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Lysobacter arenosi sp. nov. and *Lysobacter solisilvae* sp. nov. isolated from soil[§]

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Two Gram-stain negative, yellow-pigmented, and mesophilic bacteria, designated strains R7^T and R19^T, were isolated from sandy and forest soil, South Korea, respectively. Both strains were non-motile rods showing catalase- and oxidase-positive activities. Both strains were shown to grow at 10–37°C and pH 6.0–9.0, and in the presence of 0–1.5% (w/v) NaCl. Strain R7^T contained iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (comprising $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ *ω9c*), whereas strain R19^T contained iso-$C_{11:0}$ 3-OH, $C_{16:1}$ *ω7c* alcohol, iso-$C_{11:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (comprising $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ *ω9c*) as major cellular fatty acids (> 5%). Both strains contained ubiquinone-8 as the sole isoprenoid quinone and phosphatidylglycerol, phosphatidylethanolamine, and an unidentified phospholipid as the major polar lipids. The DNA G + C contents of strains R7^T and R19^T calculated from their genomes were 66.9 mol% and 68.9 mol%, respectively. Strains R7^T and R19^T were most closely related to *Lysobacter panacisoli* C8-1^T and *Lysobacter niabensis* GH34-4^T with 98.7% and 97.8% 16S rRNA sequence similarities, respectively. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains R7^T and R19^T formed distinct phylogenetic lineages within the genus *Lysobacter*. Based on phenotypic, chemotaxonomic, and molecular features, strains R7^T and R19^T represent novel species of the genus *Lysobacter*, for which the names *Lysobacter arenosi* sp. nov. and *Lysobacter solisilvae* sp. nov. are proposed. The type strains of *L. arenosi* and *L. solisilvae* are R7^T (= KACC 21663^T = JCM 34257^T) and R19^T (= KACC 21767^T = JCM 34258^T), respectively.

Keywords: *Lysobacter arenosi*, *Lysobacter solisilvae*, taxonomy, new taxa, soil

Introduction

The genus *Lysobacter,* meaning literally "the lysing rod", was first proposed as a member of the family *Xanthomonadaceae* of the class *Gammaproteobacteria* with *Lysobacter* enzymogenes as the type species by Christensen and Cook (1978). At the time of writing this paper, the genus *Lyosbacter* includes 63 species with validly published names (https://lpsn.dsmz.de/genus/lysobacter). Members of the genus *Lysobacter* form a highly mucoid, cream-colored, pink, or yellow-brown colony and their cells have been reported to be Gram-negative, gliding, flexing rod-shaped, and generally non-flagellated (some *Lysobacter* species such as *L. spongiicola* [Romanenko *et al*., 2008], *L. arseniciresistens* [Luo *et al*., 2012], and *L. mobilis* [Yang *et al*., 2015] are flagellated), and contain ubiquinone-8 (Q-8) as the major respiratory quinone and relatively high G + C contents of 61.7–70.7 mol% (Christensen and Cook, 1978; Reichenbach, 2006; Weon *et al*., 2007). Members of this genus often degrade diverse polysaccharides such as chitin and agar, but not filter-paper cellulose, and have lytic effects on a variety of microorganisms, including Gram-negative and Gram-positive bacteria, actinomycetes, blue-green and green algae, yeasts, filamentous fungi, nematodes, and oomycetes (Christensen and Cook, 1978; Reichenbach, 2006). These lytic effects have been attributed to the production of various extracellular enzymes, including protease and endopeptidase (Lapteva *et al*., 2012), glucanase (Palumbo *et al*., 2003), lipase (Ko *et al*., 2009), and chitinase (Qian *et al*., 2012).

Members of the genus *Lysobacter* have been isolated from various terrestrial habitats, including the rhizosphere of plants, plant-cultivated soil, geothermal soil, field soil, iron-mined soil, cave, and lake sediment (Aslam *et al*., 2009; Srinivasan *et al*., 2010; Luo *et al*., 2012; Choi *et al*., 2014; Du *et al*., 2015; Singh *et al*., 2015a, 2015b; Chen *et al*., 2016; Xie *et al*., 2016; Huo *et al*., 2018; Jang *et al*., 2018; Im *et al*., 2020; Li *et al*., 2020; Xu *et al*., 2020; Lee and Whang, 2021), and aquatic habitats, including sea water, estuary sediment, sludge, and deep-sea sponges (Ye *et al*., 2015; Jeong *et al*., 2016; Choi *et al*., 2018; Chhetri *et al*., 2019; Xu *et al*., 2021). Efforts have been made to isolate and characterize members of the bacterial community from soil environments in Korea. In this study, we isolated two putative novel species belonging to the genus *Lysobacter*, designated strains R7^T and R19^T, from soil samples collected in Yeongwol, Gangwon Province of South Korea and characterized them taxonomically using a polyphasic approach.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and genome sequences of strains R7^T and R19^T are MN197915 and CP071517 and MW173243 and CP071518, respectively.

