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ORIGINAL PAPER

Lysobacter helvus sp. nov. and *Lysobacter xanthus* sp. nov., isolated from Soil in South Korea

Inhyup Kim · Jiwon Choi · Geeta Chhetri · Taegun Seo

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Abstract Two bacterial strains, designated D10^T and U8^T, were isolated from soil samples from the Dong-angyeong cave and Geommeolle wharf seacoast, Udo-Island, Jeju, South Korea. Both novel bacterial strains are yellow-pigmented, Gram-stain negative, motile by means of monotrichous flagella, short rod shaped and strictly aerobic. A phylogenetic tree was reconstructed based on their 16S rRNA gene sequences, which indicated that these two strains belong to the genus *Lysobacter* within the family *Xanthomonadaceae*. Strain D10^T showed high 16S rRNA gene sequence similarities with *Lysobacter humi* FJY8^T (99.0%), *Lysobacter xinjiangensis* RCML-52^T (98.9%) and *Lysobacter mobilis* 9NM-14^T (97.2%), whereas strain U8^T showed high sequence similarities to *L. mobilis* 9NM-14^T (97.9%), *L. xinjiangensis* RCML-52^T (97.8%), *L. humi* FJY8^T (97.5%) and *Lysobacter bugurensis* ZLD-29^T (97.1%). The 16S rRNA gene sequence similarity between D10^T and U8^T was 97.0%. Strain D10^T showed low DNA–DNA relatedness to U8^T (57.7 ± 3.4%), *L. humi* FJY8^T (48.8 ± 4.3%), *L. xinjiangensis* RCML-52^T (60.1 ± 2.4%) and *L. mobilis* 9NM-14^T (55.9 ± 1.9%). The level of DNA–DNA relatedness for strain U8^T with respect to D10^T, *L. mobilis* 9NM-14^T, *L. xinjiangensis* RCML-52^T, *L. humi* FJY8^T, and *L. bugurensis* ZLD-29^T was 55.5 ± 0.5%, 54.5 ± 2.1%, 58.1 ± 0.8%, and 51.9 ± 3.4%, respectively. The major polar lipids for both strains were identified as diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. The major cellular fatty acids for both strains were identified as iso-$C_{15:0}$, iso-$C_{16:0}$ and summed feature 9 (iso-$C_{17:1}$ ω9c/$C_{16:0}$ 10-methyl), and ubiquinone (Q-8) as the only isoprenoid quinone for both strains. The DNA G + C contents of the strains D10^T and U8^T were determined to be 70.2 mol% and 70.6 mol%. On the basis of phenotypic, genotypic, chemotaxonomic, and phylogenetic analysis, both strains D10^T and U8^T represent a novel species in the genus *Lysobacter*, for which the names *Lysobacter helvus* sp. nov. and *Lysobacter xanthus* sp. nov. are proposed, respectively. The type strain of *L. helvus* is D10^T (= KCTC 62111^T = JCM 32364^T) and the type strain of *L. xanthus* is U8^T (= KCTC 62112^T = JCM 32365^T).

Keywords DNA relatedness · *Lysobacter* · Taxonomy · Strictly aerobic · Phylogenetic analysis

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I. Kim · J. Choi · G. Chhetri · T. Seo (✉)
Department of Life Science, Dongguk University-Seoul, Goyang 10326, South Korea
e-mail: tseo@dongguk.edu

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Introduction

The genus *Lysobacter* was first proposed by Christensen and Cook (1978) for non-fruiting, gliding bacteria. According to the LPSN (www.bacterio.net/lysobacter.html), the genus *Lysobacter* currently comprises nearly 50 species with validly published names including the recently described species *Lysobacter psychrotolerans* (Luo et al. 2019), *Lysobacter silvisoli* (Zhang et al. 2018), *Lysobacter silvestris* (Margesin et al. 2018), and *Lysobacter tabacisoli* (Xiao et al. 2018). *Lysobacter* species have been found in diverse environments such as soil (Singh et al. 2015a, b; Srinivasan et al. 2010; Zhang et al. 2011), plants (Singh et al. 2015a, b), fresh water (Fukuda et al. 2013; Siddiqi and Im 2016), and estuary sediments (Jeong et al. 2016). Most of the species belonging to the genus *Lysobacter* are Gram-negative rods that contain a high G + C content in their DNA of 61.7 to 70.7 mol% (Reichenbach 2006). Also, they contain ubiquinone Q-8 as the predominant respiratory quinone. A large number of *Lysobacter* do not contain flexirubin-type pigments although a few of them show positive reactions in tests for these (Lin et al. 2014; Singh et al. 2015a, b; Ngo et al. 2015).

