**Lysobacter humi** sp. nov., isolated from soil

Dongwook Lee, Jun Hyeong Jang, Seho Cha and Taegun Seo

**Abstract**

A yellow-pigmented and strictly aerobic bacterial strain, designated FJY8T, was isolated from the soil of Goyang, South Korea. The cells of FJY8T were Gram-reaction-negative, non-motile rods. Colonies were circular, convex and transparent. Strain FJY8T grew optimally at 30 °C, with 0% (w/v) NaCl and at pH 8. Phylogenetic analysis of the 16S rRNA gene sequence of FJY8T 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 22558T 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}ω9c 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 FJY8T with *Lysobacter xinjiangensis* RCML-52T and *Lysobacter mobilis* 9NM-14T 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 FJY8T (=KCTC 42810T=JCM 31019T) 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 firmicitumachus* [2], *Lysobacter erysipheiresistens* [3], *Lysobacter hankyangensis* 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 FJY8T that represents a novel species within the family Lysobacteraceae.

Strain FJY8T 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 FJY8T was grown on R2A agar. *Lysobacter xinjiangensis* RCML-52T and *Lysobacter mobilis* 9NM-14T 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 FJY8T (=KCTC 42810T=JCM 31019T) represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter humi* sp. nov. is proposed.

**Author affiliation:** Department of Life Science, Dongguk University – Seoul, Goyang 10326, Republic of Korea.

**Correspondence:** Taegun Seo, tseo@dongguk.edu

**Keywords:** DNA–DNA relatedness; Lysobacter; taxonomy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FJY8T 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 was in the same cluster as *Lysobacter xinjiangensis* RCML-52. Sequence comparisons based on the 16S rRNA gene revealed that the strain FJY8 was most closely related to *Lysobacter xinjiangensis* RCML-52 (98.7 % sequence similarity), *Lysobacter mobilis* 9NM-14 (97.4 %), *Lysobacter bugurensis* ZLD-29 (95.8 %) and *Lysobacter korlensis* ZLD-17 (95.4 %). The 16S rRNA gene sequence comparisons by means of NCBI BLAST searches showed that strain FJY8 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 were investigated by transmission electron microscopy (LIBRA 120, Carl Zeiss), and the 0.4 % toluidine blue content was determined by using the 0.4 % toluidine blue method of Buck [21]. Morphological characteristics of strain FJY8 were investigated by transmission electron microscopy (LIBRA 120, Carl Zeiss), and the 0.4 % toluidine blue content was determined by using the 0.4 % toluidine blue method of Buck [21].

**Fig. 1.** Neighbour-joining phylogenetic tree reconstructed using the 16S rRNA gene sequences of strains FJY8 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.
Total lipids [22] extracted from strain FJY8\textsuperscript{T} and \textit{Lysobacter xinjiangensis} RCML-52\textsuperscript{T} were examined by two-dimen-
sional TLC with two developing solvents, chloroform/meth-
anol/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 methyl-
ated 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 com-
pared 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\textsuperscript{T} contained
phosphatidylethanolamine, phosphatidylglycerol, di-
phosphatidylglycerol, an unidentified phospholipid and two
unidentified aminophospholipids. Strain FJY8\textsuperscript{T} and \textit{Lysobacter xinjiangensis} RCML-52\textsuperscript{T} had phosphatidylethanol-
amine, phosphatidylglycerol, diphosphatidylglycerol and
two unidentified aminophospholipids in common. How-
ever, strain FJY8\textsuperscript{T} differed from the reference strain by the
absence of the unidentified lipid (see Fig. S2). This result
supported affiliation of strain FJY8\textsuperscript{T} to the genus \textit{Lysobacter};
in particular, all members of this genus produce phosphati-
dyethanolamine, phosphatidylglycerol and diphosphatidyl-
glycerol as major polar lipids [5, 7, 27]. The major fatty
acids of strain FJY8\textsuperscript{T} were identified as iso-C\textsubscript{16:0}, summed
feature 9 (iso-C\textsubscript{17:1}ω9c and/or C\textsubscript{16:0} 10-methyl) and iso-
C\textsubscript{15:0}. Minor amount of fatty acids with less than 0.5 % are
C\textsubscript{14:0}, C\textsubscript{16:0} N-alkanol, iso-C\textsubscript{17:0} 3-OH, Summed feature 1
(iso-C\textsubscript{15:1}ω H and/or iso-C\textsubscript{15:1} ω6). iso-C\textsubscript{16:0} was the most
abundant fatty acid in all strains, indicating that the fatty
acid profile of strain FJY8\textsuperscript{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\textsuperscript{T} was ubiquinone 8 (Q-8), which corresponds to
the description of the genus \textit{Lysobacter} [9, 27, 28].

