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Lysobacter zhanggongensis sp. nov. Isolated from a Pit Mud

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Abstract The Gram-stain-negative, rod-shaped and non-motile bacterial strain, designated ZGLJ7-1^T, was isolated from a pit mud. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain ZGLJ7-1^T was related to the genus *Lysobacter* and had the highest 16S rRNA gene sequence similarity with the type strain of *Lysobacter arseniciresistens* ZS79^T (97.4%). The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{11:0}$ and iso-$C_{11:0}$3-OH. Strain ZGLJ7-1^T had Q-8 as the predominant ubiquinone. The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid, two unidentified aminolipids and two unidentified lipids. The genomic DNA G+C content of strain ZGLJ7-1^T was 69.5 mol%. Strain ZGLJ7-1^T shared DNA relatedness with 35% *Lysobacter arseniciresistens* CGMCC 1.10752^T. Combined data from phenotypic, phylogenetic and DNA–DNA relatedness studies demonstrated that the strain ZGLJ7-1^T is a representative of a novel species of the genus *Lysobacter*, for which we propose the name *Lysobacter zhanggongensis* sp. nov. (type strain ZGLJ7-1^T = KACC 18547^T = CGMCC 1.15404^T).

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Introduction

The genus *Lysobacter* was established by Christensen and Cook [1] and classified within the family Xanthomonadaceae [2]. Later its description was emended by Park et al. [3]. At the time of writing, the genus *Lysobacter* contains 40 species with validly published names, with *Lysobacter* enzymogenes as the type species. The members of the genus *Lysobacter* have been isolated from various natural environments, including soil, ore, sludge and water samples. Members of the genus *Lysobacter* contain ubiquinone Q-8 as the major respiratory quinone, show a predominance of iso-branched fatty acids and have a high DNA G+C content [2, 4–8].

In 2015, in the course of our study on the diversity of culturable bacteria in aged pit mud, a bacterial strain designed ZGLJ7-1^T was isolated from white wine enterprise. Phenotypic, genotypic and phylogenetic characteristics suggested that it represents a novel species of the genus *Lysobacter*.

Materials and Methods

Isolation of the Bacterial Strain and Culture Conditions

Strain ZGLJ7-1^T was isolated from a pit mud of Zhanggong white wine enterprise in Henan Province of China. There grow varieties of microbial groups of different functions in pit mud which are helpful for wine-making. Sample was suspended with sterile saline solution (0.8%) immediately and appropriate dilutions were then plated on LB agar plate at 30 °C. After incubation at 30 °C for 4 days, single colonies were selected and subcultured on LB to achieve purity. One of the pure cultures was designated ZGLJ7-1^T.

Strain *Lysobacter arseniciresistens* ZS79^T (CGMCC 1.10752^T) [6] was obtained from CGMCC for comparison as a reference species. Strain ZGLJ7-1^T and *Lysobacter arseniciresistens* CGMCC 1.10752^T were routinely cultured on LB at 30 °C and stored as a suspension with 25% (v/v) glycerol at −80 °C.

Morphological, Physiological and Biochemical Analyses

Gram staining was tested using the bioMérieux Gram stain kit. Cell morphology was examined by phase contrast microscopy (×1000; Zeiss Axiostar Plus) and scanning electron microscopy (Oxford INCA 250) of cells grown on LB at 30 °C.

Growth at 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C was assessed on LB. The pH range for growth was determined at 30 °C in LB at pH 4.0–10.0 (with intervals of 0.5 units) using the following buffers: citrate/Na2HPO4 buffer (pH 4.0–7.0), Tris buffer (pH 7.5–9.0) and NaHCO3/Na2CO3 buffer (pH 9.5–10.0); growth was evaluated by measuring OD600 after 3 days of incubation. Growth with 0–10% (in 1% increments, w/v) NaCl was investigated after 14 days of cultivation at 30 °C in LB liquid medium. Physiological and biochemical characteristics and enzyme activities were determined using API 20 NE, API 20 E and API ZYM systems (bioMérieux) at 30 °C according to the manufacturers' instructions.

Determination of DNA G+C Content, 16S rRNA Gene Sequencing and Phylogenetic Analyses

DNA was extracted and purified as described by Sambrook and Russell [9]. The 16S rRNA gene was amplified and sequenced according to previous protocols [10]. The EzTaxon-e database was used to identify the nearest taxa [11]. Multiple sequence alignments were performed using the CLUSTAL-W program integrated in the MEGA version 6 [12]. The phylogenetic trees were reconstructed using maximum-likelihood (ML), maximum-parsimony (MP) and neighbour-joining (NJ) algorithms in the MEGA version 6.0. The resultant tree topology generated was evaluated by bootstrap analysis based on 1000 replicates.