The present study is a continuation of our bacterial diversity and bioprospecting studies in different regions of South Korea. The characterisation and classification of two strains, designated D10^T and U8^T, that each represent a novel species within the genus *Lysobacter* is reported.

Materials and methods

Isolation of bacterial strains and culture conditions

Strains D10^T and U8^T were isolated from soil samples taken from the Dong-angyeong cave (33°29′48.4″N 126°58′00.1″E) and Geommeolle wharf sea-coast (33°29′47.3″N 126°58′06.9″E), Udo-Island, Jeju, South Korea. For isolation, 10 g of each soil sample was immersed in 10 ml of sterile 0.85% saline and serially diluted by a standard dilution method. A 100-µl aliquot of the resulting suspensions was spread onto Reasoner's 2A agar (R2A agar; Difco) and then incubated at 30 °C for 2 days. Selection of the colonies was subsequently carried out and strains D10^T and U8^T were isolated. The isolates were routinely cultured on R2A agar at 30 °C and stored in glycerol suspensions (25%, w/v in distilled water) at − 80 °C. *Lysobacter bugurensis* ZLD-29^T and *Lysobacter xinjiangensis* RCML-52^T were obtained from the Korean Collection for Type Cultures (KCTC/Daejeon, South Korea), *Lysobacter mobilis* 9NM-14^T was obtained from the German Collection of Microorganisms and Cell Cultures (DSM/Braunschweig, Germany) and *Lysobacter humi* FJY8^T was obtained from our previous study (Lee et al. 2017). All reference strains except *L. bugurensis* (cultured on half strength Marine agar at 30 °C for 2 days) were cultured under the same conditions, as described above, for comparative testing.

16S rRNA gene sequencing and phylogenetic analysis

The 16S rRNA gene sequences of strains D10^T and U8^T were amplified using the universal bacterial primer sets 27F, 785F, 800R, and 1492R (Weisburg et al. 1991). Assembly and compilation to obtain the full sequences of the 16S rRNA genes were performed using the SeqMan software (DNASTAR Inc., Madison, WI, USA). To compare the 16S rRNA gene sequences with those of other taxa, the EzTaxon-e service (http://www.ezbiocloud.net/; Yoon et al. 2017) and NCBI BLAST searches (Altschul et al. 1997) were used. Multiple alignments were performed with 16S rRNA gene sequences of related taxa using the ClustalW program (Larkin et al. 2007) and the gaps were edited with the BioEdit program (Hall 1997). The phylogenetic trees were constructed using the software MEGA 7 (Kumar et al. 2016) by computing evolutionary distances with the Kimura 2-parameter method (Kimura 1980) in the neighbor-joining algorithm (Saitou and Nei 1987). For comparisons of the neighbor-joining phylogenetic tree, the maximum likelihood tree based on the Jukes-Cantor model and the maximum-parsimony tree using the min-mini heuristic method were constructed using MEGA 7. The bootstrap values were calculated to show the probability of each node (1000 replicates) (Felsenstein 1985). The *Bacillus subtilis* DSM 10^T (AJ276351) sequence was used as an out-group.

DNA G + C mol% and DNA–DNA hybridization of genomic analysis

Total genomic DNA was extracted and purified to evaluate the G + C mol% content and DNA–DNA hybridizations, following the method described by Ausubel et al. (1995). The DNA G + C content of strains D10^T and U8^T were determined using a simple fluorimetric method (Gonzalez and Saiz-Jimenez 2002) with SYBR Green 1 (Life Technologies, Waltham, USA) and a real-time PCR thermocycler (Roter-Gene Q, Qiagen, Hilden, Germany). The genomic DNA of *Bacillus licheniformis* KACC 10476^T, *B. subtilis* subsp. *subtilis* KACC 17796^T, *Corynebacterium glutamicum* KACC 20786^T, *Escherichia coli* KACC 14818^T, *Lactococcus lactis* subsp. *lactis* KACC 13877^T, *Micrococcus luteus* KACC 13377^T and *Pseudomonas aeruginosa* ATCC 15442^T were used as references for the calibration.

