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Lysobacter lacus sp. nov., isolated from from lake sediment

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Abstract

An aerobic and Gram-stain-negative bacterial strain, designated UKS-15^T, was isolated from lake water in the Republic of Korea. Results of 16S rRNA gene sequence and phylogenetic analyses indicated that the novel isolate belongs to the genus *Lysobacter* and was most closely related to *Lysobacter xinjiangensis* RCML-52^T (98.0 %), *Lysobacter mobilis* 9 NM-14^T (97.4 %) and *Lysobacter humi* FJY8^T (97.2 %). The DNA G+C content was 69.1 mol%. Strain UKS-15^T possessed ubiquinone-8 (Q-8) as the sole respiratory quinone and the fatty acid profile comprised iso-$C_{15:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{16:0}$ 10-methyl) as its major components. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and one unidentified aminophospholipid. Moreover, the physiological and biochemical results and low level of DNA–DNA relatedness (<22.0 %) allowed the phenotypic and genotypic differentiation of strain UKS-15^T from other *Lysobacter* species. Therefore, on the basis of the data from this polyphasic taxonomic study, strain UKS-15^T should represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter lacus* sp. nov. is proposed. The type strain is UKS-15^T (=JCM 30983^T=KACC 18719^T).

The genus *Lysobacter* was first described by Christensen and Cook in 1978 [1], and its description was emended by Park *et al.* in 2008 [2]. Members of the genus *Lysobacter* contain ubiquinone Q-8 as the major respiratory quinone and have a high DNA G+C content [2, 3]. Species of the genus *Lysobacter* are Gram-stain-negative, aerobic, non-fruiting, gliding organisms. The colonies are very mucoid and cream, pink or yellow-brownish in colour. Members of *Lysobacter* can also be separated from other related microbes due to high G+C content (usually ranging between 65–72 %), lytic activity against other micro-organisms and the absence of flagella [4]. At the time of writing, there are 46 recognized species in the genus *Lysobacter* (www.bacterio.net) including the recently described species *Lysobacter mobilis* [5], *Lysobacter hankyongensis* and *Lysobacter sediminicola* [6], *Lysobacter pocheonensis* [7], *Lysobacter humi* [8] and *Lysobacter caeni* [9].

In this study, we report the taxonomic characterization of a novel strain, designated as UKS-15^T, which appeared to be member of the genus *Lysobacter*. Strain UKS-15^T was isolated from lake sediment collected in Ungok (35° 28′ 16.3″ N 126° 39′ 21.6″ E) in Gochang Province, Republic of Korea. The temperature (25±2.5 °C) and pH (7.5±0.5) of the water were measured *in situ* with a portable pH/VORP/ meter [Ph-20N, KEITI (Korea Environmental Industry and Technology Institute)]. A large number of bacterial strains were isolated using the standard dilution-plating technique on Reasoner's 2A (R2A) agar (Difco) at room temperature. The isolated strains were mostly reported previously except for one strain, designated as UKS-15^T, which appeared to be a member of the genus *Lysobacter*. Strain UKS-15^T was routinely cultured on R2A agar plates at 30 °C and preserved as a suspension in R2A broth with 20 % (w/v) glycerol at −80 °C.

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Keywords: 16S rRNA gene; draft genome; polyphasic taxonomy; *Lysobacter lacus*.
Abbreviations: EDTA, Ethylenediaminetetraacetic acid; IPTG, Isopropyl β-D-1-thiogalactopyranoside; NADP, Nicotinamide adenine dinucleotide phosphate; PBS, Phosphate Buffered Saline; PE, Phophatidylethanolamine; SDS, Sodium Dodecyl Sulphate; SEM, Scanning Electron Micrograph; UV, Ultraviolet.
The NCBI/EMBL/DDBJ accession numbers for the 16S rRNA and draft genome sequences of strain UKS-15^T are KP893900 and VTRV00000000, respectively.
Two supplementary figures and two supplementary tables are available with the online version of this article.

