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Lysobacter panacihumi sp. nov., isolated from ginseng cultivated soil[§]

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A Gram-negative, non-motile, aerobic, catalase-, and oxidase-positive bacterial strain, designated DCY117^T, was isolated from ginseng cultivated soil in Gochang-gun, Republic of Korea, and was characterized taxonomically using a multi-faceted approach. 16S rRNA gene sequence analysis revealed that strain DCY117^T showed highest similarity to *Lysobacter ruishenii* CTN-1^T (95.3%). Phylogenetic analysis revealed that closely related relatives of strain DCY117^T were *L. aestuarii* S2-C^T (95.1%), *L. daejeonensis* GH1-9^T (95.0%), and *L. caeni* BUT-8^T (94.9%). Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) were the major polar lipids of strain DCY117^T. The major isoprenoid quinone was Q-8. The major cellular fatty acids of strain DCY117^T were iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (comprising iso-$C_{17:1}$ *ω9c* and/or 10-methyl-$C_{16:0}$). Genomic DNA G + C content was 61.8 mol%. On the basis of our findings, strain DCY117^T is a novel species in the genus *Lysobacter*. We propose the name *Lysobacter panacihumi* sp. nov., and the type strain is DCY117^T (= KCTC 62019^T = JCM 32168^T).

Keywords: *Lysobacter panacihumi*, ginseng cultivated soil, 16S rRNA gene

Introduction

The genus *Lysobacter* was first described by Christensen and Cook (1978) based on the type species of the genus *L. enzymogenes,* and an amended description of the genus was provided by Park *et al.* (2008). Currently, there are 45 *Lysobacter* species and subspecies with valid names (http://www.bacterio.net/lysobacter.html), including the recently published species *L. solanacearum* (Kim *et al.*, 2017) and *L. humi* (Lee *et al.*, 2017). Members of the genus *Lysobacter* are Gram-negative, aerobic rods with a G + C content in the range of 61.7–70.7 mol% (Ye *et al.*, 2015; Jeong *et al.*, 2016; Choi *et al.*, 2018). *Lysobacter* species have been isolated from various sources, including activated sludge and freshwater sediment (Siddiqi and Im, 2016a), the rhizopshere (Kim *et al.*, 2017), soil (Kim *et al.*, 2017), and spongin (Choi *et al.*, 2018). In particular, several *Lysobacter* species have been isolated from ginseng cultivated soil, including *L. panacisoli* (Choi *et al.*, 2014) and *L. pocheonensis* (Siddiqi and Im, 2016b). Here, we report a novel species in the genus *Lysobacter*, designated DCY117^T, that was isolated from ginseng cultivated soil.

Materials and Methods

Isolation and culture conditions

Soil samples were obtained from a ginseng field in Gochang-gun (35° 26′ 89″ N 126° 42′ 740″ E), Republic of Korea. Briefly, soil samples were serially diluted to 10^{-5} using 0.85% (w/v) NaCl solution, spread on Reasoner's 2A (R2A, MB cell) agar media, and incubated at 30°C for 5 days. Based on colony morphology, color, and margins, purified colonies were obtained from subcultures. DNA was extracted from isolated colonies, and the 16S rRNA gene was sequenced by Genotech. Among the purified isolates, a novel *Lysobacter* species was identified, named DCY117^T, and was characterized taxonomically using a multifaceted approach. Bacterial stock was stored at -80°C as a suspension in 20% (w/v) glycerol for long-term preservation. Strain DCY117^T was deposited in the Korean Collection for Type Cultures (KCTC 62019^T) and the Japan Collection of Microorganisms (JCM 32168^T). Type strains of *Lysobacter* spp. were obtained for comparative purposes: *L. ruishenii* (KCTC 23715^T) was procured from the Korean Collection for Type Cultures (KCTC), while *L. aestuarii* (KACC 18502^T), *L. daejeonensis* (KACC 11406^T), and *L. caeni* (KACC 17141^T) were obtained from the Korean Agricultural Culture Collection (KACC).

16S rRNA sequencing and construction of a phylogenetic tree

The 16S rRNA gene sequence of strain DCY117^T was sequenced by Genotech using the universal bacterial primer sets 27F/1492R (Lane, 1991) and 518F/800R (Weisburg *et al.*, 1991). Seq-Man software version 4.1 (DNASTAR, Inc.)