Materials and Methods

Isolation of bacterial strains and culture conditions

Strains R7^T and R19^T were isolated from sand (37°10′36.6″N, 128°28′03.8″E) and forest (37°15′4.1″N, 128°32′26.8″E) soil from Yeongwol in Gangwon Province, South Korea, respectively, as described previously (Feng *et al.*, 2019). In brief, soil samples were serially diluted in 0.9% (w/v) saline and aliquots of each serial dilution were spread on R2A agar (BD). The agar plates were aerobically incubated at 30°C for 5 days, and colonies were purified and maintained on R2A agar. The 16S rRNA genes of colonies were PCR-amplified using the universal bacterial primers F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′). The PCR products were double-digested with *Hae*III and *Hha*I, and their restriction fragment patterns were compared. Representative PCR products showing unique fragment patterns were partially sequenced using the universal bacterial primer 340F (5′-CCT ACG GGA GGC AGC AG-3′) and their sequences were compared with those of all type strains using the Nucleotide Similarity Search program (http://www.ezbiocloud.net/identify/) (Yoon *et al.*, 2017). Eventually, two putative novel strains belonging to the genus *Lysobacter* were selected. Strains R7^T and R19^T were routinely cultured aerobically on R2A and 1/2 diluted R2A agar at 30°C for 3 days and were preserved at -80°C in R2A and 1/2 diluted R2A broths, respectively, containing a final concentration of 15% (v/v) glycerol unless otherwise described. *Lysobacter enzymogenes* KACC 11382^T, *Lysobacter oryzae* KACC 14553^T, *Lysobacter niabensis* KACC 11587^T, *Lysobacter panacisoli* KACC 17502^T, and *Lysobacter soli* KACC 15381^T were obtained from the Korean Agricultural Culture Collection (KACC) center and used as reference strains for the comparison of phenotypic properties and fatty acid analysis. The properties of strains R7^T and R19^T and the reference strains were investigated under optimal growth conditions.

16S rRNA sequencing and phylogenetic analysis

The 16S rRNA genes of strains R7^T and R19^T that were amplified using the F1 and R13 primers were further sequenced using the primers 518R (5′-ATTACCGCGGCTGCTGG-3′), and 805F (5′-GATTAGATACCCTGGTAGTC-3′), and near complete 16S rRNA gene sequences of strains R7^T (1468 nucleotides) and R19^T (1473 nucleotides) were obtained through the assembly of 518R-, 805R-, and 340F-based sequences. The 16S rRNA gene sequence similarity values between strains R7^T and R19^T and their closely related type strains were calculated using the Nucleotide Similarity Search program. The 16S rRNA gene sequences of strains R7^T and R19^T and closely related type strains were aligned using the fast secondary-structure aware infernal aligner available at the Ribosomal Database Project (https://pyro.cme.msu.edu/aligner/form.spr) (Nawrocki and Eddy, 2007). Phylogenetic trees based on the neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) algorithms with bootstrap values (1,000 replications), based on the Kimura two-parameter model, the nearest-neighbor-interchange heuristic search method, and the pairwise deletion options, respectively, were constructed using the MEGA7 software (Kumar *et al.*, 2016).

Phenotypic and biochemical characterization

The growth of strains R7^T and R19^T was investigated at 30°C for 3 days on R2A, and 1/2 diluted R2A agar, LB agar (BD), marine agar (MA; BD), nutrient agar (NA; BD), and tryptic soy agar (TSA; BD). In addition, the growth of strains R7^T and R19^T was assessed on R2A and 1/2 diluted R2A agars, respectively, at different temperatures (4, 10, 15, 20, 25, 30, 37, 40, and 45°C). The growth of strains R7^T and R19^T was evaluated in R2A and 1/2 diluted R2A broths with different pH values (3.5–10.0 at 0.5 pH unit intervals) at 30°C for 3 days. R2A and 1/2 diluted R2A broths with pH 3.5–6.0, pH 6.5–7.0, pH 7.5–9.0, and pH 9.5–10.0 were prepared using the sodium citrate, Na_2HPO_4/NaH_2PO_4, Tris-HCl, and $Na_2CO_3/NaHCO_3$ buffers, respectively (Gomori, 1955). After sterilization (121°C for 15 min), the pH values were readjusted where necessary. The salt tolerance of strains R7^T and R19^T was tested by culturing the strains in R2A and 1/2 diluted R2A broths containing different concentrations of NaCl (0%, no addition; 0.5%; and 1.0–7.0% at 1.0% intervals). Gram staining was performed using a Gram stain kit (bioMérieux) according to the manufacturer's instructions. Cell morphology was observed using phase-contrast microscopy (Zeiss Axio Scope. A1; Carl Zeiss) and transmission electron microscopy (JEM-1010, JEOL). To evaluate the anaerobic growth of strains R7^T and R19^T, the strains were streaked on R2A and 1/2 diluted R2A agar and their growth was checked after 3 weeks of incubation at 30°C under anaerobic conditions formed by the GasPak Plus system (BBL). The phenotypic properties of strains R7^T and R19^T and the reference strains were investigated in parallel under the same conditions. Catalase and oxidase activities were determined by assessing the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution (Junsei) and the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively (Smibert and Krieg, 1994). Hydrolysis of casein, starch, aesculin, tyrosine, Tween 20, and Tween 80 by strains R7^T and R19^T was investigated on R2A and 1/2 diluted R2A agar, respectively, according to the methods described previously (Lányi, 1988; Smibert and Krieg, 1994). Additional tests on carbon-source utilization and enzyme activities were performed using API 20NE and API ZYM kits (bioMérieux), respectively, according to the manufacturer's instructions, at their optimal growth temperatures.

