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Lysobacter silvisoli sp. nov., isolated from forest soil

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Abstract

A yellow-pigmented, Gram-stain-negative, gliding and rod-shaped bacterial strain, designated zong2l5^T, was isolated from a forest soil sample at Dinghu Mountain, Guangdong Province, PR China. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain zong2l5^T belongs to the genus *Lysobacter*, and was most closely related to *Lysobacter enzymogenes* KCTC 12131^T (97.7 %) and *Lysobacter soli* KCTC 22011^T (97.6 %). The novel strain showed an average nucleotide identity (ANI) value of 81.5 % and a digital DNA–DNA hybridization (dDDH) value of 25.3 % with *L. enzymogenes* KCTC 12131^T based on draft genome sequences, followed by *L. soli* KCTC 22011^T with ANI and dDDH values of 79.4 % and 22.7 %, respectively. The DNA G+C content of strain zong2l5^T based on the whole genome sequence was 69.2 mol%. The major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$). Strain zong2l5^T contained Q-8 as the major isoprenoid quinone and the major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl-*N*-methylethanolamine, phosphatidylethanolamine, three unidentified phospholipids and an unidentified aminolipid. The phenotypic, genotypic and chemotaxonomic anlyses clearly showed that strain zong2l5^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter silvisoli* sp. nov. is proposed. The type strain is zong2l5^T (=GDMCC 1.1489^T =KCTC 52923^T).

The genus *Lysobacter* was proposed by Christensen and Cook [1] with *Lysobacter enzymogenes* as the type species, which belongs to the family *Xanthomonadaceae* in *Gammaproteobacteria*. Members of the genus *Lysobacter* are characterized by Gram-stain-negative, rod-shaped, non-fruiting, gliding cells and having ubiquinone 8 (Q-8) as the major respiratory quinone [2, 3]. Most species of the genus *Lysobacter* have a genome size of about 6.0 Mb with a high DNA G+C content (61–70 mol%) [4, 5]. In addition, due to their fascinating secondary metabolites, *Lysobacter* species have become a new source of bioactive natural products and new antibiotics [6, 7]. There are 48 species with valid names in the genus *Lysobacter* (http://www.bacterio.net). Most of them were isolated from soil [3, 8–13] and other environments such as fresh water [14, 15], rhizosphere of different plants [16–18], lead–zinc ore [19], estuary sediment [20], pit mud [21] and cave [22].

During the isolation of myxobacteria by using filter baiting on CNST agar [KNO_3 0.05 % (w/v), Na_2HPO_4 0.025 % (w/v), $MgSO_4{\bullet}7H_2O$ 0.1 % (w/v), $FeCl_3$ 0.001 % (w/v), agar 1.5 % (w/v), pH 7.2] [23], strain zong2l5^T was isolated from a forest soil sample collected from Dinghu Mountain National Nature Reserve (23° 09′ 5″ N 112° 30′ 46″ E), Guangdong Province, China. The strain was purified and cultured on Reasoner's 2A (R2A) agar. The genomic DNA of strain zong2l5^T was extracted as described by Kieser *et al.* [24] and the 16S rRNA gene was amplified with the universal bacterial primers 27F and 1492R [25]. Then the PCR products were cloned into the vector p-EASY-T1 (Transgen Biotech) and sequenced by Majorbio (Shanghai). The 16S rRNA gene sequence similarity search was performed by using the EzBioCloud server (www.ezbiocloud.net) [26]. Phylogenetic analyses based on 16S rRNA gene sequences were reconstructed using the software MEGA 5.0 [27] with the neighbour-joining (NJ) [28], maximum-parsimony (MP) [29] and maximum-likelihood (ML) methods [30]. The bootstrap values were set as 1000 replications and the evolutionary distances were calculated with Kimura's two-parameter model [31, 32]. An Illumina Hiseq platform was

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Keywords: *silvisoli*; forest soil; average nucleotide identities (ANI); DNA–DNA hybridization (dDDH).
Abbreviations: AL, unidentified aminolipid; DPG, diphosphatidylglycerol; GL 1-3, unidentified glycolipids; NA, nutrient agar; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PYE, peotone yeast extract agar; R2A, reasoner's 2 agar; TSA, tryptic soy agar; PME, phosphatidyl-*N*-methylethanolamine.
The GenBank accession number for the 16S rRNA gene sequence of strain zong2l5^T is MF630933. The whole genome shotgun projects for strain zong2l5^T and *Lysobacter soli* KCTC 22011^T have been deposited at GenBank/EMBL/DDBJ under the accession numbers of QTSU00000000 and QTJR00000000, respectively.
Four supplementary figures are available with the online version of this article.