DNA–DNA Hybridization Test

The DNA G+C content was determined using the thermal denaturation method [13] using Escherichia coli K12 as the calibration standard. DNA–DNA hybridizations were done by the liquid renaturation method [14] as modified by Huss et al. [15]. DNA–DNA hybridizations were carried out in 2 × SSC at 82 °C and each determination was done in triplicate. Both experiments were performed at 260 nm with a model Lambda 35 UV/VIS spectrometer equipped with a Peltier System (PTP 1 + 1) (Perkin-Elmer).

Chemotaxonomic Analysis

For fatty acid methyl ester analysis, strain ZGLJ7-1^T and the reference strain *Lysobacter arseniciresistens* CGMCC

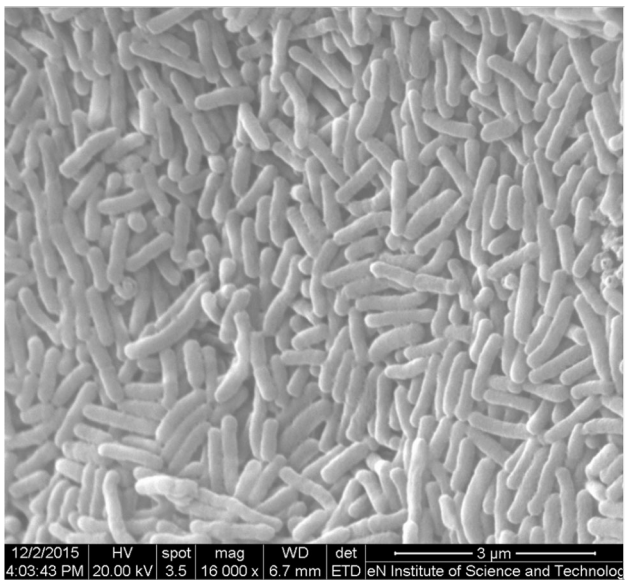

Fig. 1 Scanning electron microscopy (SEM) of strain ZGLJ7-1^T grown at 30 °C on LB agar

Table 1 Phenotypic characteristics that differentiate strain ZGLJ7-1^T from *Lysobacter arseniciresistens* CGMCC 1.10752^T

Characteristic	1	2
Source	Pit mud	Iron-mined soil
Motility	–	+
Hydrolysis of urea	+	–
Cystine arylamidase	+	–
Trypsin	+	–
α-chymotrypsin	+	–
Trisodium citrate	+	–

Strains: *1* ZGLJ7-1^T, *2* *Lysobacter arseniciresistens* CGMCC 1.10752^T (all data from this study)

+ positive, − negative

Both strains are positive for hydrolysis of gelatin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-Bi-phosphohydrolase, and negative for lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, assimilation of D-glucose, *N*-acetyl-glucosamine, D-maltose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid, indole production, glucose fermentation, arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, Voges–Proskauer and H_2S production

1.10752^T were grown on TSA at 30 °C for 3 days. All two strains shared similar growing behaviour and a sufficient amount of cells of comparable physiological age could be harvested from the third streak quadrant of the MA plates after cultivation under the applied conditions. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI, version 6.1) [16], using the data bank TSBA40 for calculation.

Respiratory quinones were extracted according to Altenburger et al. [17] and were analysed by HPLC [18]. Analysis of polar lipids of strain ZGLJ7-1^T was carried out by the Identification Service, DSMZ, Braunschweig (Germany) according to the methods of Bligh and Tindall et al. [19–22].

Results and Discussion

Morphological, Physiological and Biochemical Characteristics

Cells of strain ZGLJ7-1^T grown on LB agar plate were observed to be straight rods with 0.2–0.3 μm in width and 1.2–1.7 μm in length and devoid of flagella (Fig. 1). The strain hydrolyses urea and gelatin, but do not use esculin. Nitrate reduction and Voges–Proskauer activity are not present.

The strain showed distinct phenotypic features that discriminated it from the most closely related member in the genus *Lysobacter* as shown in Table 1. More detailed results of phenotypic features of the strain are given in the species description.