Chemotaxonomic analysis

The isoprenoid quinones of strains R7^T and R19^T were extracted according to the method of Minnikin *et al.* (1984), and analyzed using an HPLC machine (LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu), and a reversed-phase column (250 × 4.6 mm, Kromasil; Akzo Nobel) using methanol-isopropanol (2:1, v/v) as an eluent (1 ml/min), as described by Komagata and Suzuki (1988). For the analysis of cellular fatty acids, strains R7^T and R19^T and the reference strains were cultured in R2A and 1/2 R2A (only for strain R19^T) broth at 30°C and the microbial cells were harvested at the same growth stage (exponential phase, OD600 = 0.6–0.8). The fatty acids of the microbial cells were

saponified, methylated, and extracted using the standard MIDI protocol, and analyzed by gas chromatography (Hewlett Packard 6890). Cellular fatty acids were identified and quantified using the RTSBA6 database of the Microbial Identification System (Sherlock ver. 6.0B) (Sasser, 1990). The polar lipids of strains R7^T and R19^T were analyzed by two-dimensional thin-layer chromatography (TLC) using cells harvested at the exponential growth phase, according to the method described by Minnikin *et al.* (1977). The following reagents were used to detect different polar lipids: 10% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer-Lester reagent (for phospholipids), and α-naphthol (for glycolipids). The presence or absence of phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) in strains R7^T and R19^T were confirmed using standard polar lipid compounds purchased from Sigma-Aldrich.

Genomic analysis

For the whole genome sequencing of strains R7^T and R19^T, genomic DNA was extracted from cultured cells using the phenol-chloroform extraction and ethanol precipitation method (Sambrook *et al.*, 1989). The genomes of strains R7^T and R19^T were completely sequenced using a combination of the Oxford Nanopore MinION sequencer at the laboratory and an Illumina Hiseq X instrument with 151 bp paired-end reads at Macrogen. The sequencing data derived from the Nanopore and Illumina sequencing were hybrid *de novo*-assembled using the Unicycler software (ver. 0.4.7) (Wick *et al.*, 2017). Multiple rounds of polishing were performed with Pilon 1.23 in the Unicycler pipeline to correct small sequence errors. The complete genome sequences of strains R7^T and R19^T were deposited in GenBank database under the accession numbers CP071517 and CP071518, respectively and annotated using the NCBI prokaryotic genome annotation pipeline (www.ncbi.nlm.nih.gov/genome/annotation_prok/). For genome-based phylogenomic analysis, the up-to-date bacterial core gene pipeline (UBCG; https://help.ezbiocloud.net/ubcg-gene-set/, Na *et al.*, 2018) was used to extract 92 core housekeeping genes from the genomes of strains R7^T and R19^T and closely related taxa. An ML tree based on the concatenated sequences of 92 core housekeeping genes was reconstructed using the MEGA7 software. Average nucleotide identity (ANI) and *in silico* DNA-DNA hybridization (*in silico* DDH) values were calculated using Orthologous Average Nucleotide Identity Tool (OAT) software available in the EzBioCloud web server (www.ezbiocloud.net/sw/oat) (Lee *et al.*, 2016) and the server-based Genome-to-Genome Distance Calculator version 2.1 (http://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff *et al.*, 2013). Genome annotation of strains R7^T and R19^T was performed using the NCBI Prokaryotic Genome Annotation Pipeline and antimicrobial metabolite biosynthesis genes were predicted using antiSMASH 5.0 (Blin *et al.*, 2019).

