INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

NOTE

Lee *et al.*, *Int J Syst Evol Microbiol* 2017;67:951–955

DOI 10.1099/ijsem.0.001722

MICROBIOLOGY SOCIETY

Lysobacter humi sp. nov., isolated from soil

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Abstract

A yellow-pigmented and strictly aerobic bacterial strain, designated FJY8^T, was isolated from the soil of Goyang, South Korea. The cells of FJY8^T were Gram-reaction-negative, non-motile rods. Colonies were circular, convex and transparent. Strain FJY8^T grew optimally at 30 °C, with 0 % (w/v) NaCl and at pH 8. Phylogenetic analysis of the 16S rRNA gene sequence of FJY8^T revealed a clear affiliation of this bacterium to the family *Lysobacteraceae*, and it was related to members of the genus *Lysobacter*, with *Lysobacter xinjiangensis* KCTC 22558^T being its closest relative (98.7 % sequence similarity). The DNA G+C content was 68.0±0.4 mol%. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol were identified as the major polar lipids, and an unidentified phospholipid and two unidentified aminophospholipids were also detected as the minor polar lipids. The major fatty acids were iso-$C_{16:0}$, summed feature 9 (iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl) and iso-$C_{15:0}$. Only ubiquinone-8 (Q-8) was detected as the isoprenoid quinone. DNA–DNA hybridization values of strain FJY8^T with *Lysobacter xinjiangensis* RCML-52^T and *Lysobacter mobilis* 9NM-14^T were 55.8±2.0 and 45.2±4.8 %, respectively. On the basis of DNA–DNA hybridization, phylogenetic distinctiveness, and some physiological and biochemical tests, strain FJY8^T (=KCTC 42810^T=JCM 31019^T) represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter humi* sp. nov. is proposed.

Lysobacter is the type genus of the family *Lysobacteraceae*, members of which contain ubiquinone Q-8 as the predominant respiratory quinone. The genus *Lysobacter* was first proposed by Christensen and Cook [1] for non-fruiting bacteria with high guanine-plus-cytosine (G+C) contents in their deoxyribonucleic acids. According to LPSN (www.bacterio.net/lysobacter.html), at the time of writing the genus *Lysobacter* comprises 39 species with validly published names including the recently described species *Lysobacter firmicutimachus* [2], *Lysobacter erysipheiresistens* [3], *Lysobacter hankyongensis* and *Lysobacter sediminicola* [4]. Most of the species within the genus *Lysobacter* are frequently isolated from soil [5–9]. The present work is a continuation of our bacterial diversity and bioprospecting studies in different regions of South Korea. We report the characterization of a strain designated FJY8^T that represents a novel species within the family *Lysobacteraceae*.

Strain FJY8^T was isolated from a soil sample collected in an arid area in Goyang, Gyeonggi Province, South Korea. For isolation, 5 g soil was suspended with 10 ml sterile NaCl (0.85 %, w/v) and serially diluted with the standard dilution technique. A 100-µl aliquot of each dilution was plated onto R2A agar (MB cell) and then incubated at 30 °C for 3 days. Single colonies were then chosen for further purification. Purified colonies were stored at −70 °C in liquid R2A broth (MB cell) supplemented with 25 % (v/v) glycerol. For morphological and biochemical characterization, strain FJY8^T was grown on R2A agar. *Lysobacter xinjiangensis* RCML-52^T and *Lysobacter mobilis* 9NM-14^T were respectively obtained from the KCTC (Daejeon, South Korea) and DSM (Braunschweig, Germany).

The nearly complete 16S rRNA gene sequence of strain FJY8^T was amplified by colony PCR using the 27F, 785F, 800R and 1492R universal primers [10], and sequencing was performed by Solgent (Daejeon, South Korea). The 16S rRNA gene sequence of strain FJY8^T was assembled with SeqMan software (DNASTAR). The sequences obtained were compared with those of close relatives by BLAST searches [11] and by using the EzTaxon-e server (www.ezbiocloud.net/eztaxon/; [12]). Sequences were aligned by CLUSTAL W [13] within the BioEdit Sequence Alignment Editor, version 7.2.5 [14], and phylogenetic trees were reconstructed using MEGA version 6.06 [15, 16], by the neighbour-joining method with the Kimura two-parameter model [17, 18]. Bootstrap values were calculated based on 1000 replicates [19]. The min-mini heuristic algorithm was applied in maximum-parsimony analysis to compare with the neighbour-joining phylogenetic tree [20]. The sequence of *Bacillus subtilis* DSM 10^T was used as an outgroup. In the neighbour-joining phylogenetic tree, strain FJY8^T clearly

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Keywords: DNA-DNA relatedness; *Lysobacter*; taxonomy.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FJY8^T is KR698371.
One supplementary table and two supplementary figures are available with the online Supplementary Material.