The DNA–DNA hybridization analysis was performed to determine the relatedness of the novel isolates and related taxa based on denaturation principles and the equations introduced by De Ley et al. (1970) and Gillis et al. (1970) as well as an optimised procedure delineated by Loveland-Curtze et al. (2011). The DNA–DNA hybridization values were assessed with the fluorimetric method using real-time PCR. Strains *L. humi* FJY8^T, *L. mobilis* 9NM-14^T and *L. xinjiangensis* RCML-52^T were selected as the close relatives to the novel strain D10^T. Strains *L. bugurensis* ZLD-29^T, *L. humi* FJY8^T, *L. mobilis* 9NM-14^T and *L. xinjiangensis* RCML-52^T were selected as the close relatives to the novel strain U8^T. All assays were carried out in triplicate.

Phenotypic and biochemical analysis

The Gram reaction was assessed using the non-staining KOH method (3% KOH; Buck 1982). The cell morphology of strains D10^T and U8^T were observed by transmission electron microscopy (LIBRA120, Carl Zeiss, Germany) and the 0.4% agar stabbing (Tube method) technique was used to investigate cell motility at 30 °C for 7 days. Growth under anaerobic conditions was assessed using the GasPak jar (BBL, Cockeysville, MD, USA) at 30 °C for 7 days. Catalase activity was observed via the detection of oxygen bubble production using a 3% (v/v) aqueous hydrogen peroxide solution and oxidase activity was observed via the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (BioMérieux). Growth on tryptic soy agar (Difco), nutrient agar (Difco), Luria–Bertani agar (Difco), marine agar (Difco), and MacConkey agar (Difco) was investigated at 30 °C for 7 days with the purpose of determining the optimal media. The pH range of the growth was measured for 7 days in R2A broth in four buffers ranging from pH 5.0–11.0 at intervals of 1.0 pH unit. The buffers evaluated were acetate buffer (pH 5.0), phosphate buffer (pH 6.0–8.0), Tris buffer (pH 9.0–10.0), and Na_2HPO_4–NaOH buffer (pH 11.0) at final concentrations of 50 mM. The NaCl tolerance was tested by culturing the strains in R2A broth containing various concentrations of NaCl in the range of 0–4% at 0.5% concentration intervals for 7 days. Growth were assessed at temperatures of 4, 10, 15, 25, 30, 37, and 42 °C on R2A agar for 7 days. The presence of flexirubin-type pigments was investigated using 20% (w/v) KOH solution (Fautz and Reichenbach 1980).

The tests for hydrolysis were performed on R2A agar containing (w/v): casein (2% skim milk, Biopure); chitin (1%, Sigma); starch (1%, Sigma); Tween 20 (0.01% $CaCl_2{\cdot}2H_2O$, Sigma and 1% Tween 20, Biopure); Tween 80 (0.01% $CaCl_2{\cdot}2H_2O$, Sigma and 1% Tween 80, Biopure); and DNA (DNase agar, Difco). To identify the biochemical characteristics of the strains, API ZYM, API 20NE, and API 50CH systems (BioMérieux, Craponne, France) were used in accordance with the manufacturer's instructions.

Chemotaxonomic analyses

The polar lipids of strains D10^T and U8^T were extracted (Minnikin et al. 1984) using cells harvested from R2A broth for 2 days at 30 °C. The polar lipid extracts were examined via two-dimensional thin-layer chromatography (TLC) using two different development solvents with ratios of 65:25:4 (v/v/v) chloroform to methanol to water and 80:15:12:4 (v/v/v/v) chloroform to acetic acid to methanol to water. The results were visualised by spraying with phosphomolybdic acid (Sigma), molybdenum blue spray reagent (Sigma), α-naphthol and ninhydrin (Komagata and Suzuki 1987). Cells grew on the R2A agar at 30 °C for 2 days were used for the fatty acid analysis. Cellular fatty acids were acquired by saponification, methylation and extraction as previously described

(Kuykendall et al. 1988). The Sherlock Microbial Identification System V6.01 (MIS, database TSBA6, MIDI Inc., Newark, DE, USA) was used for analysis of the extracts. Isoprenoid quinones were extracted with a ratio of 2:1 (v/v) chloroform to methanol and analysed using high-performance lipid chromatography (HPLC) as described previously (Hiraishi et al. 1996; Collins and Jones 1981).

