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Lysobacter profundi sp. nov., isolated from freshwater sediment and reclassification of *Lysobacter panaciterrae* as *Luteimonas panaciterrae* comb. nov.

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Abstract

A polyphasic taxonomic study was carried out on strains CHu50b-3-2^T and CHu40b-3-1 isolated from a 67 cm-long sediment core collected from the Daechung Reservoir at a water depth of 17 m, Daejeon, Republic of Korea. The cells of the strains were Gram-stain-negative, non-spore-forming, non-motile and rod-shaped. Comparative 16S rRNA gene sequence studies showed a clear affiliation of two strains with γ-*Proteobacteria*, which showed the highest pairwise sequence similarities to *Lysobacter hankyongensis* KTce-2^T (96.5%), *Lysobacter pocheonensis* Gsoil193^T (96.3%), *Lysobacter ginsengisoli* Gsoil 357^T (96.1%), *Lysobacter solanacearum* T20R-70^T (96.1%), *Lysobacter brunescens* KCTC 12130^T (95.4%) and *Lysobacter capsici* YC5194^T (95.3%). The phylogenetic analysis based on 16S rRNA gene sequences showed that the strains formed a clear phylogenetic lineage with the genus *Lysobacter*. The major fatty acids were identified as summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{18:1}$ 10-methyl), iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$. The respiratory quinone was identified as ubiquinone Q-8. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholipid. The genomic DNA G+C content was determined to be 66.8 mol% (genome) for strain CHu50b-3-2^T and 66.4 mol% (HPLC) for strain CHu40b-3-1. Based on the combined genotypic and phenotypic data, we propose that strains CHu50b-3-2^T and CHu40b-3-1 represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter profundi* sp. nov. is proposed. The type strain is CHu50b-3-2^T (=KCTC 72973^T=CCTCC AB 2019129^T). Besides *Lysobacter panaciterrae* Gsoil 068^T formed a phylogenetic group together with strain *Luteimonas aquatica* RIB1-20^T (EF626688) that is clearly separated from all other known *Lysobacter* strains. Based on the phylogenetic relationships together with fatty acid compositions, *Lysobacter panaciterrae* Gsoil 068^T should be reclassified as a member of the genus *Luteimonas: Luteimonas aquatica* comb. nov. (type strain Gsoil 068^T=KCTC 12601^T=DSM 17927^T).

INTRODUCTION

Phylogenetically, the genus *Lysobacter* has an evolutionary lineage within the family *Xanthomonadaceae* of the class *Gammaproteobacteria*. The genus comprises 59 validly named species (https://lpsn.dsmz.de) [1], since Christensen and Cook [2] proposed *Lysobacter* as a new genus in the family *Xanthomonadaceae*. Most of the species within the genus *Lysobacter* are typically isolated from soil habitats, and some from water, sludge, sediment, tomato stems, zinc ore, deep-sea sponge and human Meibomian gland secretions [2–12] and from sites all over the world [4, 13–17]. Cells of members of the genus *Lysobacter* are Gram-stain-negative, aerobic and rod-shaped with variable motility, chemoorganotrophic, high DNA G+C contents and contain ubiquinone Q-8 as the major respiratory quinine [8, 17]. Herein, we describe two yellow-pigmented aerobic bacterial strains, CHu50b-3-2^T and CHu40b-3-1, which were recovered from freshwater sediment. Phylogenetic analysis of 16S rRNA gene sequences showed that strains CHu50b-3-2^T and CHu40b-3-1 were closely related to members of the genus *Lysobacter*. Based on a polyphasic approach including the determination of their phenotypic, chemotaxonomic properties

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Keywords: CHu50b-3-2^T; CHu40b-3-1; *Lysobacter*; *Lysobacter profundi*; *Luteimonas panaciterrae*.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridisation; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipids; UPGMA, unweighted pair group method with arithmetic mean.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains CHu40b-3-1 and CHu50b-3-2^T are MK696267 and MK696268, respectively. The GenBank accession number for the whole genome sequence of type strain CHu50b-3-2^T is VKHQ00000000.
†These authors contributed equally to this work
Four supplementary tables and three supplementary figures are available with the online version of this article.

Table 1. Phenotypic and chemotaxonomic characteristics distinguishing strains CHu50b-3-2^T and CHu40b-3-1 from some close members of the genus *Lysobacter*

Strains: 1, CHu50b-3-2^T; 2, CHu40b-3-1; 3, *Lysobacter ginsengisoli* KCTC 12602^T; 4, *Lysobacter hankyongensis* KACC 16618^T; 5, *Lysobacter solanacearum* KACC 18656^T; 6, *Lysobacter brunescens* KCTC 12130^T; 7, *Lysobacter capsici* KCTC 22007^T. All data were from this study unless indicated. All strains were observed to be negative for activities of nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, *N*-acetyl-*β*-glucosaminidase, α-fucosidase, *β*-galactosidase, *β*-glucuronidase, α-mannosidase and valine arylamidase; carbon assimilation of acetate, adipate, L-alanine, caprate, gluconate, inositol, 5-ketogluconate, malate, phenyl acetate and rhamnose. +, Positive; –, negative.

Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Isolation source	Sediment	Sediment	Soil*	Activated sludge†	Soil‡	Fresh water§	Soil‖
Gelatin hydrolysis	+	–	–	+	+	+	+
NaCl tolerance range (w/v, %)	0–1	0–1	0–2*	No tolerance†	0–1‡	0–1§	0–2‖
Carbon utilization:							
N-Acetyl-glucosamine	–	–	–	+	+	–	+
L-Arabinose	–	–	–	+	–	–	–
Citrate	–	–	–	+	–	–	+
L-Fucose	–	–	–	+	–	–	–
D-glucose	–	–	+	+	+	+	+
Glycogen	–	–	–	+	+	–	+
Histidine	–	–	–	+	–	–	–
3-Hydroxy-benzoate	–	–	–	+	–	–	–
4-hydroxy-benzoate	–	–	–	+	–	–	–
3-Hydroxy-butyrate	–	–	–	+	–	–	+
Itaconate	–	–	–	+	–	–	–
DL-Lactate	–	–	–	+	–	–	–
2-Ketogluconate	–	–	–	+	–	–	–
Malonate	–	–	–	+	–	–	–
Maltose	–	–	+	+	+	+	+
D-Mannitol	–	–	–	+	–	–	–
D-Mannose	–	–	–	–	–	+	+
Melibiose	–	–	–	+	+	–	+
L-Proline	+	+	+	+	–	–	+
Propionate	–	–	–	+	–	–	–
D-Ribose	–	–	–	+	+	–	–
Ssalicin	–	–	–	+	–	–	+
L-Serine	–	–	–	+	–	–	–
D-Sorbitol	–	–	–	+	–	+	–
Suberate	–	–	–	+	–	–	–
Sucrose	–	–	–	+	+	–	+
Valerate	–	–	–	+	–	–	–
Enzyme activity:							
Acid phospatase	+	–	+	–	+	+	+
Alkaline phosphatase	+	–	–	+	+	+	+

Continued

Table 1. Continued

Characteristics	1	2	3	4	5	6	7
α-Chymotrypsin	−	−	−	+	+	−	−
Cystine arylamidase	−	−	−	+	−	−	−
Esterase (C4)	−	−	+	−	+	+	+
Esterase lipase (C8)	+	−	+	−	+	−	+
α-Galactosidase	−	−	−	−	−	−	+
α-Glucosidase	+	+	−	−	−	−	+
β-Glucosidase	+	−	−	−	−	−	+
Leucine arylamidase	+	+	+	+	+	−	+
Lipase (C14)	−	−	−	−	−	−	+
Naphthol-AS-BI-phosphohydrolase	+	−	−	−	+	+	+
Trypsin	−	−	−	+	+	+	+
DNA G+C content (mol%)	66.8	66.4	69.4*	68.6†	63.0	67.7§	65.4‖

*Data taken from Jung *et al*. [40].
†Siddiqi and Im [8].
‡Kim *et al*. [41].
§Christensen and Cook [2].
‖Park *et al.* [42].

and a detailed phylogenetic investigation, we propose strains CHu50b-3-2^T and CHu40b-3-1 as belonging to a new species *Lysobacter profundi* sp. nov.

METHODS

Isolation and culture condition

Sediment samples were taken from a 67 cm-long sediment core collected from the Daechung Reservoir at a water depth of 17 m in Daejeon (36° 22′ 30″ N, 127° 33′ 58″ E), Republic of Korea. Then, 1 g sediment sample was initially diluted serially with a 0.85% saline solution. A 100 µl sub-sample of the suspended material was aseptically transferred and spread on modified 1/10 R2A agar (0.05 g l^{-1} peptone, 0.05 g l^{-1} yeast extract, 0.05 g l^{-1} casamino acid, 0.05 g l^{-1} dextrose, 0.05 g l^{-1} soluble starch, 0.03 g l^{-1} K_2HPO_4, 0.005 g l^{-1} $MgSO_4$, 0.03 g l^{-1} sodium pyruvate and 15 g l^{-1} agar), and the plates were incubated at 25 °C for 30 days. Two yellow-pigmented colonies that appeared on the modified Reasoner's 2A (R2A) plates were selected for further study. For long-term storage, the two isolates (CHu50b-3-2^T and CHu40b-3-1) were routinely cultured on R2A plates at 30 °C under an aerobic condition and stored frozen at −80 °C in 15% (v/v) glycerol stock solution. For most of the experiments, all strains were cultivated on an R2A agar (BD Difco) or broth (MB cell; MB-R2230) at 28 °C for 48 h. All reference strains, *Lysobacter hankyongensis* KACC 16618^T, *Lysobacter ginsengisoli* KCTC 12602^T, *Lysobacter solanacearum* KACC 18656^T, *Lysobacter brunescens* KCTC 12130^T and *Lysobacter capsici* KCTC 22007^T, were taken from the KACC (Korean Agricultural Culture Collection) and the KCTC (Korean Collection for Type Cultures).

