Lysobacter olei sp. nov., isolated from oil-contaminated soil

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TAXONOMIC DESCRIPTION

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Lysobacter olei sp. nov., isolated from oil-contaminated soil

Dhiraj Kumar Chaudhary,[1] Sang Don Lee[2] and Jaisoo Kim[1,*]

Abstract

Strain D-14^T, a brown-coloured, Gram-stain-negative, non-motile and rod-shaped bacterium, was isolated from oil-contaminated soil. It was able to grow at 20–40 °C, at pH 6.0–10.0 and at 0–1 % (w/v) NaCl concentration. Based on the 16S rRNA gene sequence analysis, strain D-14^T belonged to the genus *Lysobacter* and was closely related to *Lysobacter caeni* BUT-8^T (99.0 % sequence similarity), *Lysobacter ruishenii* CTN-1^T (98.5 %), *Lysobacter daejeonensis* GH1-9^T (98.2 %) and *Lysobacter panacisoli* CJ29^T (97.2 %). The only respiratory quinone was ubiquinone-8. The polar lipid profile revealed the presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidyl-*N*-methyl-ethanolamine. The predominant fatty acids of strain D-14^T were iso-$C_{15:0}$, iso-$C_{16:0}$, summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), iso-$C_{14:0}$, $C_{11:0}$iso 3-OH, $C_{15:1}$iso F and $C_{16:0}$. The genomic DNA G+C content of this novel strain was 68.7 mol%. The DNA–DNA relatedness values between strain D-14^T and *L. caeni* BUT-8^T, *L. ruishenii* CTN-1^T, *L. daejeonensis* GH1-9^T and *L. panacisoli* CJ29^T were 56.0, 46.3, 48.7 and 41.7 %, respectively, which fall below the threshold value of 70 % for the strain to be considered as novel. The morphological, physiological, chemotaxonomic and phylogenetic analyses clearly distinguished this strain from its closest phylogenetic neighbours. Thus, strain D-14^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacterolei* sp. nov. is proposed. The type strain is D-14^T (=KEMB 9005-572^T=KACC 19173^T=JCM 31917^T).

The genus *Lysobacter* of the family *Lysobacteraceae*, belonging to the phylum *Proteobacteria*, was first described by Christensen and Cook [1] for non-fruiting bacteria. At the time of writing, the genus *Lysobacter* comprises 43 species with validly published names (www.bacterio.net/lysobacter.html), among which *Lysobacter enzymogenes* is the type species of the genus [1].

The species of the genus *Lysobacter* are pink to yellow-brown-coloured, rod-shaped and Gram-stain-negative bacteria with gliding motility. Chemotaxonomically, species of the genus *Lysobacter* are characterized by the presence of ubiquinone-8 as the predominant respiratory quinone; iso-$C_{15:0}$, iso-$C_{16:0}$ and summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl) as the major fatty acids; phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids; and a high DNA G+C content ranging from 61.7 to 70.1 mol% [1–5]. The members of the genus *Lysobacter* have been isolated from a wide variety of habitats, including pesticide sludge, chlorothalonil-contaminated soil, greenhouse soil, ginseng soil, anaerobic granules, solid waste, a freshwater lake, deep-sea sponge, estuary sediment, rhizosphere, soil, tomato plants and lead-zinc ore [2–14].

This study describes strain D-14^T, a novel member of the genus *Lysobacter*, isolated from oil-contaminated soil of Gunsan, North Jeolla Province, Republic of Korea. Based on the results obtained from phenotypic, genotypic, chemotaxonomic and phylogenetic analyses, strain D-14^T is proposed as a novel species of the genus *Lysobacter*.

Strain D-14^T was isolated using a modified culture technique with six-well polycarbonate transwell plates as described previously [15]. For pure colonies, the bacterial growth was repeatedly streaked on R2A medium. The pure cultures obtained on R2A agar plates were stored at 4 °C for short-term maintenance and restreaked every 3–4 weeks. Before use, the strain was routinely cultivated by streaking on R2A agar incubating at 28 °C for 48 h. For long-term maintenance, the cultures were preserved at −70 °C in R2A broth supplemented with 20 % (v/v) glycerol.