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The NCBI GenBank accession number for the 16S rRNA gene sequence of strain DCY117^T is KY996487.
DPD taxon number: TA00437

was used to compile and edit the 16S rRNA sequence of strain DCY117^T. Alignment gaps were trimmed manually using the BioEdit program (Hall, 1999). Furthermore, 16S rRNA gene sequences of related reference strains were obtained from GenBank database and the EzBioCloud server [https://www.ezbiocloud.net] (Yoon *et al.*, 2017). Sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed by neighbor-joining (Saitou and Nei, 1987) with the Kimura 2-parameter model (Kimura, 1979), maximum-likelihood (Felsenstein, 1981) using the Tamura-Nei model (Tamura and Nei, 1993), and maximum-parsimony (Fitch, 1971) using the MEGA 6 program (Tamura *et al.*, 2013). Bootstrap analysis with 1,000 replicates was performed (Felsenstein, 1985).

Phenotypic and biochemical characteristics

Colony morphology of strain DCY117^T was determined after incubation at 30°C for 2 days. Gliding motility was investigated using a hanging-drop technique (Bernardet *et al.*, 2002). Gram-staining was evaluated using a Gram-stain kit (bioMérieux). Oxidase activity and catalase activity were detected using 1% (w/v) *N*,*N*,*N*,*N*-tetramethyl-*p*-phenylenediamine reagent (bioMérieux) according to the instructions of the manufacturer and by assessing the production of oxygen bubbles in 3% (v/v) hydrogen peroxide solution mixed with cells, respectively. Anaerobic growth was monitored by GasPakTM EZ Gas Generating Systems (Becton Dickinson) over a 14 days incubation at 30°C. Growth was tested in several media types, including Reasoner´s 2A (R2A, MB Cell) agar, trypticase soy agar (TSA, MB cell), lysogeny broth agar (LB, MB cell), nutrient agar (NA, Difco), potato dextrose agar (PDA, MB cell), and MacConkey agar (Difco) at 30°C for 1 week. Salinity tolerance was investigated using TSB supplemented with 0–5.0% (w/v) NaCl, at 0.5% intervals, after incubation at 30°C for 5 days. Growth at temperatures of 4, 10, 15, 20, 25, 30, 35, 37, and 40°C was evaluated for a period of one week. Growth at pH 4.0–10.0 in pH 0.5-unit intervals was evaluated in TSB broth. The pH level was adjusted using a method previously described by Kang *et al.* (2015). After 5 days of incubation at 30°C, measurement of optical density at 600 nm was performed by UV-Vis spectrophotometry (Ultrospec 2100 Pro, Amersham Biosciences). Hydrolysis of the following substrates was analyzed: tyrosine [on R2A agar containing 0.5% (m/v) tyrosine], casein [on R2A agar supplemented with 2% (m/v) skim milk], Tween 80 [on R2A agar containing 1% (v/v) Tween 80 and 0.02% (m/v) $CaCl_2$], DNA (on DNase agar medium), and gelatin [on R2A agar containing 1.2% (m/v) gelatin]. To assess plant growth-promoting characteristics, production of indole acetic acid (IAA) and siderophores (Schwyn and Neilands, 1987; Glickmann and Dessaux, 1995) was assessed. Other enzyme activities and biochemical characteristics were assessed using API ZYM, API 20NE, and API ID 32 GN kits (bioMérieux) as instructed by the manufacturer.

Table 1. Characteristics of strain DCY117^T and related *Lysobacter* type strains
Strains: 1, strain DCY117^T (data from this study); 2, *L. ruishenii* KCTC 23715^T; 3, *L. aestuarii* KACC 18502^T; 4, *L. daejeonensis* KACC 11406^T; 5, *L. caeni* KACC 17141^T. Data are from this study, except for DNA G + C content, which is from Wang *et al.* (2011), Jeong *et al.* (2016), Weon *et al.* (2006), and Ye *et al.* (2015). +, positive; -, negative.