Morphological, physiological and biochemical characterization

Gram-staining, cell morphology, motility and biochemical properties were determined with cells grown on R2A agar at 28 °C for 48 h. The cell growth was checked on R2A agar, trypticase soy agar (TSA; Difco), Luri–Bertani (LB; Difco) medium and nutrient agar (NA; Difco). The Gram reaction was carried out using a Gram-stain kit (Becton Dickinson) following the manufacturer's instructions. Cell morphology and motility were observed under a phase-contrast microscope (Optiphot, Nikon; ×1000 magnification). Oxidase and catalase activities were investigated using 1% tetramethyl-*p*-phenylenediamine [18] and 3% H_2O_2, respectively. Growth was studied on R2A agar at different temperatures (4, 8, 15, 25, 30, 37 and 45 °C). The pH range (pH 5–10 at intervals of 1 unit) for growth was investigated in R2A broth and different buffer systems were applied, as previously described [19]. NaCl tolerance for growth was carried out in R2A agar using different NaCl concentrations from 1 to 4% (w/v). Carbon-source utilization, enzyme activity and additional physiological tests were performed using API 20NE, ID 32 GN and API ZYM kits (bioMérieux) according to the manufacturer's instructions (bioMérieux).

Chemotaxonomic characterization

For quantitative analysis of whole-cell fatty acid profiling, strains CHu50b-3-2^T, CHu40b-3-1, *L. hankyongensis* KACC 16618^T,

L. ginsengisoli KCTC 12602^T and *L. capsici* KCTC 22007^T were cultured on R2A agar at 28 °C for 48 h. To extract the fatty acids, we harvested the cell mass when the cells reached the late exponential phase. Separation and identification of the fatty acids were done by GC (Hewlett Packard 6890), and the TSBA6 database provided by Sherlock software 6.1. Extraction of ubiquinone was completed as described by Komagata and Suzuki [20], and the analysis was done by HPLC (Shimadzu) with an YMC-Pack ODS-A column. Extraction and identification of polar lipids were done following the method described by Tindall using cells harvested from R2A broth for 2 days at 28 °C [21].

Molecular characterization

Phylogenetic positions of strains CHu50b-3-2^T and CHu40b-3-1 were determined with via 16S rRNA gene sequence analysis. Genomic DNA was extracted using the FastDNA SPIN kit for soil DNA Extraction MP. Extracted DNA was then examined for purity on an ND2000 spectrometer (Nanodrop Technologies). To amplify the 16S rRNA gene, we used the universal bacterial primer sets 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′; *Escherichia coli* position 8–27) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′; *E. coli* position 1492–1510 [22]. The purified PCR products then were sequenced with the BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems). To get the full 16S rRNA gene, we used the sequencing primers 27F, 785F (5′-GGATTAGATACC-CTGGTA-3′), 800R (5′-TACCAGGGTATCTAATCC-3′) and 1492R for the sequence analysis [18]. To reconstruct phylogenetic trees, sequence alignment and editing were carried out using CLUSTAL X [23] and BIOEDIT [24] software, respectively. Neighbour-joining, maximum-parsimony and maximum-likelihood [25–27] algorithms were applied in the MEGA7 software [28]. Bootstrap values were calculated on 1000 resamplings of the sequences [29]. Whole-genome sequencing of strain CHu50b-3-2^T was performed using the Illumina HiSeq platform. The genome was assembled by the CLC assembler (CLC-Assembly-Cell-5.1.1), and annotated by the PATRIC 3.5.36 (www.patricbrc.org). The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were calculated with the OrthoANI tool in the EZBioCloud server and the Genome-to-Genome Distance Calculator (GGDC 2.1) based on draft genome sequences [30, 31]. The UPGMA dendrogram was generated using calculated ANI values by the unweighted method. DNA G+C contents (mol%) of genomic DNA was analysed using HPLC after hydrolysis, following standard methods described by Tamaoka and Komagata [32]. Non-methylated λ DNA (Sigma) was used as a standard.

RESULTS AND DISCUSSION

Morphological, physiological and biochemical characteristics

Strains CHu50b-3-2^T and CHu40b-3-1 were observed to form visible colonies within 48 h on an R2A agar when incubated at 30 °C. Growth was found to occur at temperatures ranging from 4 to 30 °C, but no growth was observed at 37 °C. Growth was found to occur at pH 6–8, but no growth was observed at pH 5 or 9. The colonies were observed to be yellow, smooth, convex and circular with entire edges. The cells were found to be Gram-stain-negative, catalase-positive, oxidase-negative, non-motile and rod-shaped. The strains did not assimilate glucose, *N*-acetyl-glucosamine, glycogen, melibiose or sucrose, which could differentiate the novel strains from *L. ginsengisoli* KCTC 12602^T, *L. hankyongensis* KACC 16618^T, *L. solanacearum* KACC 18656^T, *L. brunescens* KCTC 12130^T and *L. capsici* KCTC 22007^T (Table 1).