Genomic DNA of strain D-14^T was extracted according to the procedure described by Marmur [16]. The 16S rRNA gene was amplified by PCR using forward-primer 27F and reverse-primer 1492R [17]. The PCR product was purified by using a multiscreen-filter plate (Millipore), and was then sequenced with an Applied Biosystems 3730XL DNA

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Keywords: *Lysobacter olei* sp. nov.; *Lysobacteraceae*; *Proteobacteria*; oil-contaminated soil.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain D-14^T is KY313413.

Three supplementary figures are available with the online Supplementary Material.

analyser utilizing the BigDye terminator cycle sequencing kit version 3.1 (Applied Biosystems). A nearly complete sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR). The nearly complete 16S rRNA gene sequence of the strain D-14^T was 1483 bp long. The closest phylogenetic neighbours of this sequence were identified by using the EZBioCloud server [18]. The 16S rRNA gene sequence of strain D-14^T was subjected to multiple alignment with the sequences of closely related bacteria using CLUSTAL X 2.1 [19]. After multiple alignment, gaps at the 5′ and 3′ ends were deleted using the software package BioEdit [20]. Phylogenetic trees were reconstructed by three different methods: the neighbour-joining method [21], the maximum-parsimony algorithm [22] and the maximum-likelihood algorithm [23] using the MEGA6 program [24]. During the phylogenetic analysis, evolutionary distances were calculated using the Kimura two-parameter model [25], and bootstrap values were calculated based on 1000 replications [26]. The comparison of the resulting 16S rRNA gene sequence with the available 16S rRNA gene sequences from GenBank by using the EZBioCloud server (http://ezbiocloud.net/) [18] revealed that strain D-14^T belonged to the genus *Lysobacter* and was most closely related to *Lysobacter caeni* BUT-8^T (99.0 % sequence similarity), *Lysobacter ruishenii* CTN-1^T (98.5 %), *Lysobacter daejeonensis* GH1-9^T (98.2 %), *Lysobacter panacisoli* CJ29^T (97.2 %), *Lysobacter aestuarii* S2-C^T (96.6 %) and *Lysobacter cavernae* YIM C01544^T (96.3 %). The pairwise sequence similarities to all other *Lysobacter* type strains were below 96.3 %. These values are at the level suggested to allocate a strain to a new species [27, 28]. Furthermore, phylogenetic trees analyses [maximum-likelihood (Fig. 1); neighbour-joining; and maximum-parsimony (Figs S1 and S2, available in the online Supplementary Material)] showed that strain D-14^T formed a stable distinct clade with *L. caeni* BUT-8^T and *L. ruishenii* CTN-1^T within the genus *Lysobacter*. Based on the 16S rRNA gene sequence similarities and the phylogenetic tree analysis, *L. caeni* BUT-8^T (=KACC 17141^T), *L. ruishenii* CTN-1^T (=DSM 22393^T), *L. daejeonensis* GH1-9^T (=KACC 11406^T) and *L. panacisoli* CJ29^T (=KACC 17502^T) were selected as reference strains for DNA–DNA hybridizations, physiological tests, biochemical studies and chemotaxonomic analysis.

