Lysobacter panacisoli sp. nov., isolated from ginseng soil

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A novel bacterial strain, designated CJ29^T, was isolated from ginseng soil of Anseong in South Korea. Cells of strain CJ29^T were Gram-stain-negative, facultatively anaerobic, rod-shaped and non-motile. Strain CJ29^T grew optimally at 28–30 °C and pH 7.0. Based on 16S rRNA gene sequence analysis, strain CJ29^T was shown to belong to the genus *Lysobacter* within the class *Gammaproteobacteria* and was related most closely to *Lysobacter soli* DCY21^T (98.5 % similarity) and *Lysobacter niastensis* GH41-7^T (98.2 %). DNA–DNA relatedness between strain CJ29^T and its closest relatives was below 55.6 %. The predominant cellular fatty acids of strain CJ29^T were iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:1}\omega9c$. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. The major isoprenoid quinone was ubiquinone 8 (Q-8). The G+C content of the genomic DNA was 65.6 mol%. Phenotypic, genotypic and phylogenetic characteristics strongly supported the differentiation of strain CJ29^T from related species of the genus *Lysobacter*. On the basis of data from this polyphasic taxonomic study, strain CJ29^T is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter panacisoli* sp. nov. is proposed. The type strain is CJ29^T (=KACC 17502^T=JCM 19212^T).

The genus *Lysobacter* was first proposed by Christensen & Cook (1978), and classified within the family *Xanthomonadaceae* belonging to the class *Gammaproteobacteria*. At the time of writing, the genus *Lysobacter* comprises 26 species with validly published names (http://www.bacterio.cict.fr/). Species of the genus *Lysobacter* are commonly found in diverse geographical and environmental habitats, especially in agricultural soil (Liu *et al.*, 2011; Srinivasan *et al.*, 2010; Ten *et al.*, 2009). Members of the genus are generally characterized as Gram-negative, aerobic, non-fruiting and gliding bacteria with a high DNA G+C content typically ranging from 61.7 to 70.1 mol% (Christensen & Cook, 1978; Weon *et al.*, 2006). Species of the genus *Lysobacter* contain ubiquinone 8 (Q-8) as the major respiratory quinone and show a predominance of iso-branched fatty acids (Fukuda *et al.*, 2013; Luo *et al.*, 2012; Saddler & Bradbury, 2005; Wei *et al.*, 2012). Some members of the genus have potential for development of biocontrol agents based on their ability to lyse a variety of micro-organisms such as Gram-negative and Gram-positive bacteria, filamentous fungi, yeasts, green algae and nematodes (Ahmed *et al.*, 2003; Folman *et al.*, 2004; Kilic-Ekici & Yuen, 2003; Ryazanova *et al.*, 2005).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CJ29^T is JQ806748.

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

Strain CJ29^T was isolated on R2A agar (BBL) from a soil sample taken from a ginseng field of Anseong in South Korea. The isolate was subsequently cultivated on R2A agar at 30 °C for 3 days and stored as a stock culture in R2A broth (MBCell) supplemented with 30 % (v/v) glycerol. In this study, we describe the morphological, biochemical and phylogenetic characteristics of strain CJ29^T, employing the most closely related type strains, *Lysobacter soli* KCTC 22011^T and *Lysobacter niastensis* KCTC 22750^T, as reference strains in parallel tests.

Genomic DNA of strain CJ29^T and the reference strains was extracted using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol. The 16S rRNA gene of strain CJ29^T was amplified using the universal bacterial primers 27F and 1492R (Baker *et al.*, 2003) and the products were purified using a PCR purification kit (Qiagen). Sequencing of the 16S rRNA gene was performed at Solgent (Daejeon, Korea) using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and an automated DNA analyser (PRISM 3730XL; Applied Biosystems). The 16S rRNA gene sequences of related taxa were obtained from the ExTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (Kim *et al.*, 2012). The 16S rRNA gene sequence of strain CJ29^T was aligned with those of the type strains of related species belonging to the genus *Lysobacter* by using the multiple sequence alignment program CLUSTAL X version 1.83 (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei,

1987) and maximum-likelihood (Kluge & Farris, 1969) methods implemented in the MEGA 5 program (Tamura *et al.*, 2011). Evolutionary distances were calculated according to the model of Jukes & Cantor (1969). The number of bootstrap replicates was set to 1000. Analysis of the 16S rRNA gene sequences indicated that the closest relatives of strain CJ29^T were *L. soli* DCY21^T (98.5 % similarity) and *L. niastensis* GH41-7^T (98.2 %), and the type strains of other species of the genus showed similarity values of <96.6 %. The phylogenetic trees showed that strain CJ29^T formed a robust cluster with the type strains of *L. soli* and *L. niastensis*, forming a distinct phylogenetic lineage within the genus *Lysobacter* (Fig. 1).