Phylogenetic analysis

The almost-complete 16S rRNA gene sequences of strains CHu50b-3-2^T and CHu40b-3-1 were compared with the 16S rRNA gene sequences of representative species within the genus *Lysobacter* and related genera in the EzTaxon-e server [33]. The results showed that strains CHu50b-3-2^T (1496 bp) and CHu40b-3-1 (1501 bp) had 96.5% pairwise similarity to *L. hankyongensis* KTce-2^T, 96.3% to *L. pocheonensis* Gsoil193^T, 96.1% to *L. ginsengisoli* Gsoil 357^T, 96.1% to *L. solanacearum* T20R-70^T, 95.4% to *L. brunescens* KCTC 12130^T and 95.3% to *Lysobacter capsici* YC5194^T, and less than 95.3% to all other species of genus *Lysobacter*. Strains CHu50b- 3-2^T and CHu40b-3-1 shared 100% 16S rRNA gene sequence similarity. According to the neighbour-joining phylogenetic tree, strains CHu50b-3-2^T and CHu40b-3-1 clearly clustered with the genus *Lysobacter* (Fig. 1), this was also supported in phylogenetic trees reconstructed with the maximum-parsimony and maximum-likelihood algorithms (Fig. S1, available in the online version of this article). The evolutionary result was also supported by the UPGMA dendrogram based on the ANI values of genomic sequences, type strain CHu50b-3-2^T formed an evolutionary lineage clustering with the species of *Lysobacter*, but independent of other genera (Fig. 2).

Whole-genome sequence analysis

The draft genome sequence of strain CHu50b-3-2^T was deposited at DDBJ/EMBL/GenBank with the accession number VKHQ00000000. Paired-end sequencing (2×150 bp) of a fragment library resulted in about 1.9×10^7 quality filtered reads. Assembly of reads resulted in 19 contigs with a total sequence length of 2714881 bp with the G+C content of 66.8%. The assembled draft genome sequence of strain CHu50b-3-2^T consists of 2570 coding genes (CDS) and 49 RNAs (Table S1). The ANI values of strain CHu50b-3-2^T with *L. capsici* 55 (CP011130), *L. spongiicola* DSM 21749^T (NZ_FUXP01000000), *L. antibioticus* ATCC 29479^T (CP013141), *L. dokdonensis* DS-58^T (NZ_JRKJ01000000) and *L. gummosus* 3.2.11 (CP011131) were 76.2, 74.8, 76.3, 75.4 and 76.1%, respectively (Table S2). These ANI values were far below the species discrimination (95–96%) indicating strain CHu50b-3-2^T should be classified as a distinct species in the genus *Lysobacter* [34, 35], although full genome sequences of the closest members are not available.