Cell size and morphology of strain D-14^T grown on R2A agar for 3 days at 28 °C were studied by transmission electron microscopy (Bio-TEM, Hitachi). Colony characteristics were observed after incubation of the bacterial cells at 28 °C for 5 days on R2A agar. Gram-staining type was determined by following the method described by Doetsch [29] and by the KOH-staining testing technique [30]. Motility testing was performed in SIM medium (Oxoid) and R2A medium supplemented with 0.4 % agar. Growth on different bacteriological culture media was assessed by using R2A agar (MB Cell), nutrient agar (NA; Oxoid), tryptone soya agar (TSA; Oxoid), sorbitol MacConkey agar (Oxoid), marine agar 2216 (Difco), Luria–Bertani agar (Oxoid) and brain heart infusion agar (BD Bacto). Growth at different temperatures was observed on R2A plates incubated at 10–42 °C (10, 15, 20, 25, 28, 30, 32, 35, 37, 40 and 42 °C). Growth in different NaCl concentrations was examined by using R2A broth as the basal medium supplemented with 0–2 % NaCl (w/v, at intervals of 0.5 %) [31]. The optimum pH for growth was assessed in R2A broth and the medium was adjusted to pH 4.0–12.0 (at intervals of 0.5 pH unit) prior to autoclaving using 0.1 M citrate/NaH_2PO_4 buffer (for pH range 4.0–5.5), 0.1 M phosphate buffer (for pH range 6–7.5), 0.2M Tris buffer (for pH range 8–10) [32] and 5 M NaOH (for pH range 10.5–12.0). Testing after autoclaving revealed only minor pH changes. Anaerobic growth was tested on R2A agar incubated at 28 °C for 14 days by using a BBL anaerobic jar with a GasPak EZ Gas Generating Container (Becton Dickinson) [15]. Endospore formation was examined according to the Schaeffer–Fulton method by staining bacterial cells with malachite green [31]. Catalase and oxidase tests were performed as described by Lin *et al.* [33]. Indole testing was conducted by adding Kovac's reagent, and hydrogen sulphide production was tested by observing the presence or absence of blackening in SIM medium (Oxoid). The methyl red–Voges–Proskauer (MR-VP) test was performed in MR-VP broth and incubated at 28 °C for 48 h [34]. The DNA degradation test was performed by flooding growth on the DNA agar plate with 1 M HCl [35]. Starch hydrolysis was performed as described previously [36], and Tween 40, Tween 80 and aesculin hydrolysis tests were conducted according to Smibert and Krieg [37]. Gelatin and casein hydrolysis were assessed according to the procedure described by Cowan and Steel [38]. Hydrolysis tests for CM-cellulose, tyrosine and chitin were conducted using R2A media supplemented with CM-cellulose (1 % w/v), tyrosine (0.1 % w/v) and chitin (1 % w/v) [35]. Other physiological, biochemical and enzymatic activities were examined by using API 20NE, API ID 32GN, and API ZYM test kits (bioMérieux) according to the manufacturer's instructions.

The polar lipids were analysed using freeze-dried cells as described by Minnikin *et al.* [39]. Two-dimensional thin-layer chromatography, using chloroform–methanol–water (65 : 25 : 4; v/v/v) in the first dimension and chloroform–methanol–acetic acid–water (40 : 7.5 : 6 : 2; v/v/v/v) in the second, was used to analyse the polar lipid profile. Appropriate detection reagents [39, 40] were used to visualize the spots: phosphomolybdic acid reagent, 5 % w/v solution in ethanol (Sigma-Aldrich) was used to detect total polar lipids; ninhydrin reagent (0.2 % (w/v) solution; Sigma Life Science) was used to detect amino lipids; Zinzadze reagent (molybdenum blue spray reagent, 1.3 %; Sigma Life Sciences) was used to detect phospholipids; and α-naphthol reagent was used to detect glycolipids. The respiratory quinones were extracted with chloroform:methanol (2 : 1; v/v), and analysed by high-performance liquid chromatography [41, 42]. For analysis of fatty acids, strain D-14^T and its reference strains were cultured on TSA plates at 28 °C for 3 days. Biomass of all strains was harvested after the same growth phase (late log phase) and subjected to saponification and methylation, and were extracted using the standard