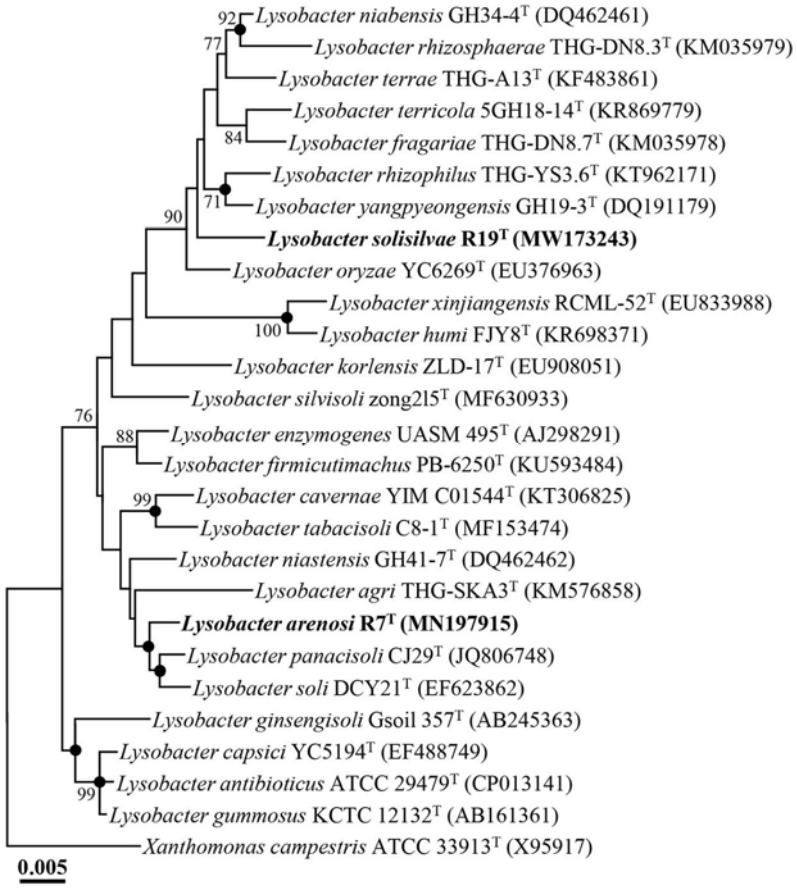

Fig. 1. A neighbor-joining tree showing the phylogenetic relationships between strains R7^T and R19^T and their closely related type strains of the genus *Lysobacter*, based on 16S rRNA gene sequences. Only bootstrap values over 70% are given on the nodes in percentages of 1,000 replicates. Filled circles (l) show the corresponding nodes that were also recovered in the trees reconstructed by the maximum-likelihood and maximum-parsimony algorithms. *Xanthomonas campestris* ATCC 33913^T (X95917) is used as the outgroup. The scale bar equals 0.005 changes per nucleotide position.

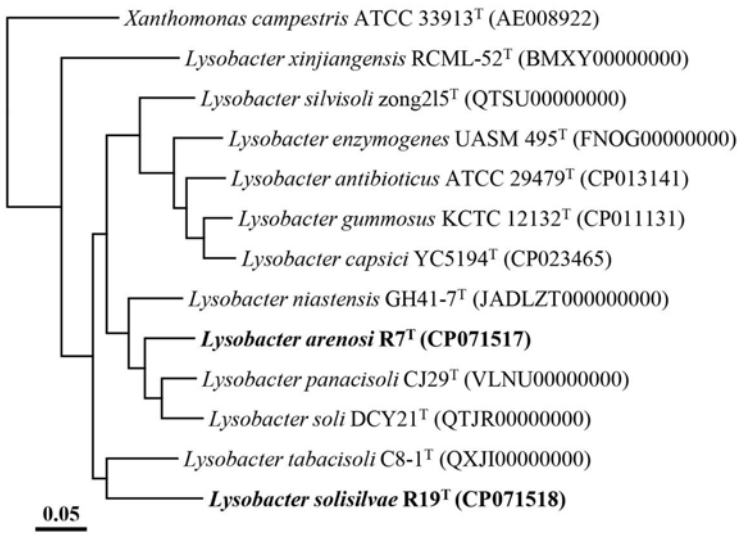

Fig. 2. A phylogenomic tree showing the phylogenetic relationships between strains R7^T and R19^T and closely related taxa, based on the concatenated 92 housekeeping core genes. *Xanthomonas campestris* ATCC 33913^T (AE008922) was used as the outgroup. Bar, 0.05 substitutions per nucleotide.

Results and Discussion

Phylogenetic analysis

Phylogenetic analysis of 16S rRNA gene sequences based on the NJ algorithm revealed that strains R7^T and R19^T formed distinct phylogenetic lineages within the genus *Lysobacter* (Fig. 1). Phylogenetic trees based on the MP and ML algorithms also supported the data that strains R7^T and R19^T formed distinct phylogenetic lineages within the genus *Lysobacter* (Supplementary data Fig. S1). The genome-based phylogenetic analysis also showed that strains R7^T and R19^T formed distinct phylogenetic lineages within the genus *Lysobacter* (Fig. 2), supporting that strains R7^T and R19^T represent two different members of the genus *Lysobacter*. Comparative an-

Table 1. Comparison of phenotype characteristics of strains R7^T and R19^T and their related taxa in the genus *Lysobacter*