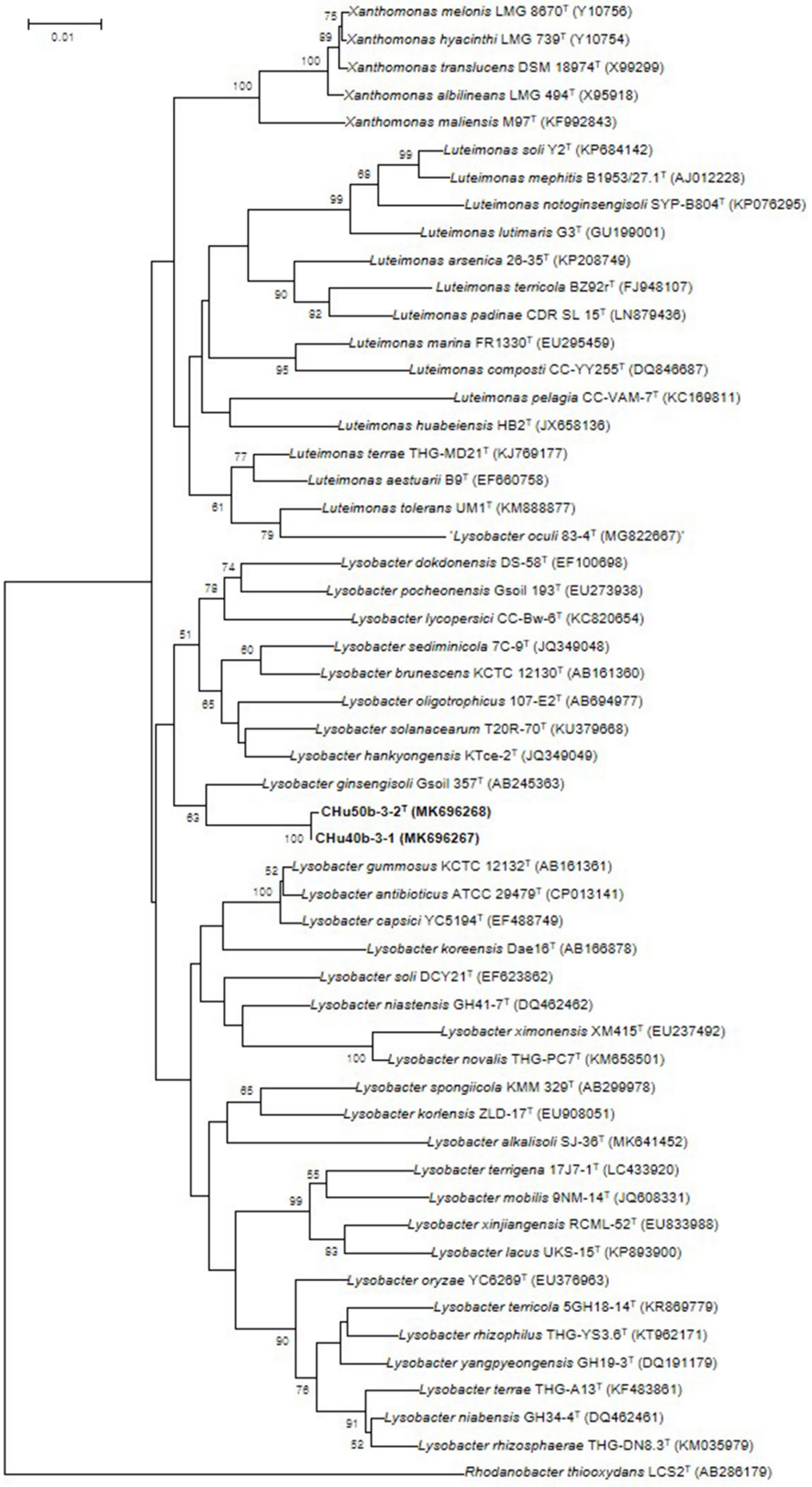

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences using the neighbour-joining method showing the positions of strains CHu50b-3-2^T and CHu40b-3-1 among type strains within the genus *Lysobacter*. Only bootstrap values over 50% are shown. Bar, 0.01 substitution per nucleotide position.

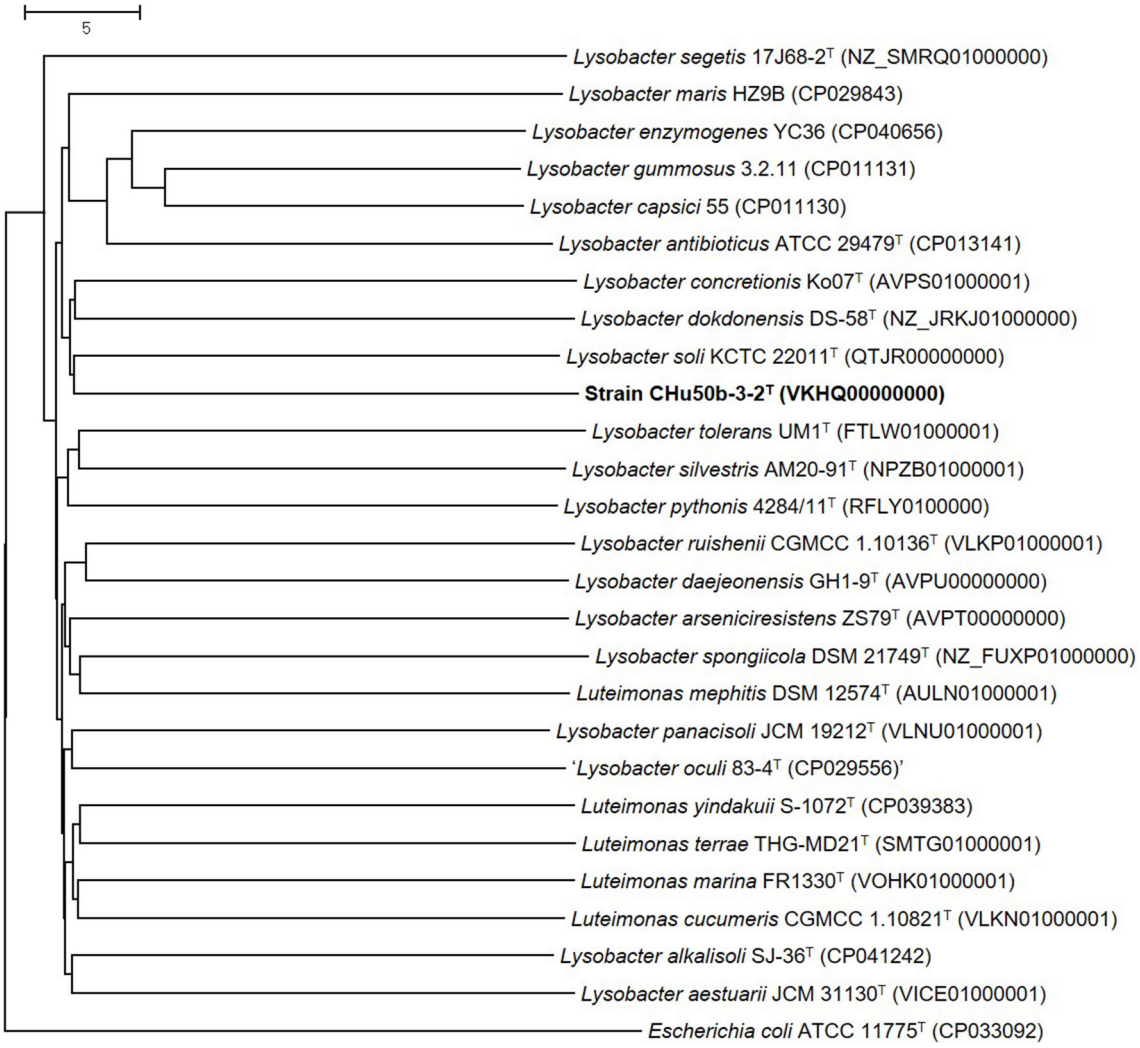

Fig. 2. UPGMA dendrogram based on ANI values of genomic sequences showing the position of strain CHu50b-3-2^T and some other related taxa. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 5% difference in ANI value.