Results and discussion

Phylogenetic analysis

The nearly complete 16S rRNA gene sequences of strains D10^T and U8^T consist of 1479 bp and 1475 bp, respectively (Genbank accession numbers MF417836 and MF417835). According to the EzTaxon server, based on their 16S rRNA gene sequence, strain D10^T is closely related to *L. humi* FJY8^T (99.0%), *L. xinjiangensis* RCML-52^T (98.8%) and *L. mobilis* 9NM-14^T (97.1%), whilst strain U8^T shows high similarity to *L. mobilis* 9NM-14^T (97.8%), followed by *L. xinjiangensis* RCML-52^T (97.7%), *L. humi* FJY8^T (97.4%) and *L. bugurensis* ZLD-29^T (97.0%). The 16S rRNA gene sequence similarity between strains D10^T and U8^T is 97.0%. In addition, the phylogenetic tree reconstructions using the neighbor-joining tree compared with the maximum-likelihood and maximum parsimony tree algorithms indicated that strains D10^T and U8^T form distinct lineages associated with these members of the genus *Lysobacter* of the family *Xanthomonadaceae* (Fig. 1; Supplementary data Fig. S1).

DNA G + C mol% of genomic analysis and DNA–DNA hybridization

The G + C content of strains D10^T and U8^T was determined to be 70.2 mol% and 70.6 mol% which is high, although within the range for other species within the genus *Lysobacter*. Strain D10^T showed a low DNA–DNA relatedness to U8^T (57.7 ± 3.4%), *L. humi* FJY8^T (48.8 ± 4.3%), *L. xinjiangensis* RCML-52^T (60.1 ± 2.4%) and *L. mobilis* 9NM-14^T (55.9 ± 1.9%). The level of DNA–DNA relatedness for strain U8^T with respect to D10^T, *L. mobilis* 9NM-14^T, *L. xinjiangensis* RCML-52^T, *L. humi* FJY8^T and *L. bugurensis* ZLD-29^T was 55.5 ± 0.5%, 54.5 ± 2.1%, 58.1 ± 0.8%, and 51.9 ± 3.4%, respectively. These values are below the 70% cut-off point for identification of genomic prokaryotic species (Wayne et al. 1987; Stackebrandt and Goebel 1994). DNA–DNA relatedness between the two strains and their close relatives demonstrated that strains D10^T and U8^T differ genetically from *Lysobacter* type strains at the species level. This finding supports the conclusion that strains D10^T and U8^T each represent a novel species of genus *Lysobacter* and they are genotypically distant from each other.

Phenotypic and biochemical characteristics

Both strains D10^T and U8^T were found to be Gram-negative, aerobic, motile by means of monotrichous flagella, and short rod or rod-shaped (Supplementary data Fig. S2). Morphological observations of the colonies of strain D10^T on R2A agar revealed that they were pale yellow, raised and smooth, while the colonies of strain U8^T on R2A agar were observed to be pale yellow, raised and slightly sticky after incubation at 30 °C for 2 days. Cells of strain D10^T were determined to have an approximate cell size of 0.3–0.6 μm wide and 0.5–1.7 μm long, and those of strain U8^T to be 0.3–0.6 μm wide and 0.8–1.5 μm long. Strains D10^T and U8^T were found to grow well on R2A agar and nutrient agar, weakly grow on tryptic soy agar, and do not grow on MacConkey agar, Luria–Bertani agar, marine agar and half-strength marine agar. The growth of strain D10^T on R2A agar was found to occur over a temperature range of 25–42 °C (optimum 30 °C), pH range of 6–10 (optimum 7), and at 0% concentration of NaCl, while strain U8^T was found to grow on R2A over a temperature range of 25–42 °C (optimum 30 °C), pH range of 6–11 (optimum 7) and at 0% concentration of NaCl. Both strains D10^T and U8^T were observed to be catalase positive. Strains D10^T and U8^T were found to hydrolyse casein, chitin, starch, Tween 20 and Tween 80 but were unable to hydrolyse DNA. The tests for oxidase activity was positive for strain D10^T and negative for strain U8^T. Comparisons of the characteristics between strains D10^T, U8^T and other selected species of the genus *Lysobacter* are detailed in Table 1.