Taxa: 1, strain R7^T (this study); 2, strain R19^T (this study); 3, *L. enzymogenes* KACC 11382^T (Christensen and Cook, 1978); 4, *L. oryzae* KACC 14553^T (Aslam *et al.*, 2009); 5, *L. niabensis* KACC 11587^T (Weon *et al.*, 2007); 6, *L. panacisoli* KACC 17502^T (Choi *et al.*, 2014); 7, *L. soli* KACC 15381^T (Srinivasan *et al.*, 2010). All strains are positive for the following characteristics: enzyme activity* of naphthol-AS-BI-phosphohydrolase, alkaline phosphatase, esterase (C4), esterase lipase (C8), and acid phosphatase. All strains are negative for the following characteristics: indole production, fermentation of glucose, hydrolysis* of starch and tyrosine, enzyme activity* of urease, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase, and assimilation* of L-arabinose, capric acid, adipic acid, potassium gluconate, D-mannitol, and phenylacetic acid. Symbols: +, positive; –, negative.

Characteristic	1	2	3	4	5	6	7
Colony color	Pale yellow	Pale yellow	Yellow	Pale yellow	Yellow	Bright yellow	Yellow
Optimum growth:							
Temperature (°C)	30	30	28–30	28	28	28–30	30
pH	7–8	8	7	7–8	6–7	7	7–7.5
Catalase*	+	+	+	+	+	+	-
Hydrolysis* of:							
Aesculin	+	-	+	-	-	+	+
Tween 20, Tween 80	-	-	+	-	-	-	-
Casein	+	+	+	-	+	+	+
Nitrate reduction to nitrite	+	-	-	-	+	+	-
Enzyme activity (API ZYM)* of:							
Lipase (C14), α-Chymotrypsin	-	-	+	-	-	-	+
Arginine dihydrolase	+	-	-	-	-	-	-
Leucine arylamidase	+	+	+	-	+	+	+
Valine arylamidase, α-Glucosidase	+	-	-	-	+	+	+
β-Glucosidase	+	-	-	-	-	+	+
N-Acetyl-β-glucosaminidase	-	-	+	-	-	-	+
Cysteine arylamidase, malic acid, trisodium citrate	-	-	-	-	-	-	+
Trypsin	+	-	+	-	-	-	+
Assimilation (API 20NE)* of:							
Aesculin	+	-	+	+	-	+	+
D-Glucose, D-annose, *N*-acetyl-glucosamine, D-maltose	+	-	+	-	-	+	+

* Data from this study.

alysis of the 16S rRNA gene sequences showed that strains R7^T and R19^T were most closely related to *L. panacisoli* C8-1^T and *L. niabensis* GH34-4^T, with 98.7% and 97.8% 16S rRNA sequence similarities, respectively. The 16S rRNA gene sequence similarity between strains R7^T and R19^T was shown to be 95.8% 16S rRNA sequence similarity, which was lower than the 98.5–98.6% guideline value for species differentiation based on 16S rRNA gene sequence similarity (Stackebrandt and Goebel, 1994; Chun *et al*., 2018), which suggests that the two strains are different species of the genus *Lysobacter*. The 16S rRNA gene sequence similarities between strain R19^T and other validly published type strains were lower than 97.8%, suggesting that strain R19^T represents a novel species of the genus *Lysobacter*.

Phenotypic and morphological characteristics

Strains R7^T and R19^T grew well on R2A agar, 1/2 diluted R2A agar, TSA agar, and LB agar, but did not grow well on MA. The optimal growth medium of strains R7^T and R19^T was R2A agar and 1/2 diluted R2A agar, respectively. Strain R7^T grew at 10–37°C (optimum, 30°C) on R2A agar, at pH 6.0–9.0 (optimum, pH 7.0–8.0) in R2A broth, and in R2A broth supplemented with 0–1.5% NaCl (optimum, 0%). Strain R19^T grew at 10–37°C (optimum, 30°C) on 1/2 diluted R2A agar, at pH 6.0–9.0 (optimum, pH 8.0) in 1/2 diluted R2A broth, and in 1/2 diluted R2A broth supplemented with 0–1.5% NaCl (optimum, 0%). Both strains exhibited pale yellow, raised, and entire colonies after 3 days of incubation at 30°C. The cells of both strains were Gram-negative, non-motile, and rod-shaped, with sizes of approximately 0.35–0.4 × 1.0–1.2 μm and 0.25–0.35 × 0.85–1.8 μm for strains R7^T and R19^T, respectively (Supplementary data Fig. S2). Strain R7^T was able to grow under anaerobic conditions, suggesting that strain R7^T is a facultative aerobe, whereas strain R19^T was not able to grow under anaerobic conditions, suggesting that strain R19^T is an obligate aerobe. Physiological and biochemical characteristics of strains R7^T and R19^T are additionally described in the species descriptions and compared with those of closely related *Lysobacter* type strains in Table 1.