Characteristic	1	2	3	4	5
Isolation source	Ginseng cultivated soil	Contaminated soil	Estuary sediment	Greenhouse soil	Sludge of a pesticide
Colony color	Yellow	Pale yellow	Deep yellow-cream	Yellow	Yellow-green
Gliding motility	-	+	-	-	-
Temperature range for growth (°C)	15–37	15–37	15–40	10–37	15–37
pH range for growth	6.0–9.5	6.0–9.0	5.5–9.0	6.0–8.0	6.0–9.0
NaCl range for growth (%)	0–3	0–1	0–7	0–3	0–1
Hydrolysis of					
Tween 80	-	-	+	+	+
Tyrosine	+	+	+	+	-
Casein	-	+	+	+	+
Enzyme activity (API ZYM)					
Lipase	-	-	+	+	+
Cystine arylamidase	-	-	-	-	+
Trypsin	-	-	+	+	+
α-Glucosidase	+	-	-	-	-
Assimilation (API 20NE and API ID 32 GN)					
D-Glucose	-	-	+	+	+
D-Maltose	-	-	+	+	+
Sodium acetate	-	+	+	+	+
D-Mannitol	+	-	+	-	-
D-Sorbitol	+	-	-	-	-
L-Arabinose	+	-	+	+	-
Propionic acid	-	-	-	-	+
Valeric acid	+	-	+	+	+
DNA G + C content (mol%)	61.8	67.1	63.8	61.7	70.6

Determination of DNA G + C content

Genomic DNA G + C content of strain DCY117^T was determined by degradation of the DNA into nucleosides by P1 nuclease (Sigma) and alkaline phosphatase (Sigma) enzymes according to the method described by Mesbah *et al.* (1989).

Chemotaxonomic analysis

Isoprenoid quinones of strain DCY117^T were extracted and identified as reported by Hiraishi *et al.* (1996). Ubiquinone was detected by high performance liquid chromatography (HPLC) analysis [model, NS-6000A, Futecs; reverse-phase column YMC-Triart C18 (250 × 4.6 mm × 0.5 μm), wavelength 270 nm, and a flow-rate of 1.0 ml/min]. Polar lipids of strain DCY117^T and *L. ruishenii* CTN-1^T were extracted and analyzed by two-dimensional thin layer chromatography (TLC) as described by Minnikin *et al.* (1984). To determine cellular fatty acids, extraction and methylation of cells cultured in R2A at 30°C for 24 h were performed according to the MIDI (Sherlock Microbial Identification System) protocol, and fatty acid methyl esters were analyzed by gas chromatography (Agilent GC 6890) using Sherlock MIDI software 6.1 and the TSBA 6.1 database (Hiraishi *et al.*, 1996).

Results and Discussion

Physiological characteristics

Cell morphology of strain DCY117^T is shown in Supplementary data Fig. S1. Colonies were circular, convex, and yellow with a diameter of about 0.8–0.9 mm after incubation for 2 days at 30°C on R2A agar plates. These physiological characteristics of strain DCY117^T are consistent with the description of the genus *Lysobacter*. Strain DCY117^T grew well on R2A, TSA, NA, and LB agar, but did not grow on PDA and MacConkey agar. Growth occurred at 15–37°C (optimum 30°C) and was observed in the presence of up to 3% (w/v) NaCl (optimum 0.5% [w/v]). The pH range for growth was pH 6.0–9.5 (optimum pH 8.0). Strain DCY117^T produced siderophores after incubation for 3 days. Siderophore production by bacteria is good for plant growth in contaminated soils, because it can remove or reduce heavy metals and inhibit the growth of plant pathogens (Sharma and Johri, 2003). However, IAA was not produced by this strain. Physiological and biochemical characteristics of strain DCY117^T are generalized in the species description, and selective characteristics are compared with those of related type strains in Table 1. Strain DCY117^T can be differentiated from strain *L. ruishenii* CTN-1^T by the following characteristics: positive for α-glucosidase activity and assimilation of D-mannitol, D-sorbitol, L-arabinose, and valeric acid.

Phylogenetic analysis

Strain DCY117^T shared the highest 16S rRNA gene sequence similarity with *L. ruishenii* CTN-1^T (95.3%), followed by *L. aestuarii* S2-C^T (95.1%), *L. daejeonensis* GH1-9^T (95.0%), and *L. caeni* BUT-8^T (94.9%). Phylogenetic trees constructed by neighbor-joining, maximum-likelihood, and maximum-parsimony methods showed that strain DCY117^T clustered within the genus *Lysobacter* (Fig. 1).