In the API ZYM tests, strain D10^T was found to give positive results for the production of alkaline phosphatase, esterase (C4), esterase (C8), lipase (C14), leucine arylamidase, trypsin, acid phosphatase,

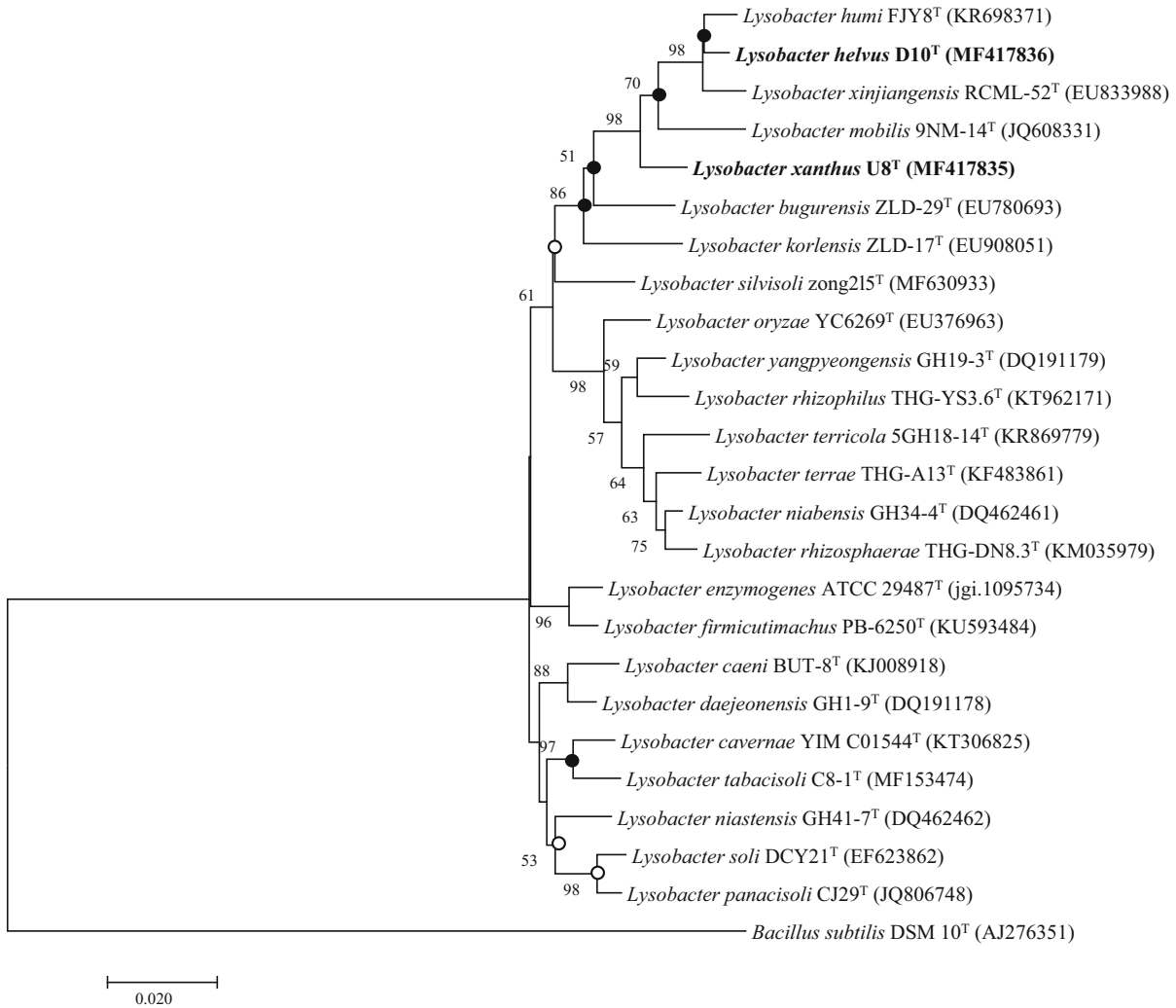

Fig. 1 Neighbor-joining tree reconstructed using 16S rRNA gene sequences showing the phylogenetic relationships between strains D10^T and U8^T and related taxa. Bootstrap values are shown as percentages of 1000 replicates (only values > 50%). Empty circles indicate the corresponding nodes were recovered using the maximum-likelihood algorithm. Filled circles indicate the corresponding nodes were recovered in trees generated with the maximum-parsimony algorithm. The scale bar represents 0.02 substitutions per nucleotide position