Genomic DNA was extracted with a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Siddiqi *et al.* [10]. Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database or www.ezbiocloud.net/eztaxon [11]. Multiple alignments were performed by the CLUSTAL_X program [12]. Gaps were edited in the BioEdit program [13]. Evolutionary distances were calculated using the Kimura two-parameter model [14]. Phylogenetic trees were reconstructed by using the neighbour-joining, maximum-likelihood and maximum-parsimony [15, 16] methods in the MEGA6 program [17] with bootstrap values based on 1000 replications [18].

Complete genome sequence analysis plays a key role in the description of novel bacterial strains. Therefore, the genomic DNA of strain UKS-15^T was extracted and sequenced through the Pacific Biosciences RS II platform. A library was reconstructed according to the instructions of the Pacific Biosciences RS II manual. Furthermore, genome annotation was performed using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAP; www.ncbi.nlm.nih.gov/books/NBK174280/). The project information is available from the Genomes OnLine Database and the complete genome sequence has been submitted to GenBank.

The complete genome of strain UKS-15^T consists of one circular chromosome of 2 855 702 bp with a 66.3 mol% G+C content. Of the 2836 genes predicted, 2757 were protein-coding genes CDS and 52 RNAs; 27 pseudogenes were also determined. Furthermore, the genomic features of strain UKS-15^T are shown in Table S1 (available in the online version of this article).

The nearly complete 16S rRNA gene sequence of strain UKS-15^T (1461 nt) was determined and subjected to comparative analysis. Phylogenetic analysis using the neighbour-joining method based on 16S rRNA gene sequences indicated that strain UKS-15^T is clustered within the genus *Lysobacter* (Fig. 1). The highest degrees of sequence similarity determined were to *Lysobacter xinjiangensis* RCML-52^T (97.8 %), *Lysobacter mobilis* 9 NM-14^T (97.4 %) and *Lysobacter humi* FJY8^T (97.2 %).

On the basis of these phylogenetic results, *L. xinjiangensis* KCTC 22558^T, *L. mobilis* DSM 27574^T and *L. humi* KCTC 42810^T were selected as the closest recognized neighbours of