DNA–DNA relatedness studies were performed fluorimetrically (Ezaki *et al.*, 1989) between strain CJ29^T and the type strains of the most closely related species (>97 % 16S rRNA gene sequence similarity) using the DIG-High Prime DNA Labelling and Detection Starter kit II according to the manufacturer's instructions (Roche). Hybridization was conducted in triplicate with reciprocal probes. The DNA G+C content was determined by HPLC analysis with a reversed-phase column (Capcell Pak C18 UG 120; Shisheido) according to the method of Mesbah *et al.* (1989). DNA–DNA relatedness between strain CJ29^T and *L. soli* KCTC 22011^T and *L. niastensis* KCTC 22750^T was 50.1 % (55.6 %, reciprocal analysis) and 19.1 % (35.1 %, reciprocal analysis), respectively. These values are below the 70 % cut-off recommended for genomic species delineation by Wayne *et al.* (1987), clearly suggesting that strain CJ29^T represents a novel species of the genus *Lysobacter*. The DNA G+C content of

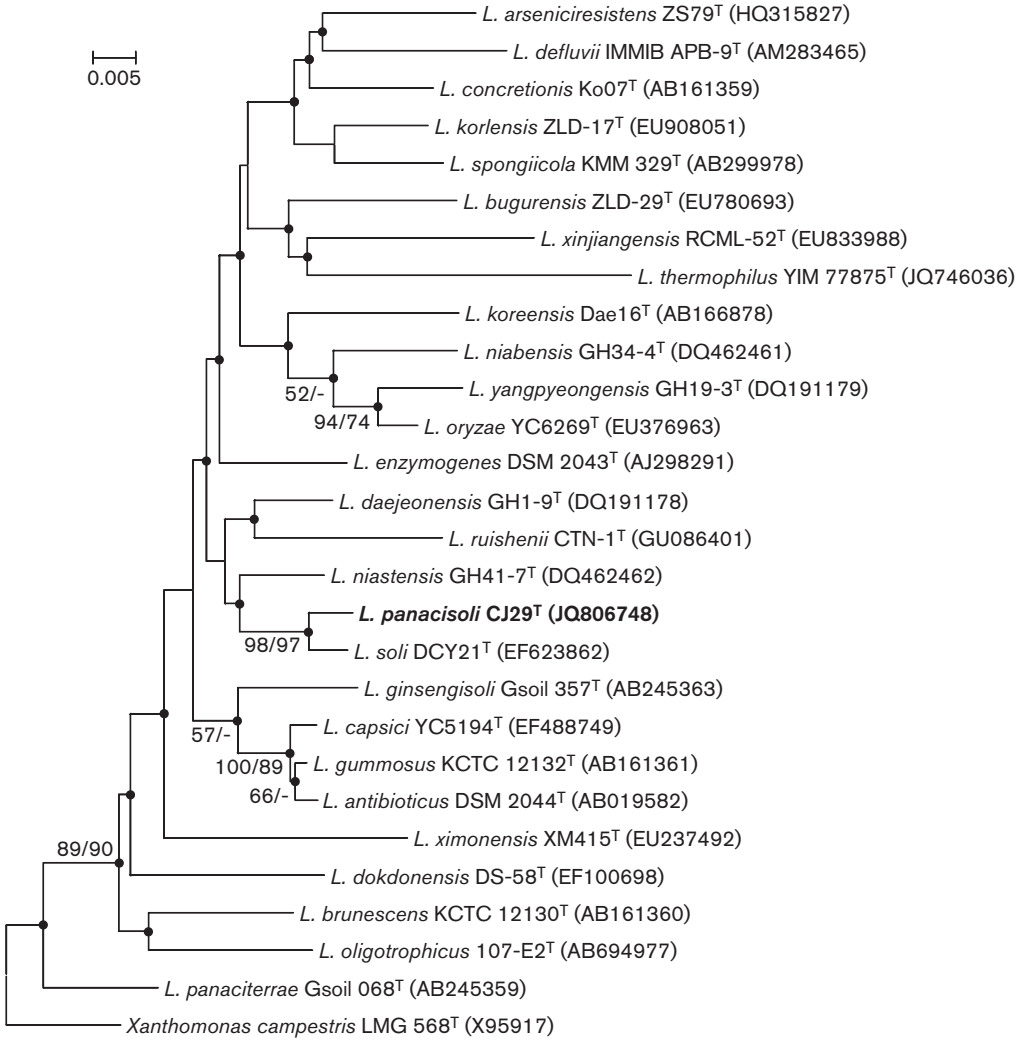

Fig. 1. Phylogenetic tree of strain CJ29^T and the type strains of related taxa based on 16S rRNA gene sequences. The tree was reconstructed using the neighbour-joining (NJ) and maximum-likelihood (ML) methods and numbers at nodes represent bootstrap percentages (NJ/ML, based on 1000 resamplings). Filled circles indicate genetic branches that were present in both the NJ and ML trees. GenBank accession numbers are given in parentheses. *Xanthomonas campestris* LMG 568^T was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