used for whole genome sequencing of strain zong2l5^T and *Lysobacter soli* KCTC 22011^T by the Personal Biotech (Shanghai). The genomic DNA G+C content of strain zong2l5^T and the closely reference type strains were calculated from the whole genome sequence. The average nucleotide identities (ANI) values were calculated by using the ANI calculator (www.ezbiocloud.net/tools/ani) and the digital DNA–DNA hybridization (dDDH) values were calculated using an online software tool (http://ggdc.dsmz.de/).

The 16S rRNA gene sequence of strain zong2l5^T was 1506 bp in length with the GenBank accession number of MF630933. The similarity analysis showed that strain zong2l5^T shared the highest similarity to *Lysobacter enzymogenes* KCTC 12131^T (97.7 %), followed by *Lysobacter soli* KCTC 22011^T (97.6 %), and lower values (<97.0 %) with other species of the genus *Lysobacter*. The phylogenetic trees based on the ML, MP and NJ methods all showed that strain zong2l5^T fell into the genus *Lysobacter* and was closely related to *L. soli* KCTC 22011^T (Figs 1, S1 and S2, available in the online version of this article). Considering the threshold values of 98.7–99.0 % and 98.65 % in 16S rRNA gene sequence similarity suggested by Stackebrandt and Ebers [33] and Kim *et al.* [34], it was indicated that strain zong2l5^T might represent a novel species of the genus *Lysobacter*. The raw reads (1 243 118 976 bp) of strain zong2l5^T were assembled into 7 contigs with a coverage of 249× and N50 of 2 558 937 bp. The draft genome of strain zong2l5^T was 4 536 546 bp in length with a DNA G+C content of 69.2 mol% (GenBank accession no. QTSU00000000). The whole genome sequence of *L. soli* KCTC 22011^T was 3 953 742 bp in length with 27 contigs, a mean coverage of 258×, N50 of 285 382 bp and a DNA G+C content of 67.7 mol% (QTJR00000000). The genome sequence of *L. enzymogenes* KCTC 12131^T (FNOG00000000) was downloaded from GenBank. Strain zong2l5^T shared a 79.4 % ANI value and a 22.7 % dDDH value with *L. soli* KCTC 22011^T, followed by ANI and dDDH values of 81.5% and 25.3 % with *L. enzymogenes* KCTC 12131^T, respectively. Based on the recommendation threshold values of 95–96 % for ANI and dDDH of 70 % for species discrimination [35–38], these data suggested that strain zong2l5^T represents a new member of the genus *Lysobacter*.