Chemotaxonomic characteristics

The G+C content of the genomic DNA was determined to be 66.4–66.8 mol%, which is within the range value for the genus *Lysobacter* (61.7–70.7 mol%) [36–38]. The major fatty acids were identified as summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{18:1}$ 10-methtyl), iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$ (Table 2). In the fatty acid comparison among strains CHu50b-3-2^T and CHu40b-3-1 and their related *Lysobacter* species, the major fatty acids in strains CHu50b-3-2^T and CHu40b-3-1 were consistent with the major fatty acid components in species from the genus *Lysobacter*, which are common in this genus. However, some qualitative and quantitative differences in the fatty acid profiles were observed (Table 2). The major respiratory quinone was qubiquinone-8 (Q-8). The polar lipids were composed of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and unidentified phospholipids (PL1–4 for strain CHu40b-3-1; PL1–7 for strain CHu50b-3-2^T) (Fig. S2).

Based on phenotypic and phylogenetic characteristics, our new isolates are considered to be members of the genus *Lysobacter*. Some physiological evidence was found differentiating the two strains from their closest formally described relatives. The capacity for carbon utilization, enzyme activities and polar lipid compositions differentiated the isolates from the closest member of the genus *Lysobacter*. Therefore, we suggest that strains CHu50b-3-2^T and CHu40b-3-1 represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter profundi* sp. nov. is proposed.

The 16S rRNA gene sequence similarity of strain *Lysobacter panaciterrae* Gsoil 068^T (EMBL accession number AB245359) with almost all the representative species

Table 2. Cellular fatty acid compositions (%) of strains CHu50b-3-2^T and CHu40b-3-1 with related type strains

Strains: 1, CHu50b-3-2^T; 2, CHu40b-3-1; 3, *Lysobacter ginsengisoli* KCTC 12602^T; 4, *Lysobacter hankyongensis* KACC 16618^T; 5, *Lysobacter solanacearum* KACC 18656^T [40]; 6, *Lysobacter brunescens* KCTC 12130^T [40]; 7, *Lysobacter capsici* KCTC 22007^T. All data were from present study unless indicated. Cells of all strains were harvested after growth on R2A agar at 28 °C for 48 h. –, Not detected.

Fatty acids	1	2	3	4	5	6	7
iso-$C_{10:0}$	0.3	0.2	0.6	–	–	–	–
iso-$C_{11:0}$	7.1	5.4	4.1	4.1	4.6	4.9	4.8
anteiso-$C_{11:0}$	0.3	0.3	0.4	–	–	–	–
$C_{10:0}$ 2-OH	–	–	–	–	–	–	0.4
$C_{10:0}$ 3-OH	–	–	–	–	–	–	1.2
iso-$C_{12:0}$	–	–	0.4	0.6	–	–	–
iso-$C_{11:0}$ 3-OH	8.8	6.0	6.7	4.3	6.1	5.5	9.2
iso-$C_{13:0}$	–	0.3	–	–	–	–	0.2
iso-$C_{12:0}$ 3-OH	–	–	0.3	–	–	–	0.1
iso-$C_{14:0}$	1.3	1.3	2.0	10.4	3.6	1.2	0.6
$C_{14:1}$ ω5*c*		–	0.2	1.3		–	–
$C_{14:0}$	1.3	1.3	0.6	1.6	0.7	1.1	1.3
iso-$C_{15:1}$ ω9*c*	–	–	–	–	–	–	2.0
iso-$C_{15:1}$ F	0.6	0.6	0.6	1.5	1.5	1.7	–
iso-$C_{15:0}$	15.4	15.2	9.1	18.9	25.4	26.3	28.4
anteiso-$C_{15:0}$	3.7	3.7	4.2	2.0	1.2	3.5	2.4
$C_{15:1}$ ω6*c*	–	–	0.2	–	–	–	–
$C_{16:1}$ ω7*c* alcohol	–	–	–	0.9	–	–	2.0
iso-$C_{16:1}$ h	–	–	1.5	1.5	1.0	–	–
iso-$C_{16:0}$	15.3	16.7	37.2	26.8	22.3	12.3	3.2
$C_{16:1}$ ω5*c*	–	–	–	–	–	–	–
$C_{16:1}$ ω11*c*	–	–	–	–	–	–	4.2
$C_{16:0}$	4.8	5.1	2.8	–	1.8	3.6	7.1
iso-$C_{17:0}$	9.7	11.1	4.9	–	2.7	7.7	3.7
anteiso-$C_{17:0}$	0.6	0.6	1.0	–	–	–	0.3
$C_{17:1}$ ω8*c*	–	–	–	–	–	–	0.1
$C_{16:0}$ 3-OH	–	–	–	–	–	–	0.2
iso-$C_{18:0}$	–	0.4	0.8	–	–	–	–
$C_{18:0}$	–	–	0.6	–	–	–	–
iso-$C_{17:0}$ 3-OH	–	0.5	–	–	–	–	0.2
Summed feature 3*	4.5	4.5	3.5	16.9	11.6	9.1	16.8
Summed feature 8†	–	–	–	–	–	–	2.1
Summed feature 9‡	26.3	26.9	18.2	4.6	14.0	18.8	8.8