Chemotaxonomic analysis

The only respiratory quinone detected in strains R7^T and R19^T was Q-8, which is in good agreement with those detected in other strains of the genus *Lysobacter* (Christensen and Cook, 1978; Reichenbach, 2006; Weon *et al*., 2007; Aslam *et al*., 2009). The major cellular fatty acids (> 5% of the total fatty acids) of strains R7^T and R19^T were iso-$C_{14:0}$ (6.58%), iso-$C_{15:0}$ (20.53%), iso-$C_{16:0}$ (28.01%), and summed feature 9 (comprising $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ *ω9c*, 7.21%), and $C_{16:1}$ *ω7c* alcohol (5.02%), iso-$C_{11:0}$ (7.04%), iso-$C_{15:0}$ (21.80%),

Table 2. Cellular fatty acid compositions (%) of strains R7^T and R19^T and their related taxa of the genus *Lysobacter*

Taxa: 1, strain R7^T; 2, strain R19^T; 3, *L. enzymogenes* KACC 11382^T; 4, *L. oryzae* KACC 14553^T; 5, *L. niabensis* KACC 11587^T; 6, *L. panacisoli* KACC 17502^T; 7, *L. soli* KACC 15381^T. All data were obtained from this study. Data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 1% in all strains are not shown. Major components (> 5.0%) are highlighted in bold. tr, trace amount (< 1%); –, not detected.

Fatty acid	1	2	3	4	5	6	7
Saturated:							
$C_{16:0}$	2.65	1.51	**5.89**	3.54	2.11	2.00	1.01
$C_{18:0}$	1.85	tr	1.14	tr	tr	tr	tr
Unsaturated:							
$C_{16:1}$ *ω7c* alcohol	tr	**5.02**	tr	4.97	3.28	tr	-
$C_{16:1}$ *ω11c*	tr	tr	-	2.19	tr	-	-
iso-$C_{15:1}$ *ω9c*	-	2.72	-	tr	-	-	-
Branched:							
iso-$C_{10:0}$	tr	tr	tr	1.52	2.14	1.57	1.29
iso-$C_{11:0}$	4.41	**7.04**	3.18	**6.59**	**6.57**	**6.22**	**6.03**
iso-$C_{12:0}$	tr	tr	tr	1.21	1.84	-	tr
iso-$C_{14:0}$	**6.58**	4.43	1.79	3.07	**6.48**	3.22	**5.09**
iso-$C_{15:0}$	**20.53**	**21.80**	**32.08**	**10.73**	**12.21**	**33.27**	**23.38**
iso-$C_{16:0}$	**28.01**	**26.84**	**12.90**	**26.23**	**34.29**	**15.79**	**27.39**
iso-$C_{17:0}$	2.72	3.39	3.04	**6.32**	2.85	**5.15**	2.54
iso-$C_{16:1}$ H	tr	1.15	tr	tr	1.03	tr	1.33
iso-$C_{15:1}$ F	tr	-	tr	-	1.12	1.77	tr
Anteiso-$C_{15:0}$	2.41	1.60	3.24	**5.66**	2.55	1.04	2.94
Anteiso-$C_{17:0}$	tr	tr	tr	1.85	tr	tr	tr
Cyclo-$C_{17:0}$	4.75	-	4.44	-	tr	tr	tr
Hydroxy							
iso-$C_{11:0}$ 3-OH	3.26	**6.13**	4.28	3.13	4.51	3.28	2.48
Summed feature*:							
3	1.76	tr	**12.70**	1.37	1.24	3.07	4.81
8	tr	-	1.69	tr	tr	tr	tr
9	**7.21**	**11.75**	**5.63**	**12.41**	**11.38**	**15.17**	**11.61**

*Summed features represent groups of fatty acids that cannot be separated by gas–liquid chromatography with the MIDI system. Summed feature 3, $C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω6c* /$C_{16:1}$ *ω7c*, summed feature 8, $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*, and summed feature 9, $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ *ω9c*.

iso-$C_{16:0}$ (26.84%), iso-$C_{11:0}$ 3-OH (6.13%), and summed feature 9 (comprising $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ *ω*9*c*, 11.75%), respectively. Although the overall fatty acid profiles of strains $R7^T$ and $R19^T$ were similar to those of the species of the genus *Lysobacter*, there were some differences in the respective compositions of some fatty acid components, as shown in Table 2. Both strains $R7^T$ and $R19^T$ contained PG, phosphatidylethanolamine (PE), and an unidentified phospholipid as the major polar lipids (Supplementary data Fig. S3). The presence of PG and PE in strains $R7^T$ and $R19^T$ was in agreement with other *Lysobacter* species members (Aslam *et al*., 2009; Zhang *et al*., 2019; Jin *et al*., 2020; Xu *et al*., 2021). However, DPG that has been reported as a major polar lipid in many *Lysobacter* species (Jeong *et al*., 2016; Im *et al*., 2020; Jin *et al*., 2020; Xu *et al*., 2021) was not detected in strains $R7^T$ and $R19^T$ (Supplementary data Fig. S4). However, because the absence of DPG has been also reported in some *Lysobacter* species (Aslam *et al*., 2009; Siddiqi and Im, 2016), which suggests that the presence of DPG is different depending on *Lysobacter* species.