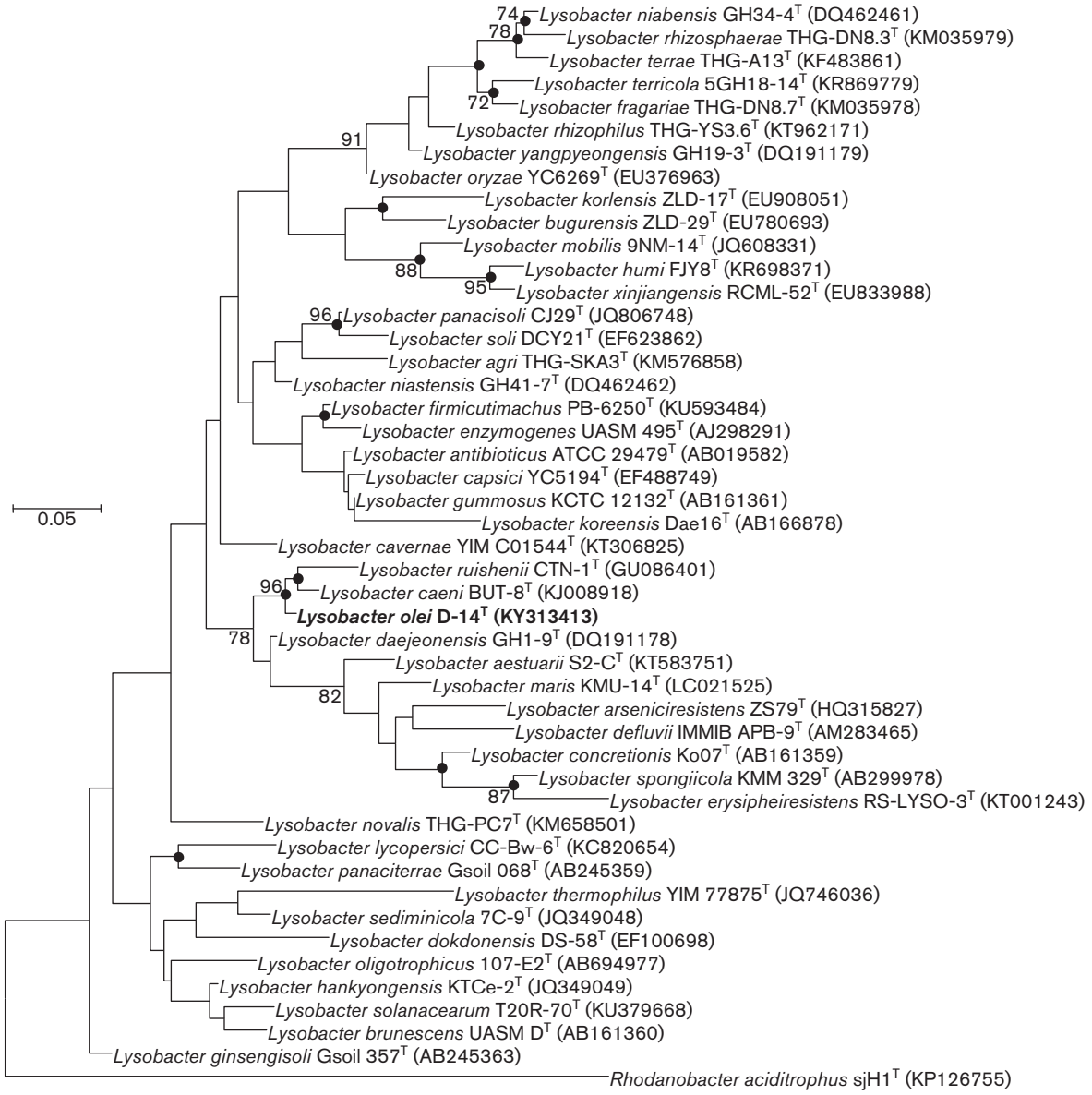

Fig. 1. Maximum-likelihood tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic position of strain D-14^T among closely related taxa. Filled circles indicate nodes recovered by using the maximum-likelihood and neighbour-joining methods. The numbers at the nodes indicate the percentage of 1000 bootstrap replicates; only values >70 % are shown. GenBank accession numbers are given in parentheses *Rhodanobacter aciditrophus* SjH1^T was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

MIDI protocol (Sherlock Microbial Identification System, version 6.0B). Analysis of fatty acids was performed with a gas chromatograph (HP 6890 Series GC System; Hewlett Packard) and the fatty acids were identified using the TSBA6 database of the Microbial Identification System [43].

The measurement of the G+C content of the chromosomal DNA of strain D-14^T was performed according to the procedure described by Mesbah *et al.* [44]. DNA–DNA hybridization was performed between strain D-14^T and four reference strains (*L. caeni* BUT-8^T, *L. ruishenii* CTN-1^T, *L. daejeonensis* GH1-9^T and *L. panacisoli* CJ29^T) according to the method described by Ezaki *et al.* [45]. Salmon sperm was used as the negative control and photobiotin was used as a probe to label the DNA of strain D-14^T. The values of DNA–DNA relatedness were determined fluorometrically in microplate wells using a 1420 Multilabel Counter (Perkin Elmer). Additionally, for reverse hybridization, each reference strain was labelled with photobiotin and used as a probe to strain D-14^T. All the assays were carried out in triplicate.