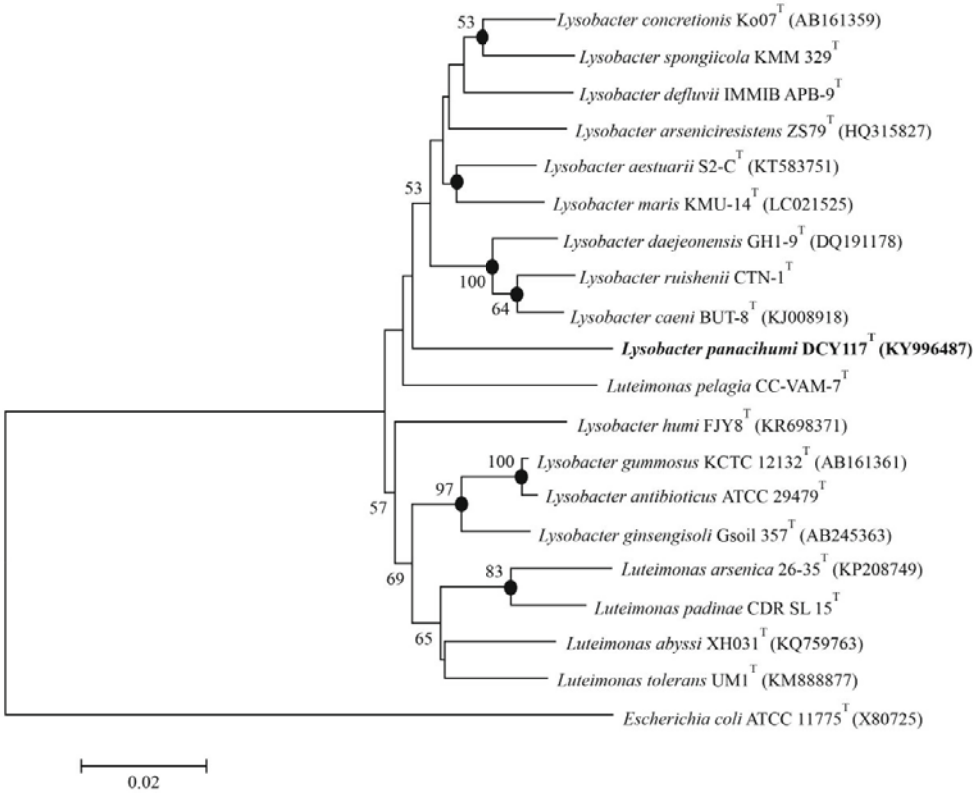

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the taxonomic position of strain DCY117^T in the genus *Lysobacter*. Filled circles indicate that the corresponding nodes were also recovered in the trees generated by the maximum-parsimony and maximum likelihood algorithms. Bootstrap values > 50% based on 1,000 replications are shown at branching points. Bar, 0.02 substitutions per nucleotide position.

Table 2. Cellular fatty acid profile of strain DCY117^T and related type strains
Strains: 1, strain DCY117^T; 2, *L. ruishenii* KCTC 23715^T; 3, *L. aestuarii* KACC 18502^T; 4, *L. daejeonensis* KACC 11406^T; 5, *L. caeni* KACC 17141^T. All data were obtained in this study. -, not detected; tr, trace amount (< 0.5%).

Fatty acid	1	2	3	4	5
iso-$C_{11:0}$	4.2	3.9	11.8	3.7	4.6
iso-$C_{12:0}$	2.5	0.8	1.5	2	tr
iso-$C_{11:0}$3-OH	7.8	3.9	10.2	6	6.1
iso-$C_{12:0}$3-OH	tr	tr	tr	0.6	tr
iso-$C_{14:0}$	3.1	3.6	7.5	11.2	2.5
$C_{14:0}$	tr	tr	tr	0.9	tr
iso-$C_{15:1}$F	2.4	2.8	tr	3.2	0.9
iso-$C_{15:0}$	10.2	19.5	17.8	13.1	28.1
anteiso-$C_{15:0}$	0.6	1.9	1.1	3.2	0.9
iso-$C_{16:1}$H	0.7	-	tr	tr	tr
iso-$C_{16:0}$	30.5	26.0	22.3	33.7	20.9
iso-$C_{16:0}$H	-	0.8	-	2.6	-
$C_{16:0}$	6.9	6.8	tr	1.4	4.8
iso-$C_{17:0}$	2.2	3.7	1.6	0.6	7.7
Summed feature 3*	0.9	4.9	5.5	6.1	1.9
Summed feature 9**	21.7	17.2	16.3	6.7	17.5