belonged to the lineage defined by the genus *Lysobacter* of the family *Lysobacteraceae* (Fig. 1). Also, strain FJY8^T was in the same cluster as *Lysobacter xinjiangensis* RCML-52^T. Sequence comparisons based on the 16S rRNA gene revealed that the strain FJY8^T was most closely related to *Lysobacter xinjiangensis* RCML-52^T (98.7 % sequence similarity), *Lysobacter mobilis* 9NM-14^T (97.4 %), *Lysobacter bugurensis* ZLD-29^T (95.8 %) and *Lysobacter korlensis* ZLD-17^T (95.4 %). The 16S rRNA gene sequence comparisons by means of NCBI BLAST searches showed that strain FJY8^T had 93.9~98.7 % sequence similarity with type strains of validly named species of the genus *Lysobacter*.

The Gram-staining reaction was determined following the non-staining method of Buck [21]. Morphological characteristics of strain FJY8^T were investigated by transmission electron microscopy (LIBRA 120, Carl Zeiss), and the 0.4 % agar method was used to determine cell motility. The NaCl tolerance of the isolate was assessed by growing the strain in R2A medium supplemented with 0–10 % (w/v) NaCl, in increments of 0.5 %, for 10 days at 30 °C. The ability to grow in acid and alkaline media was assessed by adjusting media to pH 4–11 with acetic acid/sodium acetate (pH 4.0), 10 mM MES (pH 5.0–6.0), 10 mM Tris (pH 7.0–9.0) and sodium carbonate/sodium bicarbonate (pH 10.0–11.0). Growth at 30 °C was observed for 10 days on Luria–Bertani (Difco) agar, MacConkey agar (Difco), marine agar (Difco), nutrient agar (Difco) and tryptic soy agar (Difco). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 42 and 45 °C) was assessed using R2A agar (MB cell) after a 10-day incubation period. Tetramethyl-*p*-phenylenediamine and H_2O_2 (3 %, w/v) were used to test for oxidase and catalase activity, respectively. A GasPak jar was used for anaerobic incubation (BBL). To identify additional biochemical characteristics, the API 20NE, API 32GN and API ZYM systems (bioMérieux) were used according to the manufacturer's instructions. After 2 days of incubation at 30 °C, strain FJY8^T cultured on R2A agar yielded colonies that were circular, convex, transparent and yellow-coloured. The cells of strain FJY8^T were Gram-reaction-negative, non-motile, strictly aerobic and rod-shaped, with dimensions in the range 0.7–1.0×1.3–1.5 µm (see Fig. S1 available in the online Supplementary Material). The phenotypic characteristics of strain FJY8^T are given in the species description, and a comparison with the characteristics of other species of the genus *Lysobacter* is presented in Table 1.