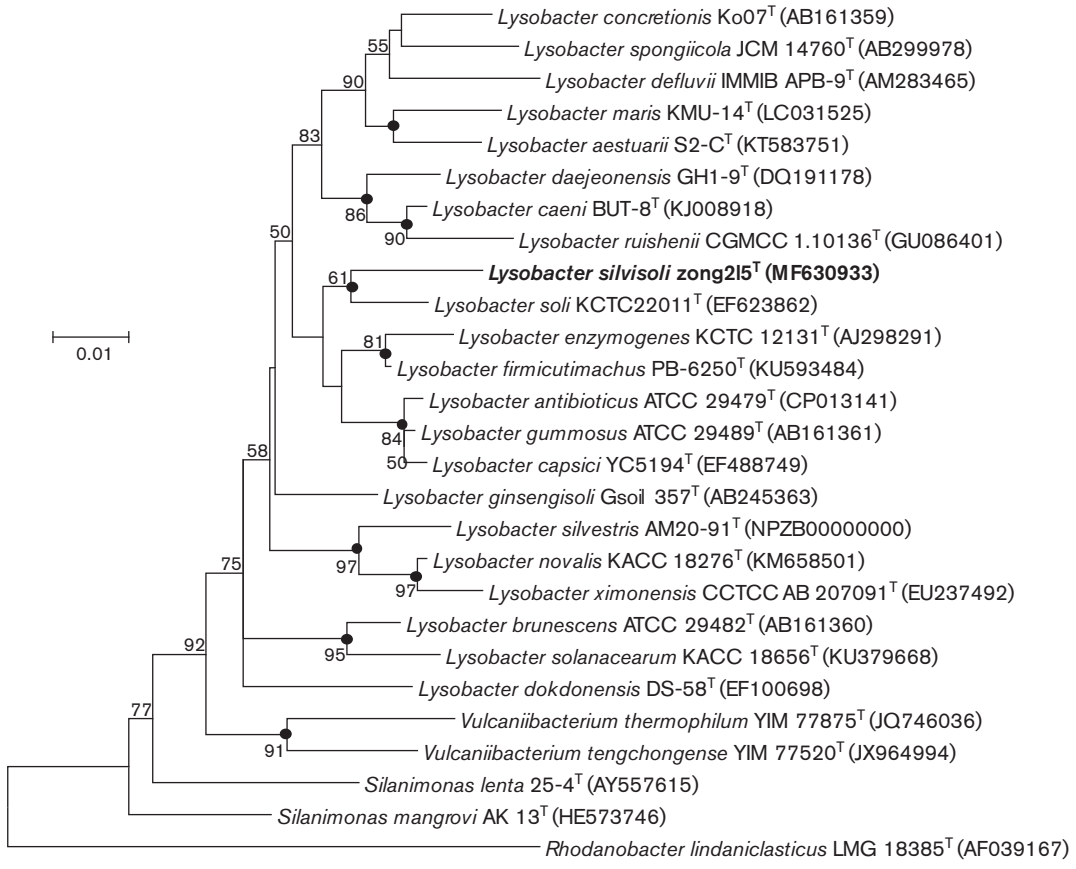

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain zong2l5^T and other type strains of the genus *Lysobacter*. Bootstrap values are expressed as percentages of 1000 replications. Only bootstrap values >50 % are shown. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate that the corresponding branches were also recovered in the trees generated with the NJ and MP methods.

Colonies of strain zong2l5^T were observed on R2A agar at 30 °C after 3 days. Cells morphology of the new strain was observed by using a transmission electron microscope (H7650, Hitachi) with cells grown in R2A broth at 30 °C for 3 days. Gram-staining test was performed using a Gram-stain kit according the protocol of the manufacturer (Huankai). Growth tests were examined on nutrient agar (NA; Huankai), tryptic soy agar (TSA; Hope) and PYE agar [39] at 30 °C for 7 days. Anaerobic growth was tested in serum bottles containing R2A broth supplemented with thioglycolate (0.1 %, w/v) and $NaNO_3$ (0.1 %, w/v), in which the upper air layer was substituted with nitrogen gas. Then they were cultured at 30 °C for 7 days under anaerobic workstations (Electrotek AEP). Gliding motility was observed by following the hanging-drop method [40]. Growth at different temperatures (4, 10, 15, 25, 28, 30, 37, 40 and 45 °C) was observed on R2A agar for 7 days. The pH range (3.0–10.0, at intervals of 1.0 pH unit) for growth of the strain was determined in R2A broth at 30 °C for 7 days. The pH of the R2A broth was adjusted with citrate/sodium citrate buffer (pH 3.0–5.0), Na_2HPO_4/NaH_2PO_4 buffer (pH 6.0–8.0) and Tris/HCl buffer (pH 9.0–10.0). Tolerance to NaCl salinity was assessed in R2A broth with 1–5 % NaCl (w/v, at intervals of 1 %) at 30 °C for 7 days. Oxidase activity was determined using the commercial strip (Huankai) according the manufacturer's instructions and catalase activity was tested in 3 % (w/v) H_2O_2. Hydrolyses of starch (1 %, w/v), casein (1 %, w/v), CM-cellulose (1 %, w/v), chitin (1 %, w/v), tyrosine (0.5 %, w/v), Tween 20, Tween 40, Tween 60 and Tween 80 (1 %, w/v) were tested on R2A agar with different substrates at 30 °C. The enzyme activities were determined by using the API ZYM strip (bioMérieux). Substrates assimilation was tested by using the API 20NE (bioMérieux) and GENIII MicroPlate (Biolog) according to the manufacturers' protocols.

Strain zong2l5^T grew well on R2A agar, NA and PYE agar, but weakly on TSA. Colonies on R2A agar were bright yellow, smooth, sticky, transparent and circular. Cells were Gram-stain-negative, rod-shaped, 0.4–0.7 μm wide and 1.4–2.3 μm long (Fig. S3). Growth of strain zong2l5^T was observed at 15–40 °C (optimum, 28–30 °C), pH 6.0–10.0 (optimum, 7.0) and in the presence of 0–3 % (w/v) NaCl (optimum, without NaCl). Both the reactions for oxidase and catalase activities were positive, while nitrate reduction, indole production, arginine dihydrolase and urease were all negative. Other physiological characteristics of strain zong2l5^T are detailed in the species description. The different phenotypic characteristics between strain zong2l5^T and the closely related type strains were shown in Table 1. The differences in hydrolyses of Tween 20, Tween 60, Tween 80 and aesculin, β-galactosidase, substrate assimilation and some enzyme activities could clearly distinguish the novel strain from its reference type strains.