Genomic analysis

The *de novo* assembly of the genome sequencing data derived from the Nanopore and Illumina sequencing of strains $R7^T$ and $R19^T$ resulted in complete genomes of 3.94 Mb and 3.93 Mb with average genome coverages of 863× and 926×, respectively. The general genomic features of strain $R7^T$ and $R19^T$ were compared with those of closely related reference strains (Table 3). The genome of strain $R7^T$ had a circular chromosome encoding 3,609 total genes, and among the total genes, 3,449 coding DNA sequences (CDSs), 2 complete rRNA operons (16S, 23S, 5S), 50 tRNAs, 4 non-coding sequences, and 100 pseudo genes were identified. The genome of strain $R19^T$ had a circular chromosome encoding 3,480 total genes, and among the total genes, 3,229 CDSs, 2 complete rRNA operons (16S, 23S, 5S), 49 tRNAs, 4 non-coding sequences, and 192 pseudo genes were identified. The G + C contents of strains $R7^T$ and $R19^T$ calculated from the complete whole-genomes were 66.9 mol% and 68.9 mol%, respectively. The general features of the genomes of strains of $R7^T$ and $R19^T$, including genome size, DNA G + C contents, and the numbers of rRNA and tRNA genes, were similar with those of closely related reference strains (Table 3). The ANI and *in silico* DDH values between strain $R7^T$ and the type strain of *L. panacisoli* (JCM 19212^T, NZ_VLNU00000000), the most closely related type strain, were 81.9% and 24.7%, respectively (Table 3), which were clearly lower than the criteria for the prokaryotic species delineation thresholds (ANI, ~95%; *in silico* DDH, 70%) (Stackebrandt and Goebel, 1994; Chun *et al*., 2018), suggesting that strain $R7^T$ represents a novel species of the genus *Lysobacter*.

To survive and compete in diverse environmental habitats, *Lysobacter* species are known to produce various extracellular

Table 3. General genomic features[†] and their relatedness of strains $R7^T$ and $R19^T$ and closely related *Lysobacter* type strains
Taxa: 1, strain $R7^T$; 2, strain $R19^T$; 3, *L. enzymogenes* ATCC 29487^T; 4, *L. panacisoli* $CJ29^T$; 5, *L. soli* $DCY21^T$.

Characteristic		1	2	3	4	5
General genomic features						
Genome size (Mb)		3.94	3.93	6.26	3.88	3.95
No. of contigs		1	1	23	2	27
DNA G + C content (%)		66.9	68.9	69.2	67.5	67.7
No. of total genes		3609	3480	5135	3626	3622
No. of protein coding genes		3449	3229	5015	3546	3531
No. of pseudogenes		100	192	58	18	32
No. of rRNA genes (5S/16S/23S)		6 (2/2/2)	6 (2/2/2)	3 (1/1/1)	6 (2/2/2)	3 (1/1/1)
No. of tRNA genes		50	49	53	52	49
Extracellular enzymes						
No. of chitinase genes		-	-	2	1	-
No. of endopeptidase genes		12	9	11	10	9
No. of protease genes		24	37	33	27	26
No. of glucanase genes		-	-	1	-	-
No. of lipase genes		10	8	12	8	8
Type of secretion system		I, II	II, III	I, II, III, IV	I, II, III, IV	I, II, III, IV
Secondary metabolite biosynthesis genes						
Non-ribosomal peptide synthase		1	4	12	1	1
Polyketide synthase		1	1	3	1	1
GenBank accession number		JABCPY000000000.1	JAEKGA000000000.1	NZ_FNOG00000000.1	NZ_VLNU01000001.1	NZ_QTJR00000000.1
		dDDH value (%)[‡]				
ANI value (%)[‡]	1	-	22.3	23.9	24.7	23.9
	2	78.52	-	22.6	22.3	22.2
	3	79.95	78.22	-	24.1	23.2
	4	81.98	78.62	79.75	-	26.1
	5	81.39	78.11	79.07	83.67	-

[†] The bioinformatic analysis of the genomes was carried out using the NCBI prokaryotic genome annotation pipeline (www.ncbi.nlm.nih.gov/genome/annotation_prok/).
[‡] dDDH, digital DNA-DNA hybridization; ANI, average nucleotide identity.