The brown-coloured colonies of strain D-14^{T}grew well on R2A and TSA. The transmission electron photomicrograph (Fig. S3) of strain D-14^T showed that cells were rod-shaped. Growth was observed at temperatures 20–40 °C and at pH 6.0–10.0. Strain D-14^T could tolerate up to 1 % (w/v) NaCl concentration. Cytochrome oxidase was absent from strain D-14^T but present in most species of the genus *Lysobacter* [2, 3]. Strain D-14^T could not hydrolyse aesculin, but the closely related reference strainscould (Table 1). Other differentiating phenotypic characteristics of strain D-14^T are given in the species description and illustrated along with other closely related species in Table 1.

The only isoprenoid quinone of strain D-14^T was ubiquinone 8 (Q-8), as found in all known members of the genus *Lysobacter* [5]. Strain D-14^T contained phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidyl-N-methyl-ethanolamine as the major polar lipids. Additionally, one unidentified phospholipid and one unidentified lipid were detected (Fig. 2). The major polar lipid profile of strain D-14^T was similar to those of *L. caeni* BUT-8^T, *L. ruishenii* CTN-1^T and *L. panacisoli* CJ29^T [2, 3, 5]. In contrast, phosphatidyl-N-methyl-ethanolamine was detected in strain D-14^T, which might differentiate this strain from other related reference strains. The major fatty acids detected in strain D-14^T were iso-$C_{15:0}$ (22.2 %), iso-$C_{16:0}$ (20.1 %), summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{16:0}$ 10-methyl; 13.2 %), summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; 13.2 %), iso-$C_{14:0}$ (8.3 %), $C_{11:0}$ iso 3-OH (6.2 %), $C_{15:1}$ iso F (6.1 %) and $C_{16:0}$ (5.5 %). The fatty acid

Table 1. Differentiating characteristics of strain D-14^T and related species belonging to the genus *Lysobacter*.

Strains: 1, D-14^T; 2, *L. caeni* BUT-8^T; 3, *L. ruishenii* CTN-1^T; 4, *L. daejeonensis* GH1-9^T; 5, *L. panacisoli* CJ29^T. All data are from the present study, except for those indicated in parentheses, which were taken from the literature [2–5]. +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Colony colour	Brown	(Yellow-green)	(Yellow)	(Yellow)	(Bright yellow)
pH range	6.0–10.0	6.0–9.0	6.0–9.0	6.0–8.0	5.0–10.5
Growth at 42 °C	–	–	–	–	+
NaCl tolerance (%, w/v)	0–1	0–1	0–1	0–3	0–1
Catalase/oxidase	+/–	+/+	+/+	–/+	+/–
Hydrolysis of:					
Tyrosine	–	–	–	+	–
Aesculin	–	+	+	+	+
Ezyme activity:					
Esterase (C4)	w	w	+	+	+
Esterase lipase (C8)	w	w	+	+	+
Valine arylamidase	+	+	–	–	–
Cystine arylamidase	w	w	–	–	–
α-Chymotrypsin	w	w	–	–	–
α-Glucosidase	–	–	–	+	+
β–Glucosidase	–	–	–	–	+
Nitrate reduction	–	–	+	+	–
4-Nitrophenyl-βD-galactopyranoside	+	–	–	–	+
Assimilation of:					
D-Mannose	–	+	–	–	+
Potassium gluconate	–	–	–	–	w
N-acetyl glucosamine	–	–	+	–	+
Maltose	w	–	–	+	+
D-Glucose	+	–	+	+	–
D-Ribose	–	+	–	–	–
Sucrose	w	+	–	–	+
Maltose	+	–	–	+	+
Suberic acid	–	–	+	–	–
Sodium acetate	–	w	–	+	–
L-Alanine	–	+	–	–	–
L-Serine	–	–	+	–	–
Valeric acid	–	–	–	+	–
L-Proline	–	+	+	–	–
DNA G+C content (mol%)	68.7	(70.6)	(67.1)	(61.7)	(65.6)