*Summed feature 3, $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*.
†Summed feature 8, $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*.
‡Summed feature 9, $C_{17:1}$ iso *ω9c* and/or $C_{18:1}$ 10-methyl.

within the genus *Lysobacter*, *Luteimonas*, and *Xanthomonas* was reanalysed. The results showed that strain Gsoil 068^T shared 98.8% pairwise similarity with *Luteimonas aquatica* RIB1-20^T, 97.2% with *Luteimonas rhizosphaerae* 4-12^T, 97.2% with *Luteimonas terrae* THG-MD21^T, 97.1% with *Xanthomonas cucurbitae* LMG 690^T and 97.1% with *Lysobacter hankyongensis* KTce-2^T, and less than 96.9% with all other species of genus *Lysobacter*. A phylogenetic tree was reconstructed to better understand the phylogenic relationships of *Lysobacter panaciterrae* Gsoil 068^T to other members of the genera *Lysobacter*, *Luteimonas* and *Xanthomonas*. The phylogenetic tree based on the neighbour-joining, *Lysobacter panaciterrae* Gsoil 068^T was phylogenetically distinct from almost all species of genus *Luteimonas* and *Xanthomonas*, but clearly affiliated with *Luteimonas aquatica* RIB1-20^T, *Luteimonas cucumeris* Y4^T and *Luteimonas vadosa* KMM 9005^T (Figs 3 and S3). Same problem was also found in *Lysobacter oculi* 83-4^T (Figs 3 and S3), but the species *Lysobacter oculi* is not yet validly published, therefore, the reclassification should be done after its validation. Phynotypic differences were also found comparing to some closely related species of *Lysobacter* (Table S3). A comparative study of fatty acid compositions shows that strain Gsoil 068^T had a highly similar profile to *Luteimonas aquatica* RIB1-20^T, but differences in composition of iso-$C_{14:0}$, summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or $C_{18:1}$ 10-methyl) (Table S4). Based on the phylogenetic relationships together with phenotypic characteristics and fatty acid compositions, *Lysobacter panaciterrae* Gsoil 068^T is reclassified as a member of the genus *Luteimonas* as *Luteimonas panaciterrae* comb. nov. (type strain Gsoil 068^T=KCTC 12601^T=DSM 17927^T).

DESCRIPTION OF *LYSOBACTER PROFUNDI* SP. NOV.

Lysobacter profundi (pro.fun′di. L. gen. n. *profundi* of a deep place).

Cells are Gram-stain-negative, non-motile, rods (0.5–0.7 by 1.4–2.5 µm) when grown for 48 h at 30 °C on R2A agar. Colonies are smooth, circular, convex and yellow-coloured on R2A agar. Growth occurs at 4–30 °C (optimum, 25–30 °C) but not at 37 °C, and at pH 6.0–8.0 (optimum, pH 7.0). Cells do not tolerate NaCl. Catalase-positive and oxidase-negative. Positive for aesculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease and *β*-galactosidase activities. Positive for L-proline, but negative for acetate, *N*-acetylglucosamine, adipate, L-alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, D-glucose, glycogen, histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, lactate, malate, malonate, maltose, D-mannitol, D-mannose, melibiose, phenylacetate, propionate, L-rhamnose, D-ribose, salicin, L-serine, D-sorbitol, suberate, sucrose and valerate. Positive for the following enzyme activities: *α*-glucosidase and leucine arylamidase;