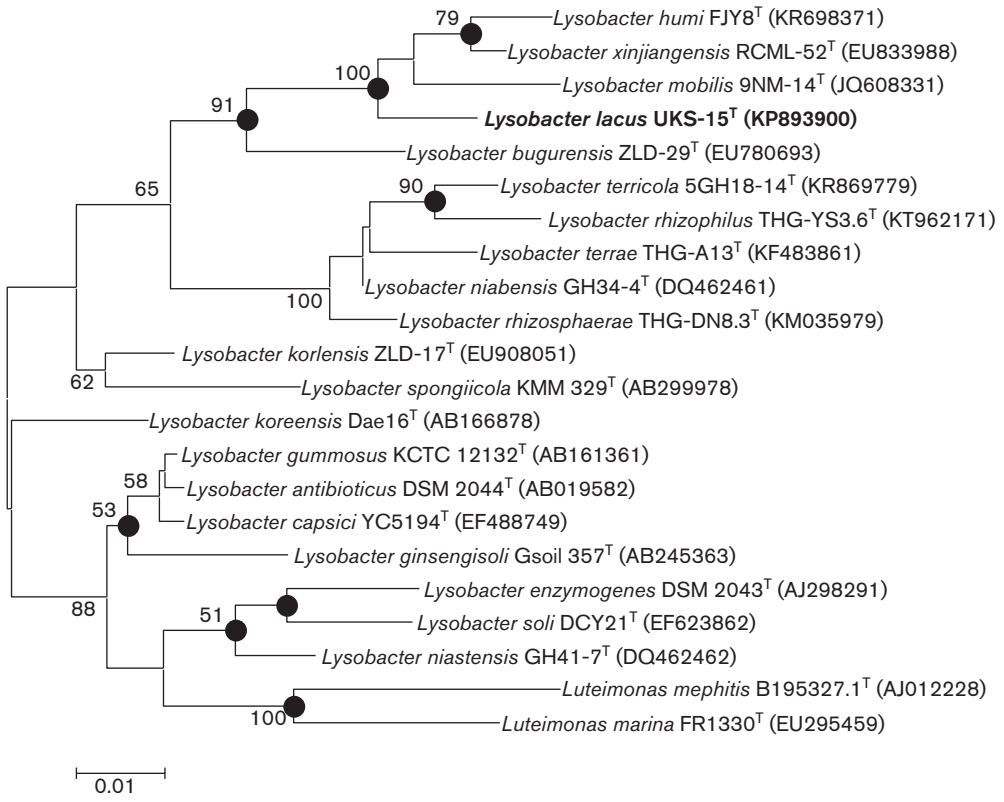

Fig. 1. Phylogenetic relationship of strain UKS-15^T with recognized species of the genus *Lysobacter*. The tree was reconstructed by using the neighbour-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. *Xanthomonas campestris* LMG568^T (X95917) was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with maximum-parsimony and maximum likelihood algorithms. Bar, 0.01 substitutions per nucleotide position.

strain UKS-15^{T}and were obtained culture collections, grown under the same conditions and used as reference strains in most of the subsequent phenotypic tests.

The Gram reaction was determined using the non-staining method, as described previously [19]. Cell morphology was examined with the scanning electron microscope (SU-3500, Hitachi), using cells grown for 2 days at 30 °C on R2A agar. Cell motility was determined using the hanging drop method [20]. Catalase and oxidase tests were performed as described previously [21]. Biochemical tests were carried out by using API 20NE, API ID 32GN and API ZYM kits according to the instructions of the manufacturer (bioMérieux). Tests for degradation of DNA, casein, starch, xylan, Tween 80 and carboxyl methyl cellulose were performed and evaluated after 7 days of incubation at 30 °C on R2A agar medium. Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40 and 42 °C) and various pH values (pH 4–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation at 30 °C using R2A agar and broth. Three different buffers (final concentration, 50 mM) were used to adjust the pH of R2A broth. Acetate buffer was used for pH 4.0–5.5, phosphate buffer was used for pH 6.0–8.0 and Tris buffer was used for pH 8.5–10.0. Salt tolerance was tested on R2A agar medium supplemented with 1–5 % (w/v at intervals of 1 % unit) NaCl and growth assessed after 7 days of incubation at 30 °C. Growth on nutrient agar, trypticase soy agar (TSA), LB agar and MacConkey agar (all from Difco) was also evaluated after 7 days of incubation at 30 °C.

Cells of strain UKS-15^T were Gram-reaction-negative, oxidase- and catalase-positive, aerobic, non-motile and rod-shaped (0.3–1.0 µm wide and 1.5–2.0 µm long; Fig. S1). Colonies of strain UKS-15^T grown on R2A agar were circular, convex and yellow-coloured after 48 h incubation at 30 °C. Strain UKS-15^T did not grow on TSA, LB and MacConkey agar, whereas it grew weakly on nutrient agar at 30 °C. The physiological and biochemical characteristics of strain UKS-15^T are summarized in the species description and a comparison of selective characteristics of strain UKS-15^T and related type strains is given in Table 1.

For the measurement of the DNA G+C content, the genomic DNA of the novel strain was extracted and purified as described previously [22], and was enzymatically degraded into nucleosides, and was determined as described by Mesbah *et al.* [23] using reverse-phase HPLC. Strain UKS-15^T was investigated for its polar lipid content; the polar lipids were extracted from 50 mg freeze-dried cells, examined by two-dimensional TLC and identified as described by Minnikin *et al.* [24]. Isoprenoid quinone was extracted with chloroform–methanol (2 : 1, v/v), evaporated under vacuum conditions, and re-extracted in n-hexane/water (1 : 1, v/v). The crude n-hexane–quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analysed by HPLC as described previously [25]. Cellular fatty acids profiles were determined for strains grown on R2A agar for 2 days at 30 °C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by a gas chromatograph (Hewlett Packard 6890) were identified by the Microbial Identification software package based on Sherlock Aerobic Bacterial Database (TSBA60) [26].

DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* [27], using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the remaining three values were used to calculate similarity values. The DNA hybridization values quoted are the means of these three values.

The DNA G+C content of the UKS-15^T was 69.1 mol%, which was similar to those of the described species of genus *Lysobacter* (Table 1). The major detected polar lipids in strain UKS-15^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminophospholipid (Fig. S2). The sole respiratory quinone was ubiquinone-8 (Q-8). The fatty acid profile of UKS-15^T was compared with those of the type strains of recognized *Lysobacter* species. The major fatty acids (>7.0 %) of strain UKS-15^T were iso-$C_{11:0}$ 3-OH (7.1 %), iso-$C_{17:0}$ (9.3 %), iso-$C_{15:0}$ (24.2 %) and summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{16:10}$ 10-methyl; 29.7 %, which is a typical profile for members of the genus *Lysobacter*, as shown in Table 2. However, some qualitative and quantitative differences (as mentioned in Table 2) in the fatty acids distinguished strain UKS-15^T from the other recognized species of the genus *Lysobacter* examined in this study.

The DNA–DNA relatedness values between strain UKS-15^T and *L. xinjiangensis* KCTC 22558^T, *L. mobilis* DSM 27574^T and *L. humi* KCTC 42810^T were 29.4±1.5 (31.2±0.8, reciprocal), 23.6±2.4 (24.8±1.3) and 8.1±1.3 (5.9±2.1), respectively. According to Wayne *et al.* [28], DNA–DNA relatedness values lower than 70 % are considered to be the threshold value for the delineation of genospecies, so the result obtained is low enough to assign strain UKS-15^T as representing a novel species of the genus *Lysobacter*.

Some common characteristics of members of the genus *Lysobacter* identified in this study were the presence of phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol as major polar lipids, ubiquinone-8 (Q-8) as a respiratory quinone, and iso-$C_{15:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{16:0}$ 10-methyl) as abundant fatty acids. However, the results of fatty acids (anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$), low values of DNA–DNA hybridization and the polyphasic study clearly showed that strain UKS-15^T can be distinguished from the closest species of the genus *Lysobacter*. Thus, strain UKS-15^T represents a novel species within the genus *Lysobacter*, for which the name *Lysobacter lacus* sp. nov. is proposed.

DESCRIPTION OF *LYSOBACTER LACUS* SP. NOV.

Lysobacter lacus (la'cus. L. gen. n. *lacus* of a lake).

Table 1. Phenotypic characteristics of UKS-15^T and other related *Lysobacter* species

Strains: 1, *Lysobacter lacus* UKS-15^T; 2, *Lysobacter xinjiangensis* KCTC 22558^T; 3, *Lysobacter mobilis* DSM 27574^T; 3, *Lysobacter humi* KCTC 42810^T. All data are from this study unless indicated otherwise. All strains were positive for alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Negative for α-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase, α-fucosidase, nitrate reduction, indole production, glucose acidification, D-mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, phenyl-acetate, L-arabinose, propionate, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, sucrose, maltose, suberate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate and L-serine. +, Positive; –, negative.