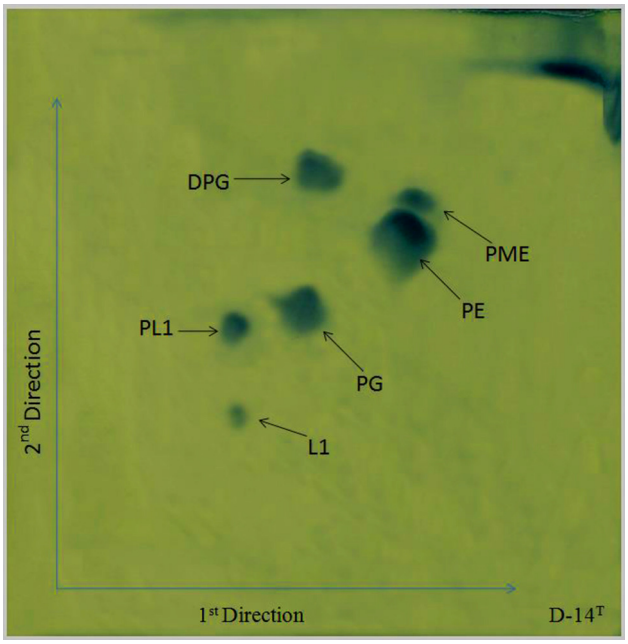

Fig. 2. Two-dimensional thin-layer chromatogram of the polar lipids from strain D-14^T detected with 5 % w/v ethanolic molybdophosphoric acid reagent. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PME, phosphatidyl-N-methyl-ethanolamine; PL1, unidentified phospholipid; L1, unidentified polar lipid.

profile of strain D-14^T was similar to that of other members of the genus *Lysobacter*, indicating that strain D-14^T is a member of the genus *Lysobacter*. However, the presence of some differences in the contents of the fatty acids clearly distinguished strain D-14^T from other closely related species of *Lysobacter* (Table 2).

The DNA G+C content of strain D-14^T was 68.7 mol%, which falls within the range of 61.7 to 70.1 mol% for the genus *Lysobacter* [5]. DNA–DNA hybridization values between strain D-14^T and *L. caeni* BUT-8^T, *L. ruishenii* CTN-1^T, *L. daejeonensis* GH1-9^T and *L. panacisoli* CJ29^T were 56.0±2.6 % (reciprocal, 41.7±7.6 %), 46.3±2.1 % (51.7 ±2.1 %), 48.7±1.5 % (35.0±4.6 %) and 41.7±3.2 % (36.7 ±2.3 %), respectively. These values were lower than the threshold value of 70 %, which clearly demonstrated that strain D-14^T differs genetically from other type strains of genus *Lysobacter* at the species level [46].

Based on the distinct phylogenetic, phenotypic, biochemical and chemotaxonomic data mentioned above, strain D-14^T represents a novel species within the genus *Lysobacter*, for which the name *Lysobacter olei* sp. nov. is proposed.

DESCRIPTION OF *LYSOBACTER OLEI* SP. NOV.

Lysobacter olei sp. nov. (o′le.i. L. gen. neut. n. *olei* of/from oil, as the organism was isolated from oil-contaminated soil).

Table 2. Cellular fatty acid profile of strain D-14^T and closely related reference strains in the genus *Lysobacter*.

Strains: 1, D-14^T; 2, *L. caeni* BUT-8^T; 3, *L. ruishenii* CTN-1^T; 4, *L. daejeonensis* GH1-9^T; 5, *L. panacisoli* CJ29^T. All data are from the present study. Values are percentages of total fatty acids. Fatty acids amounting to <0.2 % of the total fatty acids in all strains are not shown; –, not detected or <0.2 %.

Fatty acid	1	2	3	4	5
Saturated					
$C_{14:0}$	1.7	0.3	0.5	0.4	–
$C_{16:0}$	5.5	4.2	4.3	3.2	3.9
$C_{15:1}$ iso F	6.1	1.3	1.1	1.2	1.6
$C_{16:1}$ iso H	0.8	0.3	0.2	0.4	0.6
$C_{15:0}$ antesio	1.2	0.6	0.5	0.3	1.1
$C_{17:0}$ antesio	–	0.9	0.4	0.2	–
$C_{10:0}$ iso	0.5	–	0.3	0.3	–
$C_{11:0}$ iso	4.7	6.9	8.1	5.6	3.9
$C_{12:0}$ iso	0.8	0.3	0.4	0.3	0.5
$C_{13:0}$ iso	0.6	0.2	0.3	0.2	–
$C_{14:0}$ iso	8.3	2.4	0.6	1.7	2.6
$C_{15:0}$ iso	22.2	36.2	30.1	42.1	31.2
$C_{16:0}$ iso	20.1	19.2	16.3	20.5	19.9
$C_{17:0}$ iso	1.2	8.9	12.5	9.0	8.2
Unsaturated					
$C_{17:0}$ cyclo	0.6	0.2	0.4	–	–
Hydroxy					
$C_{11:0}$ iso 3-OH	6.2	6.3	8.1	5.1	3.9
$C_{12:0}$ iso 3-OH	0.3	0.2	–	–	0.3
Summed features*					
3	8.8	3.9	8.0	2.1	8.0
9	13.2	20.1	17.2	14.2	12.7

*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 contained $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; summed feature 9 contained iso-$C_{17:1}$ $\omega 9c$ and/or $C_{16:0}$ 10-methyl.