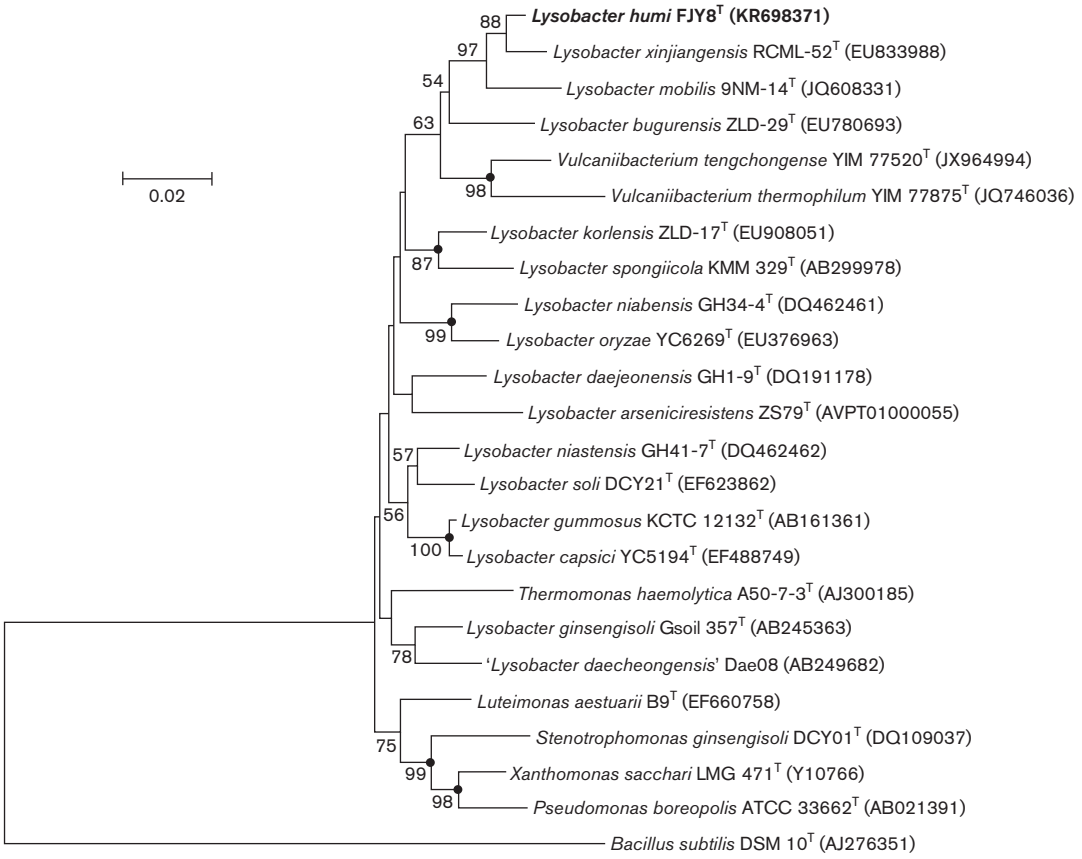

Fig. 1. Neighbour-joining phylogenetic tree reconstructed using the 16S rRNA gene sequences of strains FJY8^T and related taxa. Numbers at branch nodes present bootstrap values (>50 %) obtained as percentages of 1000 replicates. Filled circles indicate the corresponding nodes recovered by using the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position.

Table 1. Characteristics that differentiate strain FJY8^T from phylogenetically related species of the genus *Lysobacter*

Strains: 1, FJY8^T (data from this study); 2, *Lysobacter xinjiangensis* RCML-52^T (data from this study); 3, *Lysobacter mobilis* 9NM-14^T (data from this study); 4, *Lysobacter xinjiangensis* ZLD-29^T (data from [27]); 5, *Lysobacter xinjiangensis* ZLD-17^T (data from [27]). +, Positive; −, negative.

Characteristic	1	2	3	4	5
Ranges for growth					
Temperature (°C)	20–42	20–42	15–37	10–37	10–37
NaCl (%, w/v)	0–0.5	0–2	0–1	0–3	0.5–4
pH	7–9	6–10	6–8	6–11	6–11
Oxidase	+	+	−	+	+
Nitrate reduction	−	−	−	+	+
Hydrolysis of aesculin	+	+	−	+	+
Enzyme activities					
Cystine arylamidase	−	+	+	−	−
Lipase (C14)	+	+	−	−	−
Trypsin	+	+	+	−	+
Valine arylamidase	+	+	+	−	−
α-Chymotrypsin	+	+	−	+	+
Production of acid from glucose	−	−	+	+	+
DNA G+C content (mol%)	68.0	67.9	68.0	68.2	67.9

Total lipids [22] extracted from strain FJY8^T and *Lysobacter xinjiangensis* RCML-52^T were examined by two-dimensional TLC with two developing solvents, chloroform/methanol/water (65 : 25 : 4, by vol.) and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). TLC plates were visualized with appropriate detection reagents [23]. Cells for fatty acid analysis were harvested from the third quadrant on R2A agar (MB cell) after incubation at 30 °C for 3 days. Cellular fatty acids were extracted, saponified and methylated as reported by Kuykendall *et al.* [24]. The extract was analysed using the Sherlock Microbial Identification System v6.01 (MIS, database TSBA6, MIDI) and subsequently compared with those of other type species. To purify isoprenoid quinones, Sep-Pak Vac cartridges (Waters) were used, and the extract was analysed by HPLC as previously reported by Hiraishi *et al.*, and Collins and Jones [25, 26]. TLC of polar lipids showed that strain FJY8^T contained phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid and two unidentified aminophospholipids. Strain FJY8^T and *Lysobacter xinjiangensis* RCML-52^T had phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and two unidentified aminophospholipids in common. However, strain FJY8^T differed from the reference strain by the absence of the unidentified lipid (see Fig. S2). This result supported affiliation of strain FJY8^T to the genus *Lysobacter*; in particular, all members of this genus produce phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as major polar lipids [5, 7, 27]. The major fatty acids of strain FJY8^T were identified as iso-$C_{16:0}$, summed feature 9 (iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl) and iso-$C_{15:0}$. Minor amount of fatty acids with less than 0.5 % are $C_{14:0}$, $C_{16:0}$ N-alcohol, iso-$C_{17:0}$ 3-OH, Summed feature 1 (iso-$C_{15:1}$ H and/or iso-$C_{15:1}$ I). iso-$C_{16:0}$ was the most abundant fatty acid in all strains, indicating that the fatty acid profile of strain FJY8^T was in agreement with those of related type strains; detailed fatty acid compositions are given in Table S1. The only isoprenoid quinone detected in strain FJY8^T was ubiquinone 8 (Q-8), which corresponds to the description of the genus *Lysobacter* [9, 27, 28].