For cellular fatty acid analyses, strain zong2l5^T and the type strains were incubated under the same conditions on R2A agar at 30 °C for 5 days. Cellular fatty acid extraction and identification were preformed according to the protocol of the Sherlock Microbial Identification System (MIDI) with GC equipment (7890A, Agilent) [41]. The respiratory quinones were extracted and determined by using HPLC (UltiMate 3000; Dionex) as described by Collins *et al.* and

Table 1. Differential physiological characteristics between strain zong2l5^T and closely related type strains of the genus *Lysobacter*

Strains: 1, zong2l5^T; 2, *Lysobacter enzymogenes* KCTC 12131^T; 3, *Lysobacter soli* KCTC 22011^T. All data were obtained from this study under the same conditions except the DNA G+C content of strain *L. enzymogenes* KCTC 12131^T. All strains were positive for oxidase, catalase, D-glucose fermentation, hydrolysis of gelatin and tyrosine, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphtol-AS-Bl-phosphoamidase and assimilation of acetyl-glucosamine and maltose. All strains were negative for indole production, arginine dihydrolase, urease, starch hydrolysis, cystine arylamidase, α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase and assimilation of L-arabinose, D-mannitol, potassium gluconate, phenylacetic acid, stachyose, raffinose, *N*-acetyl-β-D-mannosamine, *N*-acetyl neuraminic acid, 3-methyl glucose, inosine, D-sorbitol, D-arabitol, myo-inositol, glycerol, L-pyroglutamic acid, D-gluconic acid, mucic acid, quinic acid, D-lactic acid methyl ester, L-lactic acid, γ-amino-butryric acid, α-hydroxy-butyric acid, pectin, D-saccharic acid, formic acid and D-aspartic acid. +, Positive; −, negative; W, weakly positive.

Characteristic	1	2	3
Nitrate reduction	−	−	+
Hydrolysis of:			
Aesculin	−	+	+
Tween 20	+	−	−
Tween 60	+	−	−
Tween 80	+	−	−
β-Galactosidase	+	+	−
Lipase (C14)	+	+	−
Valine arylamidase	−	−	+
α-Chymotrypsin	+	+	−
α-Glucosidase	W	W	+
β-Glucosidase	W	−	+
N-acetyl-β-glucosaminidase	+	+	W
Assimilation of:			
D-Glucose	−	+	+
D-Mannose	+	+	W
Adipic acid	−	−	W
L-Histidine	+	−	−
α-Keto-glutaric acid	+	+	−
L-Malic acid	+	+	−
Trehalose	−	+	W
Citric acid	−	+	−
Turanose	−	W	+
D-Galactose	−	−	+
DNA G+C content (mol%)	69.2	69.2	67.7*

*Data from NCBI Genome database (https://www.ncbi.nlm.nih.gov/genome).

Hiraishi *et al.* [42, 43]. For polar lipid analysis, cells of strains zong2l5^T and the reference type strains were collected in R2A broth and identified by using two-dimensional TLC described by Tindall *et al.* [44].

The major fatty acids of strain zong2l5^T (>5 % of the total amount) comprised iso-$C_{15:0}$ (40.5 %), summed feature 9 (iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$; 23.8 %) and iso-$C_{17:0}$ (17.5 %). The major fatty acid profile was similar to those of other members of the genus *Lysobacter*. However, the significant differences in the contents of summed *feature* 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), the presence of $C_{15:1}\omega9c$ and the absence of summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) could clearly distinguish strain zong2l5^T from the closely related reference strains (Table 2). It contained ubiquinone 8 (Q-8) as the major isoprenoid quinone and the polar lipids were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidyl-*N*-methylethanolamine (PME), phosphatidylethanolamine (PE), three unidentified phospholipids and an unidentified aminolipid (Fig. S4). The presence of DPG, PG and PE showed that the major polar lipids of strain zong2l5^T were in accordance with those of most species in the genus *Lysobacter*. However, PME was only found in strain zong2l5^T, *Lysobacter gummosus* KCTC 12132^T, *Lysobacter antibioticus* KCTC 12129^T, *Lysobacter capsici* KCTC 22007^T and *Lysobacter rhizophilus* KCTC 52082^T [16, 18]. Other minor amounts of unidentified phospholipids and aminolipids were distributed differently in the genus *Lysobacter*.

