some *Lysobacter* species [4, 16] was not observed in strain Gsoil 357^T. The physiological characteristics of strain

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Table 1. Comparison of characteristics of *Lysobacter ginsengisoli* sp. nov. and validly described *Lysobacter* species.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12
Gliding motility	+	+	+	–	+	+	+	+	ND	+	–	–
Nitrate reduction	–	–	+	+	+	–	–	+	–	–	–	–
Aesculin hydrolysis	+	+	+	+	–	+	+	+	–	–	–	–
Glucose acidification	–	–	+	–	–	+	–	ND	–	–	–	ND
Catalase/oxidase	+/+	+/ND	+/+	w/+	+/+	+/+	+/+	+/+	+/–	+/+	–/+	+/+
β-Galactosidase	–	+	+	–	–	+	–	+	–	–	–	–
Assimilation of												
N-Acetyl glucosamine	–	+	+	–	–	+	–	+	–	–	–	–
Maltose	–	+	+	+	–	+	–	+	–	–	–	–
Acetate	–	+	–	+	+	–	+	–	–	ND	–	–
Glycogen	–	+	+	+	+	+	–	–	–	ND	+	–
L-Serine	–	–	–	–	–	+	–	–	+	ND	–	–
D-Glucose	–	+	–	+	–	+	–	w	–	–	–	–
Salicin	–	–	–	–	–	+	–	–	–	ND	–	–
D-Melibiose	–	+	–	–	–	+	–	–	–	ND	–	–
Arabinose	–	+	–	–	–	–	–	–	+	–	–	–
Valerate	–	+	+	+	+	–	–	–	+	ND	–	–
Citrate	–	+	–	–	–	+	–	–	+	–	–	–
L-Histidine	–	+	–	–	–	–	–	+	–	ND	–	–
3-Hydroxybutyrate	–	+	+	+	+	+	–	–	–	ND	+	–
L-Proline	–	+	+	–	+	+	–	–	–	ND	–	–
D-Mannose	–	–	+	–	–	+	–	–	–	–	–	–
Malate	–	–	–	–	–	+	–	–	–	–	–	–
DNA G+C content (mol%)	69.3	65.7	69.2	61.7	63.8	69.0	67.7	66.6	68.9	67.1	67.3	62.5

Strains: 1, *Lysobacter ginsengisoli* Gsoil 357^T; 2, *L. gummosus* ATCC 29489^T; 3, *L. antibioticus* DSM 2044^T; 4, *L. daejeonensis* KACC 11406^T; 5, *L. concretionis* DSM 16239^T; 6, *L. enzymogenes* DSM 2043^T; 7, *L. brunescens* ATCC 29482^T; *L. niastensis* DSM 18481^T; 9, *L. koreensis* KCTC 12204^T; 10, *L. defluvii* DSM 18482^T; 11, *L. yangpyeongensis* KACC 11407^T; 12, *L. niabensis* DSM 18244^T; Data are from Bae *et al.* [2], Lee *et al.* [7], Weon *et al.* [16, 17], Yassin *et al.* [18], and this study. According to the API 20NE test strips, all strains are positive for gelatin hydrolysis, but negative for indole production, arginine dihydrogenase, and urease. Results are scored as +, positive; –, negative; w, weakly positive; ND, not determined.

Gsoil 357^T are summarized in the species description, and selective characteristics that differentiate strain Gsoil 357^T from other members of the genus *Lysobacter* are listed in Table 1.

For phylogenetic analysis of strain Gsoil 357^T, genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent Co. Ltd., Korea). PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* [6]. Other details of phylogenetic analysis were as reported previously [1, 13, 14]. The 16S rRNA gene sequence of the strain Gsoil 124^T determined in this study was continuous stretches of 1,494 bp. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain Gsoil 357^T fell within the *Gammaproteobacteria* and was most closely affiliated to *Lysobacter* species (Fig. 1). The closest phylogenetic neighbors of strain Gsoil 357^T are *Lysobacter antibioticus* DSM 2044^T (97.6%) and *Lysobacter gummosus* KCTC 12132^T (97.6%). The sequence similarities to other validly described species within the genus *Lysobacter* were less than 96.3%. The above-mentioned data indicate that strain Gsoil 357^T is a species that is clearly separate from other *Lysobacter* spp., with the exceptions of the above-mentioned two species of this genus. To differentiate strain Gsoil 357^T from the closely related species, DNA-DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* [5]. DNA-DNA relatedness values of strain Gsoil 357^T to *Lysobacter gummosus* KCTC 12132^T and *Lysobacter antibioticus* DSM 2044^T were 16.3% and 16.2%, respectively, which is low enough [12, 15] to show that strain Gsoil 357^T differs genetically from the two *Lysobacter* species.

Isoprenoid quinones were extracted and purified according the method reported previously [1] and analyzed by HPLC, as described by Shin *et al.* [11]. Cellular fatty acids were extracted and analyzed according to the instructions of the Sherlock Microbial Identification System (MIDI) [10]. Q-8 was the predominant ubiquinone of strain Gsoil 357^T. The quinone system supported our assignment of strain Gsoil 357^T to the family *Xanthomonadaceae*, in which the majority of species (including *Lysobacter* species) also have Q-8 as the major quinone. The cellular fatty acids of strain Gsoil 357^T were iso-$C_{16:0}$ (46.0% of total fatty acids), iso-$C_{17:1}$ ω9c (16.8%), iso-$C_{15:0}$ (11.5%), iso-$C_{17:0}$ (5.3%), anteiso-$C_{15:0}$ (4.8%), $C_{18:0}$ (4.6%), Summed