Cells are Gram-stain-negative, aerobic, non-motile, non-spore-forming and rod-shaped. The size of the cells is between 0.4 to 0.6 µm wide and 1.2 to 1.5 µm long. The size of the colonies is 0.5–1.0 mm in diameter after incubation on R2A agar at 28 °C for 5 days. Colonies on R2A are brown circular, smooth, convex and opaque with entire margins. Cells grow well on R2A and TSA; poorly on NA; and do not grow on MacConkey, Luria–Bertani, marine and brain heart infusion agar. Cell growth can be observed at temperatures of 20–40 °C (optimum, 25–35 °C) and at pH 6.0–10.0 (optimum, 6.5–8.0). The cells grow optimally in the absence of NaCl but can tolerate up to 1 % NaCl. Positive in catalase tests but negative for oxidase, H_2S production, indole production, methyl red and Voges–Proskauer tests. Hydrolyses casein, gelatin and DNA; cannot hydrolyse Tween 40, Tween 80, starch, aesculin, CM-cellulose, tyrosine and chitin. With the API ZYM test kit, positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin

and acid phosphatase; weak for esterase (C4), esterase lipase (C8), cystine arylamidase, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase; and negative for lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. During API 20NE testing, positive for gelatin and 4-nitrophenyl-β-D-galactopyranoside. Negative for nitrate reduction, L-tryptophan, fermentation of glucose, L-arginine, urea and aesculin ferric citrate. The cells weakly assimilate maltose; and cannot assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate, malic acid and phenylacetic acid. Can utilize maltose, D-glucose and 3-hydroxybutyric acid; weakly utilizes D-sucrose; and cannot utilize L-rhamnose, *N*-acetyl glucosamine, D-ribose, inositol, itaconic acids, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, salicin, melibiose, L-fucose, D-sorbitol, L-arabinose, propionic acid, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid and L-proline (API ID 32GN). Q-8 is the only respiratory quinone. The major polar lipids present are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidyl-N-methyl-ethanolamine. The major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, summed feature 9 (iso-$C_{17:1}$ $\omega 9c$ and/or $C_{16:0}$ 10-methyl), summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$), iso-$C_{14:0}$, $C_{11:0}$ iso 3-OH, $C_{15:1}$ iso F and $C_{16:0}$.