strain CJ29^T was 65.6 mol%, which was within the range described for the genus *Lysobacter* (61.7–70.1 mol%).

Cellular morphology of strain CJ29^T was observed by light (Leica; 1000×) and transmission electron (JEM 1010; JEOL) microscopy using cells grown for 1 day at 30 °C on R2A agar. Gram staining was carried out using a Gram stain kit according to the manufacturer's instructions (Sigma-Aldrich). Gliding motility was observed by production of colonies that have thin spreading edges (Spormann, 1999). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 42 and 45 °C) and pH (4.0–12.0, at intervals of 1.0 pH unit) was monitored in R2A broth for up to 5 days. NaCl tolerance was tested in R2A broth supplemented with 0–10 % (w/v) NaCl. Anaerobic growth was determined after 2 weeks of incubation at 30 °C on R2A agar using the GasPak EZ Anaerobe Pouch System (BD). Catalase and oxidase activities were determined in 3 % (v/v) hydrogen peroxide solution and 1 % (w/v) tetramethyl phenylenediamine reagent (bioMérieux), respectively. Hydrolysis of DNA and casein was tested using DNase test agar (BBL) and skimmed milk (BBL), respectively. Enzyme activities and utilization of different carbon sources were assessed using API 20NE, API ZYM and API 50CH kits (bioMérieux) at 30 °C according to the manufacturer's protocols.

Cells of strain CJ29^T were Gram-stain-negative, facultatively anaerobic, non-flagellated and rod-shaped (Fig. S1, available in the online Supplementary Material). Colonies on R2A agar were opaque, bright yellow, smooth and circular with entire edges. Gliding motility was observed. Cells grew at 4–42 °C (optimum 30 °C), at pH 5.0–11.0 (optimum pH 7.0) and with 0–1 % NaCl (optimum 1 %). Growth occurred on R2A agar, Luria–Bertani (LB) agar, trypticase soy agar (TSA), nutrient agar (NA) and marine agar (MA). Physiological and biochemical characteristics of strain CJ29^T that differentiated it from the reference strains are summarized in Table 1.

Table 1. Physiological and biochemical characteristics that differentiate strain CJ29^T from related type strains of the genus *Lysobacter*

Strains: 1, CJ29^T; 2, *L. soli* KACC 22011^T; 3, *L. niastensis* KACC 22750^T. Data are from this study unless indicated otherwise. −, Negative; +, positive; w, weak reaction.

Characteristic	1	2	3
Nitrate reduction	−	+	−
Catalase	+	+	−
Hydrolysis of aesculin	+	+	−
Enzyme activity:			
Arginine hydrolase	−	+	−
Urease	−	+	−
Galactosidase	+	−	+
Valine arylamidase	−	w	−
Trypsin	+	w	−
N-Acetyl-β-glucosaminidase	−	+	−
Utilization of carbon sources:			
Glucose	−	+	w
Mannose	+	w	−
Mannitol	−	−	w
L-Arabinose	−	+	−
Ribose	−	+	−
D-Xylose	+	+	−
Methyl β-D-xyloside	−	−	+
Galactose	−	+	−
Glucose	−	+	−
Fructose	+	−	−
N-Acetylglucosamine	−	+	−
Cellobiose	−	+	−
Sucrose	+	−	−
Trehalose	−	+	+
Gentiobiose	−	+	−
D-Lyxose	+	+	−
D-Fucose	−	+	−
DNA G+C content (mol%)	65.6	65.4*	66.6†

*Data taken from Srinivasan *et al.* (2010).
†Data taken from Weon *et al.* (2007).