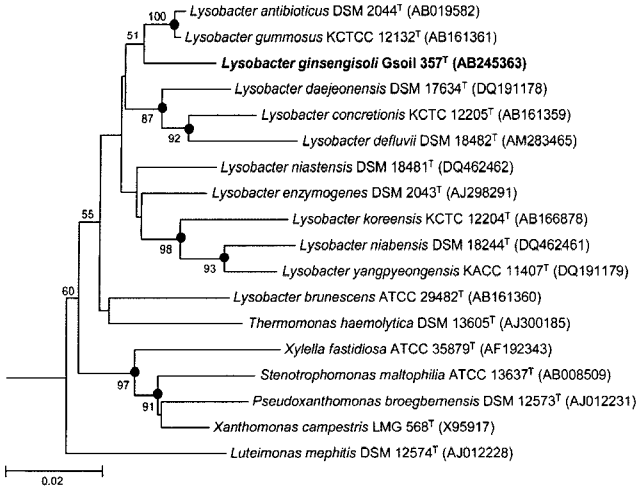

Fig. 1. Phylogenetic relationship of strain Gsoil 357^T with *Lysobacter* species and other related species of *Gammaproteobacteria*.
The tree was constructed by the neighbor-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are shown at branch points. *Escherichia coli* ATCC 11775^T was used as an outgroup (not shown). Filled circles at nodes indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bar, 0.02 substitutions per 1 nucleotide position.

feature 4 (iso-$C_{15:0}$ 2OH and/or $C_{16:1}$ ω7c, 3.2%), iso-$C_{16:0}$ H (2.0%), anteiso-$C_{17:0}$ (1.3%), Summed feature 7 ($C_{18:1}$ ω7c/ω9t/ω12t and/or $C_{18:1}$ ω7c/ω9c/ω12t, 1.3%), $C_{14:0}$ (1.1%), iso-$C_{15:1}$ F (0.8%), $C_{14:0}$ 2-OH (0.7%), and iso-$C_{18:0}$ (0.6%). These fatty acids, with some minor differences, were also detected in recognized members of the genus *Lysobacter* [2, 7, 16–18]. For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain Gsoil 357^T was extracted and purified as described by Moore [9] and enzymatically degraded into nucleotides. The G+C content was then determined as described by Mesbah *et al.* [8]. The G+C content for the genus *Lysobacter* ranges from 61.7 mol% (reported for *L. daejeonensis* KACC 11406^T) to 69.2 mol% (reported for *L. antibioticus* DSM 2044^T) [4, 16]. The DNA G+C content of strain Gsoil 357^T is 69.3 mol%.

The phenotypic and phylogenetic characteristics indicated that strain Gsoil 357^T belongs to the genus *Lysobacter*. The phylogenetic distinctiveness, together with DNA-DNA hybridization data, confirmed that strain Gsoil 357^T represents a species that is distinct from recognized *Lysobacter* species. There are some phenotypic differences between strain Gsoil 357^T and *Lysobacter* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 357^T should be classified within the genus *Lysobacter* as representing a novel species, for which the name *Lysobacter ginsengisoli* sp. nov. is proposed.

Description of *Lysobacter ginsengisoli* sp. nov.

Lysobacter ginsengisoli (gin.sen.gi.soli. N.L. n. ginsengum, ginseng; L. n. solum, soil; N.L. gen. n. ginsengisoli, of soil of a ginseng field, the source of the organism).

The colonies grown on R2A agar plate (Difco) for two days are smooth, circular, non-glossy, cream-colored coves around 2–4 mm in diameter. The colonies grown on R2A agar plate for 10 days are cream-colored circles, but turn to weak-brownish cream-colored swarming forms after one month. The optimum temperature for growth is 25°C. Grows within pH values of between 5.0 and 8.5; the optimum pH is 6.5–7.0. Tolerates 2% (w/v) NaCl, but not 3%. Nitrate is not reduced to nitrite and nitrite is not reduced to nitrogen gas. Hydrolyzes gelatin, but not chitin, starch, cellulose, xylan, lipids, and DNA. The following substrates are not utilized for growth as sole carbon source: D-glucose, D-galactose, D-mannose, D-fructose, D-arabinose, L-arabinose, D-fucose, L-rhamnose, L-sorbose, D-lyxose, D-ribose, D-xylose, *p*-nitrophenyl-β-D-galactopyranoside, *N*-acetyl-D-glucosamine, salicin, D-cellobiose, D-lactose, D-maltose, D-melibiose, D-sucrose, D-trehalose, D-raffinose, amygdalin, inulin, dextran, pyruvate, formate, acetate, propionate, DL-3-hydroxybutyrate, valerate, caprate, maleate, fumarate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, citrate, lactate, malate, malonate, succinate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, gluconate, nitrate, dulcitol, inositol, D-adonitol, D-mannitol, D-sorbitol, xylitol, methanol, ethanol, glycerol, glycogen, urea, L-alanine, L-arginine, L-asparagine, L-aspartate, L-cysteine, L-glutamate, L-glutamine, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, and L-valine. In API 20E tests, gelatinase and tryptophan deaminase are positive; the Voges-Proskauer test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, β-galactosidase, hydrogen sulfide, and indole production are all negative. Acid is not produced from D-glucose, D-melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose, and D-sucrose.

The type strain Gsoil 357^T (=KCTC 12602^T and DSM 18420^T) was isolated from a soil sample of a ginseng field in Pocheon Province (South Korea). The 16S rRNA gene sequence of strain Gsoil 357^T has been deposited in NCBI GenBank under the accession number AB245363.

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