The type strain, D-14^T (=KEMB 9005-572^T=KACC 19173^T =JCM 31917^T), was isolated from oil-contaminated soil of Gunsan, North Jeolla Province, Republic of Korea. The DNA G+C content of the type strain is 68.7 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Christensen P, Cook FD. *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* 1978; 28:367–393.
2. Ye XM, Chu CW, Shi C, Zhu JC, He Q *et al.* *Lysobacter caeni* sp. nov., isolated from the sludge of a pesticide manufacturing factory. *Int J Syst Evol Microbiol* 2015;65:845–850.
3. Wang GL, Wang L, Chen HH, Shen B, Li SP *et al.* *Lysobacter ruishenii* sp. nov., a chlorothalonil-degrading bacterium isolated from a long-term chlorothalonil-contaminated soil. *Int J Syst Evol Microbiol* 2011;61:674–679.
4. Weon HY, Kim BY, Baek YK, Yoo SH, Kwon SW *et al.* Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. *Int J Syst Evol Microbiol* 2006;56:947–951.
5. Choi JH, Seok JH, Cha JH, Cha CJ. *Lysobacter panacisoli* sp. nov., isolated from ginseng soil. *Int J Syst Evol Microbiol* 2014;64:2193–2197.
6. Bae HS, Im WT, Lee ST. *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* 2005;55:1155–1161.
7. Fukuda W, Kimura T, Araki S, Miyoshi Y, Atomi H *et al.* *Lysobacter oligotrophicus* sp. nov., isolated from an Antarctic freshwater lake in Antarctica. *Int J Syst Evol Microbiol* 2013;63:3313–3318.
8. Romanenko LA, Uchino M, Tanaka N, Frolova GM, Mikhailov VV. *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* 2008;58:370–374.
9. Yassin AF, Chen WM, Hupfer H, Siering C, Kroppenstedt RM *et al.* *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Microbiol* 2007;57:1131–1136.
10. Jeong SE, Lee HJ, Jeon CO. *Lysobacter aestuarii* sp. nov., isolated from estuary sediment. *Int J Syst Evol Microbiol* 2016;66:1346–1351.
11. Park JH, Kim R, Aslam Z, Jeon CO, Chung YR. *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* 2008;58:387–392.
12. Oh KH, Kang SJ, Jung YT, Oh TK, Yoon JH. *Lysobacter dokdonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2011;61:1089–1093.
13. Lin SY, Hameed A, Wen CZ, Liu YC, Hsu YH *et al.* *Lysobacter lycopersici* sp. nov., isolated from tomato plant *Solanum lycopersicum*. *Antonie van Leeuwenhoek* 2015;107:1261–1270.
14. Yang SZ, Feng GD, Zhu HH, Wang YH. *Lysobacter mobilis* sp. nov., isolated from abandoned lead-zinc ore. *Int J Syst Evol Microbiol* 2015;65:833–837.
15. Chaudhary DK, Kim J. *Novosphingobium naphthae* sp. nov., from oil-contaminated soil. *Int J Syst Evol Microbiol* 2016;66:3170–3176.
16. Marmur J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* 1961;3:208–218.
17. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA *et al.* Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 2008;74: 2461–2470.
18. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017; 67:1613–1617.
19. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–4882.
20. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95–98.
21. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
22. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 1971;20:406–416.
23. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
24. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
25. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
26. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
27. Yarza P, Richter M, Peplies J, Euzeby J, Amann R *et al.* The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* 2008;31:241–250.

28. **Stackebrandt E, Goebel BM.** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 1994;44:846–849.
29. **Doetsch RN.** Determinative methods of light microscopy. In: Gerhardt P (editor). *Manual of Methods for General Bacteriology*. Washington, DC, USA: American Society for Microbiology; 1981. pp. 21–33.
30. **Powers EM.** Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Appl Environ Microbiol* 1995;61:3756–3758.
31. **Chaudhary DK, Kim J.** *Arvibacter flaviflagrans* gen. nov., sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* 2016;66:4347–4354.
32. **Breznak JA, Costilow RN.** Physicochemical factors in growth. In: Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR *et al.* (editors). *Methods for General and Molecular Bacteriology*, 3rd ed. Washington, DC: American Society for Microbiology; 2007. pp. 309–329.
33. **Chaudhary DK, Kim J.** *Chryseobacterium nepalense* sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* 2017;67:646–652.
34. **Hemraj V, Diksha S, Avneet G.** A review on commonly used biochemical test for bacteria. *Innovare J Life Sci* 2013;1:1–7.
35. **Chaudhary DK, Kim J.** *Sphingomonas naphthae* sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* 2016;66:4621–4627.
36. **Tindall BJ, Sikorski J, Smibert RA, Krieg NR.** Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM *et al.* (editors). *Methods for General and Molecular Bacteriology*, 3rd ed. Washington, DC: ASM Press; 2007. pp. 330–393.
37. **Smibert RM, Krieg NR.** Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (editors). *Methods for General and Molecular Bacteriology*. Washington, DC, USA: American Society for Microbiology; 1994. pp. 607–654.
38. **Cowan ST, Steel KJ.** *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press; 1965.
39. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.*** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
40. **Komagata K, Suzuki K.** Lipids and cell wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–203.
41. **Hiraishi A, Ueda Y, Ishihara J, Mori T.** Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* 1996;42:457–469.
42. **Collins MD, Jones D.** Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* 1981;45:316–354.
43. **Sasser M.** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
44. **Mesbah M, Premachandran U, Whitman WB.** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159–167.
45. **Ezaki T, Hashimoto Y, Yabuuchi E.** Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 1989;39:224–229.
46. **Wayne LG, Moore WEC, Stackebrandt E, Kandler O, Colwell RR *et al.*** Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.