Total genomic DNA was extracted according to the method of Ausubel *et al.* [29]. DNA–DNA hybridization was performed to assess the relatedness of the new isolate and related taxa on the basis of the thermal denaturation principles and equations of De Ley *et al.* [30] and Gillis *et al.* [31] and an optimized procedure evaluated by Loveland-Curtze *et al.* [32]. These experiments were performed three times independently. Hybridization values are expressed as the mean±SD of the three values. The DNA G+C content of strain FJY8^T was determined in triplicate using a simple fluorimetric method [33] and SYBR Green 1 (Life Technologies) and a real-time PCR thermocycler (Rotor-Gene Q, Qiagen). The genomic DNAs of *Lactococcus lactis* subsp. *lactis* KACC 13877^T, *Bacillus subtilis* subsp. *subtilis* KACC 17796, *Bacillus licheniformis* KACC 10476^T, *Escherichia coli* KACC 14818, *Pseudomonas aeruginosa* ATCC 15442 and *Micrococcus luteus* KACC 13377 were used for calibration. For genomic characterization, we performed DNA–DNA hybridization using the fluorimetric method. Strain FJY8^T was found to have 55.8±2.0 and 45.2±4.8 % DNA–DNA relatedness with *Lysobacter xinjiangensis* RCML-52^T and *Lysobacter mobilis* 9NM-14^T. These values support the conclusion that strain FJY8^T represents a novel species distinct from closely related species of the genus *Lysobacter* [34, 35]. Further, the DNA G+C content of strain FJY8^T was 68.0 ±0.4 mol%, a value that is similar to those of other members of the genus *Lysobacter*.

Based on 16S rRNA gene sequence analysis, strain FJY8^T was most closely related to members of the genus *Lysobacter* in the family *Lysobacteraceae*. Common characteristics of members of the genus *Lysobacter* identified in this study were the presence of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as major polar lipids, Q-8 as a respiratory quinone and iso-$C_{16:0}$ and summed feature 9 (iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl) as abundant fatty acids. However, the results of DNA–DNA relatedness analysis and the polyphasic study clearly showed that strain FJY8^T can be distinguished from other species in the genus *Lysobacter*. Thus, strain FJY8^T represents a novel species within the genus *Lysobacter*, for which the name *Lysobacter humi* sp. nov. is proposed.

DESCRIPTION OF *LYSOBACTER HUMI* SP. NOV.

Lysobacter humi (hu′mi. L. gen. n. *humi* of soil).

Cells are Gram-reaction-negative, strictly aerobic, non-motile, non-flagellated and rod-shaped with dimensions in

the range 0.7–1.0×1.3–1.5 µm. Colonies on R2A are round, convex, transparent and yellow in colour after incubation at 30 °C for 2 days. The growth ranges by temperature, pH and NaCl concentration are 20–42 °C, pH 7.0–9.0 and 0–0.5 % (w/v) NaCl, respectively, with optimum growth at 30 °C, pH 8.0 and 0 % (w/v) NaCl, respectively. Growth occurs on nutrient agar and R2A agar. Oxidase and catalase tests are positive. According to API 20NE, cells are positive for gelatin hydrolysis and aesculin hydrolysis, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase activity, indole production and urea hydrolysis. Cells show activities for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase and α-chymotrypsin. Cells show no activity for cystine arylamidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase (ONPG), β-glucosidase or β-glucuronidase. Cells do not assimilate the following compounds: acetate, adipate, alanine, capric acid, citric acid, D-glucose, D-mannitol, D-mannose, D-sorbitol, gluconate, glycogen, inositol, itaconic acid, lactic acid, L-arabinose, L-fucose, L-histidine, L-rhamnose, L-serine, malic acid, malonic acid, maltose, melibiose, *N*-acetylglucosamine, phenylacetic acid, *p*-nitrophenyl-β-D-galactopyranoside (PNPG), potassium 2-ketogluconate, proline, propionic acid, ribose, salicin, suberic acid, sucrose, valeric acid, 3-hydroxybenzoic acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or 5-ketogluconate (API 20 NE and API ID 32GN). The only isoprenoid quinone is ubiquinone Q-8. The most abundant cellular fatty acids are iso-$C_{16:0}$, summed feature 9 (iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl) and iso-$C_{15:0}$. Polar lipids comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid and two unidentified aminophospholipids.

The type strain, FJY8^T(=KCTC 42810^T=JCM 31019^T), was isolated from an arid area in Goyang, Gyeonggi Province, South Korea. The G+C content of genomic DNA of the type strain is 68.0±0.4 mol%.

Funding information
This research was supported by the National Institute of Environmental Research, Republic of Korea. We are grateful to Dr Bernhard Schink for help with etymology.

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

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