naphthol-AS-BI-phosphohydrolase, and α-glucosidase (starch hydrolysis). Negative results were observed for the production of valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase (OPNG), β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. In the API 20NE tests, strain D10^T was found to give strong positive reactions for D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, gluconate, adipate, L-malate, citrate and weak reactions for *N*-acetyl-D-glucosamine. A negative reaction was observed for nitrate reduction, production of indole, production of acid from glucose, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), caprate and phenyl acetate. Strain D10^T was found to utilise the following as sole carbon and energy source: D-glucose, esculin ferric citrate, D-maltose, D-lactose, *d*-trehalose, D-melezitose, amidon, 5-ketogluconate (potassium) and D-ribose (weakly). Strain D10^T cannot utilise the following as sole carbon and energy source: glycerol, erythritol, D- and L-

Table 1 Characteristics differentiating strains D10^T and U8^T from phylogenetically related species of the genus *Lysobacter*

Characteristics	Species					
	1	2	3	4	5	6
Isolation source	Soil	Soil	Soil	Soil	Zinc ore	Soil
Oxidase activity	+	–	+	+	–	+
Range of growth						
NaCl (%, w/v)	0	0	0–2	0–0.5	0–0.5	0–2
pH	6–10	6–11	7–10	6–11	6–9	6–11
Temperature (°C)	25–42	25–42	25–37	25–42	15–37	10–42
Motility	+	+	+	–	+	–
Hydrolysis of						
Chitin	+	+	+	–	–	+
CM-cellulose	+	+	+	–	–	–
Starch	+	+	+	–	–	–
Enzyme activities						
Lipase (C14)	+	w	–	+	+	+
Valine arylamidase	–	w	w	+	+	+
Cystine arylamidase	–	–	–	–	–	+
α-Chymotrypsin	–	–	+	–	–	+
α-Glucosidase	+	+	–	–	–	–
Assimilation of						
Arginine dihydrolase	–	+	+	+	+	+
Urease	–	+	+	+	+	+
β-Glucosidase	–	–	+	+	–	–
Protease	–	+	+	+	+	+
D-Glucose	+	–	+	+	+	+
D-Mannitol	+	–	–	+	w	+
N-Acetyl-D-glucosamine	w	+	–	+	w	+
L-Malate	+	w	–	+	+	+
Phenyl acetate	–	+	w	–	w	+
Acid production						
D-Ribose	w	–	+	+	+	–
D-Glucose	+	–	–	+	–	+
Dulcitol	–	+	–	–	–	–
D-Maltose	+	+	–	+	–	–
D-Lactose	+	–	–	–	–	–
D-Trehalose	+	–	–	+	–	–
D-Melezitose	+	–	–	+	–	–
Amidon	+	–	–	+	–	–
D-Tagatose	–	–	–	+	+	–
L-Fucose	–	–	–	+	+	–
2-Ketogluconate	–	+	+	+	w	–
DNA G + C content (mol%)	70.2	70.6	(68.2)	(68.0 ± 0.4)	(70.7 ± 0.1)	(69.7)

Strains: 1, Strain D10^T; 2, Strain U8^T; 3, *Lysobacter bugurensis* ZLD-29^T; 4, *Lysobacter humi* FJY8^T; 5, *Lysobacter mobilis* 9NM-14^T; 6, *Lysobacter xinjiangensis* RCML-52^T. All data from this study except that indicated in the parenthesis. +, Positive; –, Negative; w, weakly positive

arabinose, D- and L-xylose, D-adonitol, methyl-β-D-xylose, D-galactose, D-fructose, D-mannose, L-sorbose, L-Rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-melobiose, D-saccharose

(sucrose), inulin, D-raffinose, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, gluconate (potassium) and 2-ketogluconate (potassium).