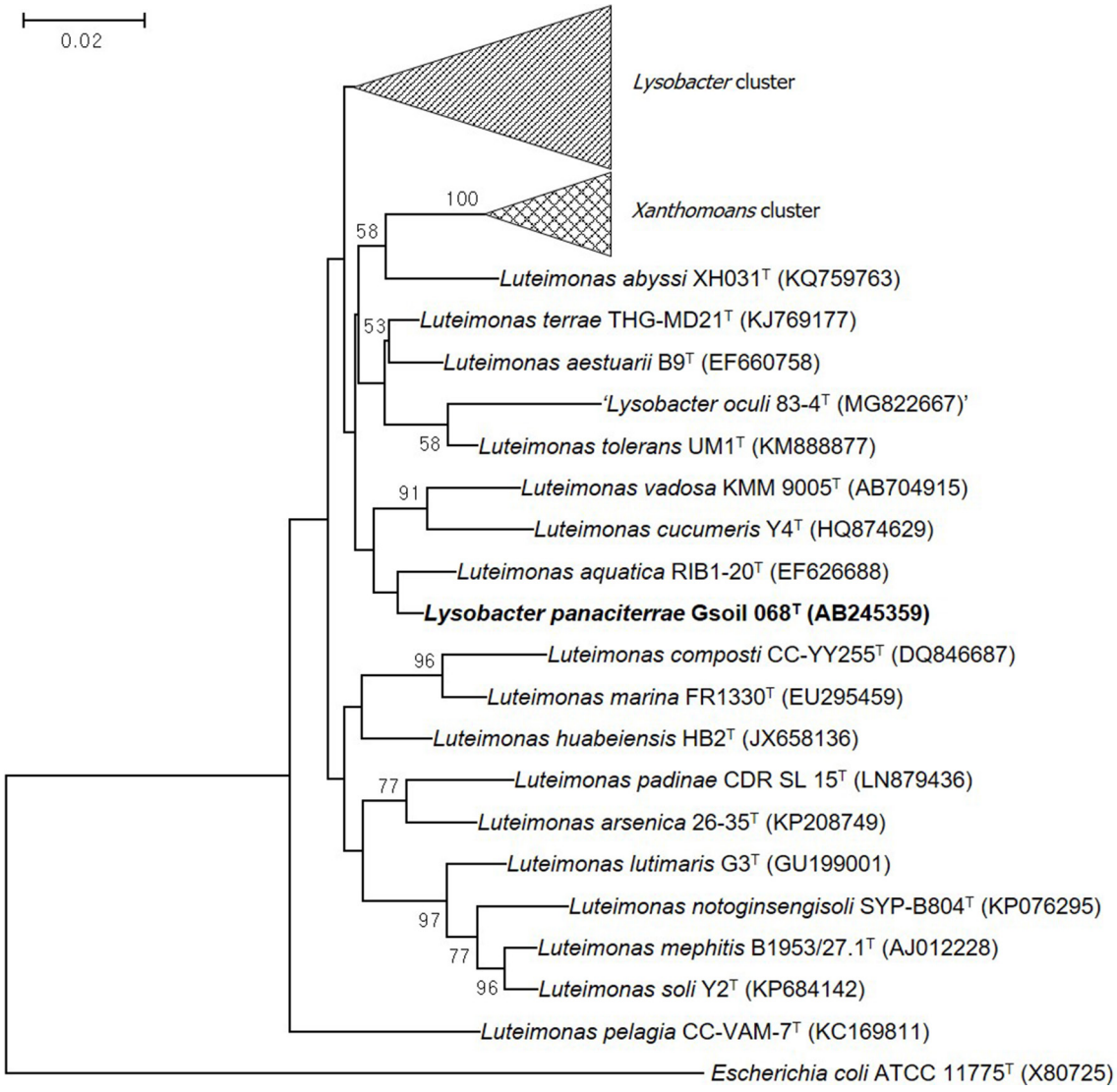

Fig. 3. Neighbour-joining tree based on almost-complete 16S rRNA gene sequence data of the genera *Lysobacter*, *Luteimonas* and *Xanthomonas*, showing the phylogenetic position of strain *Lysobacter panaciterrae* Gsoil 068^T. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 50% support. The 16S rRNA gene sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

but negative for the following enzyme activities: *N*-acetyl-*β*-glucosaminidase, *α*-chymotrypsin, cystine arylamidase, esterase (C4), *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase, trypsin and valine arylamidase; variable for acid phosphatase (positive for type strain), alkaline phosphatase (positive for type strain), esterase lipase (C8) (positive for type strain), *β*-glucosidase and naphthol-AS-BI-phosphohydrolase (positive for type strain) (Table 1). The major fatty acids are summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or $C_{18:1}$ 10-methyl), iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholipid. The predominant respiratory quinone is ubiquinone Q-8. The genomic DNA G+C content of the type strain is 66.8 mol%.

Strains CHu50b-3-2^T (=KCTC 72973^T=CCTCC AB 2019129^T) and CHu40b-3-1 (=KCTC 72972=CCTCC AB 2019128) were isolated from a 67 cm-long sediment core taken from the Daechung Reservoir, Republic of Korea. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains CHu40b-3-1 and CHu50b-3-2^T are MK696267 and MK696268, respectively. The GenBank accession number for the whole genome sequence of type strain CHu50b-3-2^T is VKHQ00000000.

DESCRIPTION OF *LUTEIMONAS PANACITERRAE* COMB. NOV.

Luteimonas panaciterrae (pa.na.ci.ter'rae. N.L. masc. n. *Panax-acis* scientific name for ginseng; L. fem. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil of a ginseng field).

Basonym: *Lysobacter panaciterrae* Ten *et al*., 2009 [39].

The description of *Luteimonas panaciterrae* is the same as that given for *Lysobacter panaciterrae*.

The type strain, Gsoil 068^T (=KCTC 12601^T=DSM 17927^T), was isolated from soil from a ginseng field in Pocheon Province, Republic of Korea.

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Conflicts of interest
The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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