On the basis of the polyphasic taxonomic data presented above, strain zong2l5^T should be considered as a novel species of the genus of *Lysobacter*, for which the name *Lysobacter silvisoli* sp. nov. is proposed.

DESCRIPTION OF *LYSOBACTER SILVISOLI* SP. NOV.

Lysobacter silvisoli (sil.vi.so'li. L. fem. n. *silva* forest; L. neut. n. *solum* soil; N.L. gen. n. *silvisoli* of forest soil, the source of isolation of the type strain).

Cells are Gram-stain-negative, strictly aerobic, rod-shaped, gliding, 0.4–0.7 µm wide and 1.4–2.3 µm long. Colonies on R2A agar are bright yellow, smooth, transparent and circular. Growth temperature is between 15–40 °C (optimum, 28–30 °C). Growth occurs at pH 6.0–10.0 (optimum, 7.0) and 0–3 % NaCl (w/v; without NaCl). Oxidase and catalase are positive, but nitrate reduction, indole production, arginine dihydrolase and urease are negative. It is able to hydrolyse Tween 20, Tween 40, Tween 60, Tween 80, gelatin and tyrosine, but not starch, aesculin, casein, chitin or cellulose. In the API-ZYM test, enzyme activities for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-AS-Bl-phosphoamidase and *N*-acetyl-β-glucosaminidase are strong. Valineary lamidase, α-glucosidase and β-glucosidase are weak. No activity for cystine arylamidase, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. Assimilates D-mannose, *N*-acetyl-glucosamine, maltose, malic acid, glycyl-L-proline, L-aspartic acid, L-glutamic acid, L-histidine, L-serine, α-keto-glutaric acid and acetoacetic acid. The following substrates are not utilized: D-glucose, L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, phenylacetic acid, L-arginine, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, lactose, melibiose, methyl β-D-glucoside, D-salicin, *N*-acetyl-β-D-mannosamine, *N*-acetyl-neuraminic acid, D-fructose, D-galactose, 3-methyl glucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-PO_4, D-aspartic acid, L-pyro-glutamic acid, pectin, D-gluconic acid, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methylester, L-lactic acid, citric acid, γ-amino-butryric acid, α-hydroxy-butyric acid, α-keto-butyric acid, propionic acid and formic acid. Contains iso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$) and iso-$C_{17:0}$ as the major fatty acids and ubiquinone 8 (Q-8) as the major isoprenoid quinone. The polar lipids are DPG, PG, PME, PE, three unidentified phospholipids and an unidentified aminolipid. The genomic DNA G+C content of the type strain is 69.2 mol%.

The type strain, zong2l5^T(=GDMCC 1.1489^T=KCTC 52923^T), was isolated from forest soil collected at the Dinghu Mountain National Nature Reserve, Guangdong Province, China.

Table 2. Fatty acid composition of strain zong2l5^T and closely related type strains

Strains: 1, zong2l5^T; 2, *Lysobacter enzymogenes* KCTC 12131^T; 3, *Lysobacter soli* KCTC 22011^T. All data was obtained from this study under the same conditions. Values are percentages of total fatty acid detected. –, ot detected. The bold represents the major fatty acid.

Fatty acid	1	2	3
iso-$C_{11:0}$	4.3	3.8	4.3
iso-$C_{11:0}$ 3-OH	1.8	2.4	2.3
iso-$C_{15:0}$	**40.5**	**41.8**	**34.3**
iso-$C_{15:1}\omega9c$	2.6	–	–
anteiso-$C_{15:0}$	1.6	0.6	0.9
$C_{16:0}$	1.5	**6.0**	1.3
iso-$C_{16:0}$	2.9	0.6	3.2
iso-$C_{17:0}$	**17.5**	**6.0**	**18.3**
Summed feature 3*	1.3	**22.4**	**5.3**
Summed feature 8*	–	1.7	1.4
Summed feature 9*	**23.8**	**11.7**	**26.4**

*Summed features are groups of two or three fatty acids which cannot be separated by using the MIDI system. Summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9, iso-$C_{17:1}\omega9c$ and/or 10-methyl iso-$C_{16:0}$.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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