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$
**Summed feature 9 comprises iso-$C_{17:1}\omega9c$ and/or 10-methyl-$C_{16:0}$

DNA G + C content and chemotaxonomic characteristics

The genomic DNA G + C content was 61.8 mol%, which a range of 61.5–62.0 mol%. Ubiquinone was identified as Q-8. This result is in accordance with the description of the genus *Lysobacter* (Wang *et al.*, 2011). Polar lipids profiles of strain DCY117^T and *L. ruishenii* CTN-1^T are listed in Supplementary data Fig. S2. The main polar lipids of strain DCY117^T are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE); minor amounts of three unidentified polar lipids (L1-3) were also identified. *L. ruishenii* CTN-1^T contains major amounts of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE), and minor amounts of two unidentified polar lipids (L1-2), mostly consistent with what has been reported for other members of the genus *Lysobacter* (Lee *et al.*, 2006; Weon *et al.*, 2006). The fatty acid profiles of strain DCY117^T and related type strains are listed in Table 2. The major cellular fatty acids of strain DCY117^T are iso-$C_{15:0}$ (10.2%), iso-$C_{16:0}$ (30.5%), and summed feature 9 (comprising iso-$C_{17:1}$ $\omega9c$ and/or 10-methyl-$C_{16:0}$, 21.7%). This major fatty acids profile is consistent with that reported for other members of the genus *Lysobacter* (Romanenko *et al.*, 2008; Jeong *et al.*, 2016). These chemotaxonomic results clearly demonstrate that strain DCY117^T has characteristics in common with the genus *Lysobacter*.

Taxonomic conclusion

Phylogenetic, physiological, and chemotaxonomic data indicate that strain DCY117^T is a member of the genus *Lysobacter*. However, the phylogenetic distance of this strain from recognized *Lysobacter* species and some unique physiological and biochemical characteristics (Table 1) indicate that isolate DCY117^T is a distinct species within the genus *Lysobacter*. We propose the name *Lysobacter panacihumi* sp. nov. for this newly isolated and characterized strain.

Description of *Lysobacter panacihumi* sp. nov.

Lysobacter panacihumi (pa.na.ci.hu'mi. N.L. n. *Panaxacis* scientific name of ginseng; L. n. *humus* soil; N.L. gen. n. *panacihumi* soil of a ginseng field).

Cells are Gram-negative, aerobic, oxidase-, and catalase-positive, non-motile (non-gliding), and rod-shaped with a width of 0.6–0.7 μm and length of 1.0–1.3 μm. Growth occurs at 15–37°C, pH 6.0–9.5, and 0–3% (w/v) NaCl. Growth occurs on R2A, TSA, and LB agar, but not on MacConkey agar. Colonies on R2A agar are circular, convex, and yellow with a diameter of about 0.8–0.9 mm after incubation under optimum conditions (30°C, pH 8.0, 0.5% NaCl). Strain DCY117^T is able to hydrolyze tyrosine and DNA, but not Tween 80, casein, starch, or gelatin. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase activity can be detected using API ZYM strips, but no lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase activity is observed. This species is positive for assimilation of L-arabinose and D-mannitol on API 20 NE strips and negative for reduction of nitrates, indole production, fermentation of D-glucose, arginine dihydrolase, urease, hydrolysis of esculin, and gelatin, assimilation of *N*-acetyl-glucosamine, potassium gluconate, D-glucose, D-maltose, D-mannose, capric acid, adipic acid, malic acid, phenylacetic acid, and trisodium citrate. This strain is also positive for assimilation of glycogen, D-mannitol, D-sorbitol, L-arabinose, valeric acid, and 3-hydroxybutyric acid using API ID 32 GN strips, but negative for assimilation of L-rhamnose, N-acetyl-glucosamine, D-ribose, myo-inositol, D-sucrose, D-maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, L-fucose, propionic acid, capric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid, and L-proline. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE). The major cellular fatty acids are iso-$C_{15:0}$ (10.2%), iso-$C_{16:0}$ (30.5%), and summed feature 9 (comprising iso-$C_{17:1}$ $\omega9c$ and/or 10-methyl-$C_{16:0}$, 21.7%). The ubiquinone is Q-8. The genomic DNA G + C content is 61.8 mol%. The type strain, DCY117^T (= KCTC 62019^T = JCM 32168^T), was isolated from ginseng cultivated soil in Gochang-gun, Republic of Korea.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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