Characteristics	**1**	**2**	**3**	**4**
Isolation sources	Sediment	Soil*	Lead–zinc ore*	Soil*
Temperature range for growth (°C)	10–30	18–42*	15–37*	20–42
NaCl range for growth (NaCl %, w/v)	0–1	0–2*	0–0.5*	0-0.5*
pH range for growth	5.5–8.5	7–11*	6–8*	7–9*
Enzymes activity				
Arginine dihydrolase	–	+	–	–
Cystine arylamidase	–	+	+	–
α-Chymotrypsin	–	+	–	–
Aesculin hydrolysis	–	+	–	–
Esterase lipase (C8)	+	–	+	–
Gelatin hydrolysis	+	+	–	–
α-Glucosidase	+	–	+	–
Lipase (C14)	–	+	–	–
Trypsin	–	+	+	+
Urease	–	+	–	–
Valine arylamidase	–	+	+	–
Assimilation of:				
N-Acetyl-D-glucosamine	–	+	–	–
L-Fucose	–	+	–	–
D-Glucose	–	+	–	–
3-Hydroxy-butyrate	–	+	–	–
Inositol	–	+	–	–
Itaconate	–	+	–	–
D-Mannitol	–	+	–	–
Melibiose	–	w	–	–
L-Rhamnose	–	+	–	–
D-Ribose	–	+	–	–
Salicin	–	+	–	+
D-Sorbitol	–	+	–	–
DNA G+C content (mol%)	69.1	69.7*	70.7*	68.0*

*Data taken from: *L. xinjiangensis* KCTC 22558^T [29], *L. mobillis* DSM 12600^T [5] and *L. humi* KCTC42810^T [8], respectively.

Table 2. Cellular fatty acid compositions (%) of strain UKS-15^T and phylogenetically related species of the genus *Lysobacter*

Strains: 1, UKS-15^T; 2, *Lysobacter xinjiangensis* KCTC 22558^T; 3, *Lysobacter mobillis* DSM 27574^T; 4, *Lysobacter humi* KCTC 42810^T. All strains were grown on R2A agar medium for 48 h at 30 °C. The data shown in this table are more than 1 % for all strains. TR, Traces (<1 %); –, not detected.

Fatty acids	1	2	3	4
Saturated:				
$C_{16:0}$	4.1	1.0	6.0	1.9
Branched:				
iso-$C_{11:0}$	5.8	7.8	6.2	7.0
iso-$C_{11:0}$ 3-OH	7.1	10.8	8.3	3.4
iso-$C_{15:0}$	24.2	11.7	31.2	9.1
iso-$C_{15:1}$ F	1.1	TR	–	–
iso-$C_{16:0}$	3.9	32.6	12.3	35.7
iso-$C_{16:1}$ h	TR	2.4	–	–
iso-$C_{17:0}$	9.3	5.9	13.4	10.2
anteiso-$C_{15:0}$	2.0	TR	–	–
anteiso-$C_{17:0}$	5.2	TR	–	–
Summed features:*				
3, $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*	3.5	1.1	3.1	5.5
9, iso-$C_{17:1}$ ω9*c* and/or $C_{16:0}$10-methyl	29.7	22.6	15.8	24.4

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system.

Cells of strain UKS-15^T are Gram-reaction-negative, non-motile, aerobic and rod-shaped. After 48 h incubation at 30 °C on R2A agar plate, the colony is smooth, circular, convex and yellow-coloured. Growth occurs between 10–30 °C (not at 4 and 35 °C) with at pH 5.5–8.5 and with 0–1.0 % NaCl. Optimum growth occurs at 30 °C at pH 7.0 without NaCl supplement. Strain UKS-15^T does not show good growth on R2A medium but grows well on 1/2-R2A and 1/4-R2A agar medium. Negative for the hydrolyses of starch, casein, CM-cellulose, xylan, Tween 80 and DNA. Nitrate is not reduced to nitrite. The substrates utilized as sole carbon sources (API ID 32 GN, API 20 NE) and the enzyme activity (API ZYM) are listed in Table 1. The list of all negative traits detected with commercial API kits is shown in Table S2. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and one unidentified aminophospholipid, while the minor polar lipids are four unidentified phospholipids. Ubiquinone-8 is the sole respiratory quinone. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}$ω9*c* and/or $C_{16:0}$ 10-methyl. The G+C content of genomic DNA is 69.1 mol%.

The type strain, UKS-15^T (=JCM 30983^T=KACC 18719^T), was isolated from Ungok lake sediment collected in Gochang Province, Republic of Korea.

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Conflicts of interest
The authors declare that they have no direct or indirect conflicts of interest.

Ethical statement
The article does not contain any studies with human participants or animals performed by any of the authors.

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