In the API ZYM tests strain U8^T gives positive results found for the production of alkaline phosphatase, esterase (C4), esterase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase (starch hydrolysis) and weakly positive results were observed for lipase (C14), and valine arylamidase. Negative results were found for the production of cystine arylamidase, α-chymotrypsin, α-galactosidase, *β*-galactosidase (OPNG), *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase and α-fucosidase. In the API 20NE tests, strain U8^T gave positive reactions for arginine dihydrolase, urease, protease (gelatin hydrolysis), L-arabinose, *N*-acetyl-D-glucosamine, D-maltose, gluconate, adipate, and phenyl acetate, and a weakly positive reaction was observed for D-mannose, L-malate and citrate. Negative reactions were detected for nitrate reduction, production of indole, production of acid from glucose, *β*-glucosidase (esculin hydrolysis), *β*-galactosidase (PNPG), D-glucose, D-mannitol and caprate. Strain U8^T was found to utilise the following as sole carbon and energy sources: dulcitol, esculin ferric citrate, D-maltose, 2-ketogluconate (potassium) and 5-ketogluconate (potassium). However, the following are not utilised: glycerol, erythritol, *d*- and *l*-arabinose, *d*-ribose, *d*- and *l*-xylose, *d*-adonitol, methyl-*β*-D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-Rhamnose, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, amygdalin, arbutin, salicin, *d*-cellobiose, D-lactose, D-melobiose, D-saccharose (sucrose), *d*-trehalose, inulin, D-melezitose, D-raffinose, amidon, glycogen, xylitol, gentiobiose, *d*-turanose, D-lyxose, D-tagatose, *d*- and *l*-fucose, *d*- and *l*-arabitol, and gluconate (potassium).

Chemotaxonomic characteristics

The TLC analysis revealed that the primary polar lipids shared between the strains D10^T and U8^T were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol, as in the reference *Lysobacter* strains (Supplementary data Fig. S3). In addition, two unidentified phosphoglycolipids and two phospholipids were detected as minor polar lipids for strain U8^T, and their chromatogram positions, differentiate strain U8^T from the reference strains (Supplementary data Fig. S3). The presence of three unidentified phosphoglycolipids, an unidentified aminophosphoglycolipid, five unidentified phospholipids, an unidentified aminolipid, and two pigments in strain D10^T differentiates it from closely related species.

Both strains were found to contain iso-branched $C_{16:0}$, summed feature 9 (comprising iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl) and iso-branched $C_{15:0}$ as predominant fatty acids (Table 2). The overall fatty acid profiles of strains D10^T and U8^T were similar to those of the type strains of phylogenetically related *Lysobacter* species (Table 2). However, qualitative

Table 2 Cellular fatty acid composition (%) of strains D10^T, U8^T and related type strains of the genus *Lysobacter*

Fatty acid	1	2	3	4	5	6
Branched-chain						
iso-$C_{12:0}$	1.1	0.9	TR	0.7	–	0.7
iso-$C_{14:0}$	4.2	1.7	–	0.9	–	0.9
iso-$C_{15:0}$	16.4	17.8	3.7	13.1	19.8	24.7
anteiso-$C_{15:0}$	1.3	TR	–	0.7	–	–
iso-$C_{16:0}$	32.2	32.1	3.4	29.2	7.9	26.3
iso-$C_{16:1}$ H	3	2.9	–	1.7	–	0.6
iso-$C_{17:0}$	–	1.6	5.6	3.7	10.3	7
iso-$C_{18:0}$	–	TR	–	0.7	–	0.7
Summed features[a]						
1	1.1	1.8	–	0.5	–	–
5	–	–	–	–	–	0.9
8	–	–	3.4	–	0.5	–
9	19.5	26.1	23.9	28.8	42.1	20.5

Strains: 1, Strain D10^T; 2, Strain U8^T; 3, *Lysobacter bugurensis* ZLD-29^T; 4, *Lysobacter humi* FJY8^T; 5, *Lysobacter mobilis* 9NM-14^T; 5, *Lysobacter xinjiangensis* RCML-52^T. All data from this study. Data are expressed as a percentage of total fatty acids. Fatty acids amounting to $< 0.5\%$ in all strains are omitted. TR, Trace amount ($< 0.5\%$); –, Not detected

[a]Summed features represent groups of two fatty acids that could not be separated by HPLC with the Microbial Identification System (MIDI, Inc.). Summed feature 1 contains $C_{13:0}$ 3-OH and/or $C_{15:1}$ i H. Summed feature 5 contains $C_{18:2}$ *ω6,9c* and/or ante-$C_{18:0}$. Summed feature 8 contains $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*. Summed feature 9 contains iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl

and quantitative differences in the proportions of the fatty acids could be observed between the two isolates and the reference strains. The only isoprenoid quinone of strains D10^T and U8^T was found to be ubiquinone-8, which is also the major respiratory quinone in other species of the genus *Lysobacter*.