enzymes and secondary metabolites, including chitinase, protease, glucanase, lipase, and antibiotics (Palumbo *et al*., 2003; Ko *et al*., 2009; Qian *et al*., 2012; Xie *et al*., 2012). Strains R7^T and R19^T possess 24 and 37 protease genes, 12 and 9 endopeptidase genes, 10 and 8 lipase genes, 1 and 4 non-ribosomal peptide synthase genes, and 1 and 1 polyketide synthase genes, respectively. *Lysobacter enzymogenes*, the type species of the genus *Lysobacter*, harbors genes encoding glucanase and chitinase (Palumbo *et al*., 2003; Qian *et al*., 2012), but no chitinase and glucanase genes were identified in the genomes of strains R7^T and R19^T. A type IV pilus operon (*pilBGMNOPQTUVWZ*) was identified from the genomes of strains R7^T and R19^T. Type I and type II secretion systems were identified from the genome of strain R7^T, whereas type II and type III secretion systems were identified from the genome of strain R19^T. Moreover, the analysis showed that other *Lysobacter* species harbor different types of secretion systems (Table 3), which suggests that *Lysobacter* species might have been independently evolved to harbor secretion systems. Genes encoding chitinases, endopeptidase, proteases, glucanases, lipases, antibiotics, and secretion systems of strains R7^T and R19^T and closely related *Lysobacter* species were compared in Table 3.

Taxonomic conclusion

The results of phylogenetic analyses based on 16S rRNA gene sequences and physiological and chemotaxonomic analyses clearly showed that strains R7^T and R19^T could be assigned to the genus *Lysobacter*. However, the formation of distinct phylogenetic lineages from the established *Lysobacter* type strains based on 16S rRNA gene sequences, 16S rRNA gene sequence similarity, and genome-based relatedness (ANI and *in silico* DDH) levels below the thresholds for novel species delineation, along with several different phenotypic characteristics clearly indicate that strains R7^T and R19^T were not affiliated with any other recognized species of the genus *Lysobacter*. In conclusion, the phylogenetic, physiological, and chemotaxonomic features suggest that strains R7^T and R19^T represent novel species of the genus *Lysobacter*, for which the names *Lysobacter arenosi* sp. nov. and *Lysobacter solisilvae* sp. nov. are proposed, respectively.

Description of *Lysobacter arenosi* sp. nov.

Lysobacter arenosi (ar.en.o′si L. gen. n. *arenosi* of a sandy place).

Cells are Gram-negative, facultative aerobic, and non-motile rods. Catalase- and oxidase-positive. Growth occurs at 10–37°C (optimum, 30°C), pH 6.0–9.0 (optimum, pH 7.0–8.0), and 0–1.5% (w/v) NaCl (optimum, 0%). Hydrolysis of starch, tyrosine, Tween 20, and Tween 80 is negative. Negative for indole production and glucose fermentation. Positive for arginine dihydrolase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase, but negative for lipase (C14), cysteine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, and urease. Assimilation of aesculin, gelatin, D-glucose, D-mannose, *N*-acetyl-glucosamine, and D-maltose is positive, but assimilation of L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid is negative. Q-8 is the sole isoprenoid quinone. Phosphatidylglycerol, phosphatidylethanolamine, and an unidentified phospholipid are detected as major polar lipids. The major fatty acids are iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (comprising $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ ω9*c*). The DNA G + C content of the type strain is 66.9 mol%.

The type strain is R7^T (= KACC 21663^T = JCM 34257^T), isolated from sandy soil of Yongwol in Gangwon Province of South Korea. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and genome sequences of strain R7^T are MN197915 and CP071517, respectively.

Description of *Lysobacter solisilvae* sp. nov.

Lysobacter solisilvae (so.li.sil′vae. L. n. *solum*, soil; L. n. *silva* forest; N.L. gen. n. *solisilvae* of/from forest soil).

Cells are Gram-negative, strictly aerobic, and non-motile rods. Catalase- and oxidase-positive. Growth occurs at 10–37°C (optimum, 30°C), pH 6.0–9.0 (optimum, pH 8.0), and 0–1.5% (w/v) NaCl (optimum, 0%). Hydrolysis of starch, aesculin, tyrosine, Tween 20, and Tween 80 is negative. Negative for the production of indole and glucose fermentation. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for arginine dihydrolase, lipase (C14), α-chymotrypsin, trypsin, valine arylamidase, α-glucosidase, cysteine arylamidase, α-galactosidase, β-glucosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, urease, and α-fucosidase. Assimilation of gelatin is positive, but assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid is negative. Q-8 is the sole isoprenoid quinone. Phosphatidylglycerol, phosphatidylethanolamine, and an unidentified phospholipid are detected as major polar lipids. The major fatty acids are $C_{16:1}$ ω7*c* alcohol, iso-$C_{11:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{11:0}$ 3-OH, and summed feature 9 (comprising $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ ω9*c*). The DNA G + C content of the type strain is 68.9 mol%.

The type strain is R19^T (= KACC 21767^T = JCM 34258^T), isolated from forest soil of Yongwol in Gangwon Province of South Korea. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and genome sequences of strain R19^T are MW173243 and CP071518, respectively.

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Conflict of Interest

The authors declare no competing financial conflict of interest.

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