For the investigation of chemotaxonomic characteristics, strain CJ29^T and the reference strains were cultured on R2A agar plates for 2 days at 30 °C and cells of these strains were harvested at a similar physiological age given that all strains showed similar growth kinetics. Cellular fatty acids were extracted and methylated according to the protocol of Sasser (1990). The fatty acid composition was identified by the standard Microbial Identification System (MIDI) version 6.1 and the RTSBA6 6.10 database (Microbial ID). Respiratory quinones were extracted as described by Minnikin *et al.* (1984) and determined by HPLC as described by Collins (1985). Polar lipids were extracted and identified by TLC according to Tindall (1990). The major fatty acids detected in strain CJ29^T were the branched compounds iso-$C_{15:0}$ (26.0 %), iso-$C_{16:0}$ (20.5 %) and iso-$C_{17:1}\omega 9c$ (19.2 %) (Table 2), which correspond to those described for recognized species of the genus *Lysobacter* (Srinivasan *et al.*, 2010; Weon *et al.*, 2007). Strain CJ29^T contained iso-$C_{15:1}$ F and a relatively higher amount of iso-$C_{17:1}\omega 9c$ and iso-$C_{17:0}$, although the major fatty acids were similar to those of the reference strains. The major respiratory quinone was ubiquinone 8 (Q-8). This quinone system is a characteristic feature of the genus *Lysobacter*. The predominant polar lipids of strain CJ29^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG), like other members of the genus *Lysobacter*. Several unidentified polar lipids including aminophospholipids, phospholipids and aminolipids were also detected (Fig. S2).

Phylogenetic analysis based on 16S rRNA sequences indicated that strain CJ29^T was clearly affiliated with the genus *Lysobacter* and formed a phyletic line distinct from the clades of related species. DNA–DNA relatedness between strain CJ29^T and the type strains of closely related species was below 70 %. Phenotypic and chemotaxonomic

Table 2. Cellular fatty acid composition of strain CJ29^T and related type strains of the genus *Lysobacter*

Strains: 1, CJ29^T; 2, *L. soli* KCTC 22011^T; 3, *L. niastensis* KCTC 22750^T. Data are from this study. TR, Trace (<1.0 %); ND, not detected. Fatty acids found in amounts <1.0 % in all strains are not shown.

Fatty acid	1	2	3
iso-$C_{11:0}$	3.0	3.2	3.9
iso-$C_{11:0}$ 3-OH	4.5	4.9	5.6
iso-$C_{12:0}$ 3-OH	TR	1.3	TR
iso-$C_{14:0}$	2.0	4.9	2.3
iso-$C_{15:0}$	26.0	19.7	33.0
anteiso-$C_{15:0}$	1.4	3.5	4.6
iso-$C_{15:1}$ F	1.9	TR	ND
$C_{16:0}$	3.1	TR	2.9
$C_{16:0}$ N alcohol	1.4	ND	TR
iso-$C_{16:0}$	20.5	34.2	17.5
iso-$C_{16:1}$ H	TR	2.6	TR
iso-$C_{17:0}$	7.9	1.4	5.1
iso-$C_{17:1}\omega 9c$	19.2	11.8	10.7
$C_{18:1}\omega 7c$	TR	1.1	TR
Summed feature 3*	4.0	5.7	6.8

*Summed feature 3 comprises $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$.

characteristics distinguished strain CJ29^T from the type strains of closely related species, in terms of carbon source utilization, enzyme activities and fatty acid composition. Based on data from the present polyphasic taxonomic study, we consider that strain CJ29^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter panacisoli* sp. nov. is proposed.

Description of *Lysobacter panacisoli* sp. nov.

Lysobacter panacisoli (pa.na.ci.so′li. N.L. n. *Panax -acis* scientific name of ginseng; L. n. *solum -i* soil; N.L. gen. n. *panacisoli* of soil of a ginseng field, the source of isolation of the type strain).

Cells are Gram-stain-negative, facultatively anaerobic and rod-shaped (0.4–0.45 μm long and 1.0–2.0 μm wide). Colonies on R2A agar are opaque, bright yellow, smooth and circular with entire edges, after incubation for 2 days at 30 °C. Cells grow at 10–42 °C (optimum 30 °C), at pH 5.0–11.0 (optimum pH 7.0) and with 0–1 % NaCl (optimum 1 %). Growth occurs on R2A agar, LB agar, TSA, NA and MA. Catalase-positive and oxidase-negative. DNA, casein, gelatin and aesculin are hydrolysed but cellulose and starch are not. Indole is not produced. Based on API ZYM test strip results, urease, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase activities are positive, but lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are negative. Mannose, acetylglucosamine, D-xylose, fructose, maltose, sucrose and D-lyxose are utilized. The predominant cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:1}\omega 9c$. The major respiratory quinone is Q-8. The predominant polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and unknown aminophospholipids.

The type strain is CJ29^T (=KACC 17502^T=JCM 19212^T), which was isolated from ginseng soil collected from Anseong in South Korea. The DNA G+C content of the type strain is 65.6 mol%.

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