Taxonomic conclusion

Based on the phylogenetic analyses, strains D10^T and U8^T were found to be affiliated with members of the genus *Lysobacter* in the family *Xanthomonadaceae*. Strains D10^T and U8^T each represent two novel species of the genus *Lysobacter*, as supported by DNA–DNA relatedness studies. In addition, phenotypic and biochemical collectively support the fact that strains D10^T and U8^T are distinguishable, whilst chemotaxonomic analyses are consistent with their affiliation with the genus *Lysobacter*. The phenotypic characterisation presented in Table 1 differentiates strains D10^T and U8^T as a separate species. The predominant respiratory quinone was Q-8, as reported for the major respiratory quinone of all members of the genus *Lysobacter*. The major fatty acids of type strains D10^T and U8^T were iso-$C_{16:0}$, summed feature 9 (comprising iso-$C_{17:1}$ ω9c and/or $C_{16:0}$ 10-methyl), and iso-$C_{15:0}$. Based on the results presented in this study, it is proposed that D10^T and U8^T represents novel members in the genus *Lysobacter*, for which the names *Lysobacter helvus* sp. nov. and *Lysobacter xanthus* sp. nov., are proposed, respectively. The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumbers for D10^T and U8^T are TA00857 and TA00858.

Description of *Lysobacter helvus* sp. nov.

Lysobacter helvus (hel'vus. L. masc. adj. *helvus*, honey-yellow, referring to the colony colour).

Cells are Gram-negative, aerobic and rod-shaped (0.3–0.6 × 0.5–1.7 μm) when grown on R2A agar at 30 °C for 2 days. Growth occurs on tryptic soy agar, nutrient agar and R2A agar (with R2A agar as the optimal medium). The temperature range for growth is 25–42 °C, pH range is 6.0–10.0 and NaCl is not tolerated. Optimal growth occurs at 30 °C, pH 7, and at 0% NaCl (w/v). The polar lipid profile contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, three unidentified phosphoglycolipids, an unidentified aminophosphoglycolipid, five unidentified phospholipids, an unidentified aminolipid and two pigments. The major fatty acids are iso-branched $C_{16:0}$, summed feature 9 (comprising iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl) and iso-branched $C_{15:0}$. The predominant quinone is Q-8. The G + C content of the genomic DNA of the type strain is 70.2 mol%.

The type strain D10^T (= KCTC 62111^T = JCM 32364^T) was isolated from a soil sample from the Dong-angyeong cave, Udo-Island, Jeju, South Korea. The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain D10^T is MF417836.

Description of *Lysobacter xanthus* sp. nov.

Lysobacter xanthus (xan'thus. N.L. masc. adj. *xanthus* [from Gr. masc. adj. *xanthos*] yellow).

Cells are Gram-negative, aerobic and rod-shaped (0.3–0.6 × 0.8–1.5 μm) when grown on R2A agar at 30 °C for 2 days. Growth occurs on tryptic soy agar, nutrient agar and R2A agar (with R2A agar being the optimal medium). The temperature range for growth is 25–42 °C, pH range is 6–11 and NaCl is not tolerated. Optimal growth is observed at 30 °C, pH 7 and 0% NaCl (w/v). Positive for catalase and negative for oxidase and flexirubin-type pigments. Casein, chitin, starch, Tween 20 and Tween 80 are hydrolysed but not DNA. The polar lipid profile contains phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol as major polar lipids, and two phosphoglycolipids, and two phospholipids as minor polar lipids. The major fatty acids are iso-branched $C_{16:0}$, summed feature 9 (comprising iso-$C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl), and iso-branched $C_{15:0}$. The predominant quinone is Q-8. The G + C content of the genomic DNA of the type strain is 70.6 ± 0.3 mol%.

The type strain U8^T (= KCTC 62112^T = JCM 32365^T) was isolated from a soil sample from the Geommeolle wharf sea-coast, Udo-Island, Jeju, South Korea. The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain U8^T is MF417835.

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Conflict of interest The authors declare that they have no conflicts of interest.

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