Total genomic DNA was extracted according to the method
of Ausubel et al. [29]. DNA–DNA hybridization was per-
formed to assess the relatedness of the new isolate and
related taxa on the basis of the thermal denaturation princi-
les 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\textsuperscript{T} was determined in triplicate using a simple
fluorimetric method [33] and SYBR Green 1 (Life Technolo-
gies) and a real-time PCR thermocycler (Rotor-Gene Q,
Qiagen). The genomic DNAs of \textit{Lactococcus lactis} subsp.
\textit{lactis} KACC 13877\textsuperscript{T}, \textit{Bacillus subtilis} subsp. \textit{subtilis} KACC
17796, \textit{Bacillus licheniformis} KACC 10476\textsuperscript{T}, \textit{Escherichia coli}
KACC 14818, \textit{Pseudomonas aeruginosa} ATCC 15442 and
\textit{Micrococcus luteus} KACC 13377 were used for calibration.
For genomic characterization, we performed DNA–DNA
hybridization using the fluorimetric method. Strain FJY8\textsuperscript{T}
was found to have 55.8±2.0 and 45.2±4.8 % DNA–DNA
relatedness with \textit{Lysobacter xinjiangensis} RCML-52\textsuperscript{T} and
\textit{Lysobacter mobilis} 9NM-14\textsuperscript{T}. These values support the con-
clusion that strain FJY8\textsuperscript{T} represents a novel species distinct
from closely related species of the genus \textit{Lysobacter} [34, 35].
Further, the DNA G+C content of strain FJY8\textsuperscript{T} was 68.0
±0.4 mol%, a value that is similar to those of other members
of the genus \textit{Lysobacter}.

Based on 16S rRNA gene sequence analysis, strain FJY8\textsuperscript{T}
was most closely related to members of the genus \textit{Lysobacter}
in the family \textit{Lysobacteraceae}. Common characteristics of
members of the genus \textit{Lysobacter} identified in this study
were the presence of phosphatidylethanalamine, phosphati-
dyglycerol and diphosphatidylglycerol as major polar lipids,
Q-8 as a respiratory quinone and iso-C\textsubscript{16:0} and summed
feature 9 (iso-C\textsubscript{17:1}ω9c and/or C\textsubscript{16:0} 10-methyl) as abun-
dant fatty acids. However, the results of DNA–DNA related-
ness analysis and the polyphasic study clearly showed that
strain FJY8\textsuperscript{T} can be distinguished from other species in the
genus \textit{Lysobacter}. Thus, strain FJY8\textsuperscript{T} represents a novel spe-
cies within the genus \textit{Lysobacter}, for which the name \textit{Lysobacter
humi} sp. nov. is proposed.

**DESCRIPTION OF LYSOBACTER HUMI SP. NOV.**

\textit{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

---

**Table 1. Characteristics that differentiate strain FJY8\textsuperscript{T} from phylogenetically related species of the genus \textit{Lysobacter}**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ranges for growth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20–42</td>
<td>20–42</td>
<td>15–37</td>
<td>10–37</td>
<td>10–37</td>
</tr>
<tr>
<td>NaCl (% w/v)</td>
<td>0–0.5</td>
<td>0–2</td>
<td>0</td>
<td>0–3</td>
<td>0.5–4</td>
</tr>
<tr>
<td>pH</td>
<td>7–9</td>
<td>6–10</td>
<td>6–8</td>
<td>6–11</td>
<td>6–11</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of aesculin</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Production of acid from glucose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>68.0</td>
<td>67.9</td>
<td>68.0</td>
<td>68.2</td>
<td>67.9</td>
</tr>
</tbody>
</table>
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 ascus 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-Bl-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, fucose, L-glycogen, inositol, itaconic acid, lactic acid, malic acid, potassium 2-ketogluconate, proline, propionic acid, xylose, α-fucosidase, β-galactosidase (ONPG), β-glucosidase or β-glucuronidase. Cannot dissolve starch. Cells do not oxidize glucose, cellobiose, lactose, maltose, melibiose, maltose, α-D-mannose, malic acid, maltose, melibiose, N-acetylgalosamininc acid, p-nitrophenyl-β-D-galactopyranoside (PNPG), potassium 2-ketogluconate, proline, propionic acid, ribose, salicin, suberic acid, succrose, valeric acid, 3-hydroxybenzoic acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or 5-ketogluconate (API 20 NE and API 32GN). The only isoprenoid quinone is ubiquinone Q-8. The most abundant cellular fatty acids are iso-C16:0 summed feature 9 (iso-C17:0 ω9c and/or C16:1 ω7c-methyl) and iso-C15:0 2-OH lysol, lysoiso-C16:0 and two unidentified aminophospholipids.

The type strain, FJYB^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.

References


---

**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.