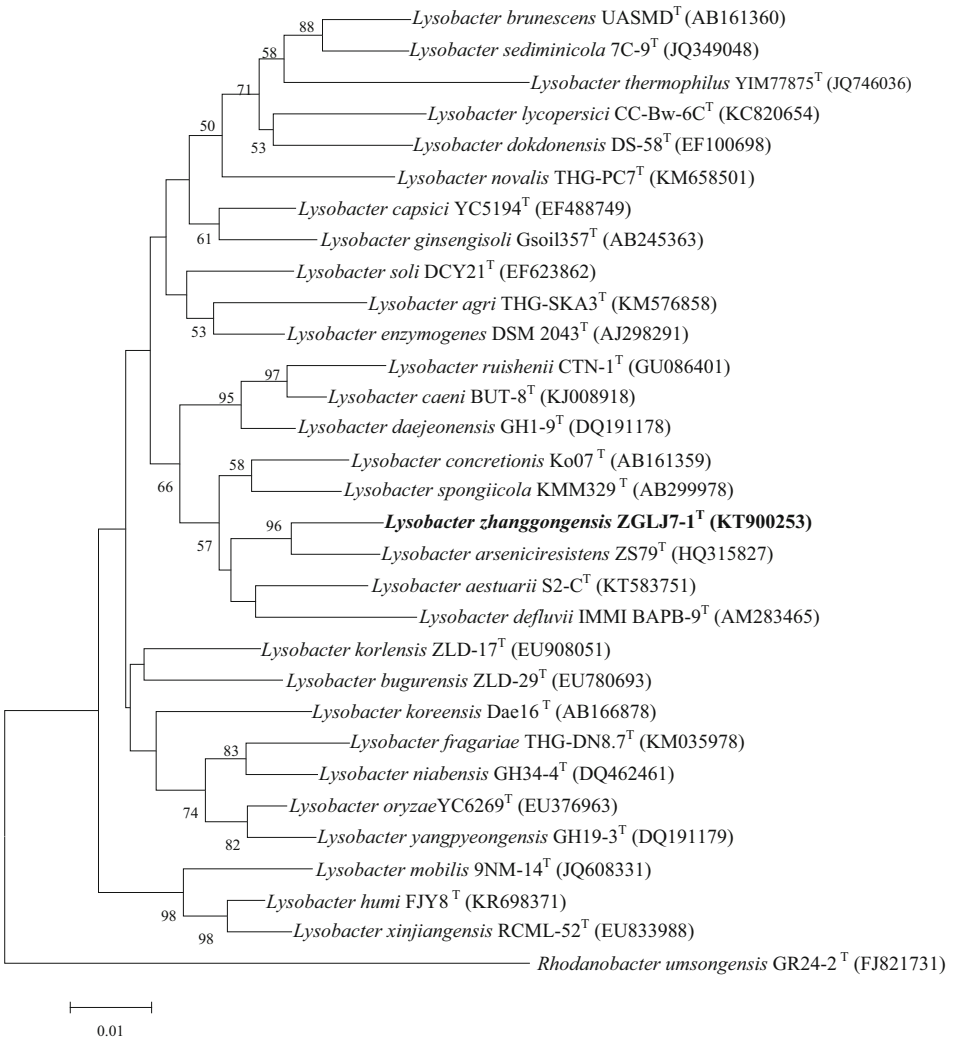

Fig. 2 Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain ZGLJ7-1^T and members of the genus *Lysobacter*. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 50% support. The 16S rRNA gene sequence of *Rhodanobacter* umsongensis GR24-2^T was used as an outgroup. GenBank accession numbers of 16S rRNA sequences are given in parentheses. *Bar* 0.01 substitutions per nucleotide position

DNA G+C Content, Phylogenetic Analysis and DNA–DNA Hybridization Test

The DNA G+C Content of strain ZGLJ7-1^T was 69.5 mol%. The 16S rRNA gene sequences of ZGLJ7-1^T were determined (GenBank/EMBL/DDBJ accession number KT900253). On the basis of pairwise comparisons of the 16S rRNA gene sequences using the recent version of the EzTaxon-e, strain ZGLJ7-1^T had the highest 16S rRNA gene sequence similarity with the type strain of *Lysobacter arseniciresistens* ZS79^T (97.4%). The reconstructed phylogenetic tree based on the NJ algorithm [23] revealed that strain ZGLJ7-1^T was grouped with the members of the genus *Lysobacter* and formed a coherent cluster with *Lysobacter arseniciresistens* ZS79^T (Fig. 2). This phylogenetic position was confirmed in the tree generated using the ML and MP algorithms.

The DNA–DNA hybridization experiments revealed that strain ZGLJ7-1^T shared DNA relatedness with 35% *Lysobacter arseniciresistens* CGMCC 1.10752^T. All these values were well below the 70% cut-off point recommended for the assignment of strains to the same genospecies [24].

Table 2 Cellular fatty acid composition of strain ZGLJ7-1^T from *Lysobacter arseniciresistens* CGMCC 1.10752^T

Fatty acid	1	2
Straight-chain		
$C_{10:0}$	0.1	0.1
$C_{14:0}$	0.2	0.1
$C_{15:0}$	0.2	0.1
$C_{16:0}$	1.3	0.9
Branched		
Iso-$C_{10:0}$	–	0.1
Iso-$C_{11:0}$	8.9	12.8
Iso-$C_{12:0}$	0.1	0.2
Iso-$C_{13:0}$	0.4	0.3
Iso-$C_{14:0}$	0.4	0.3
Iso-$C_{15:0}$	38.2	34.0
Anteiso-$C_{15:0}$	0.5	0.3
Iso-$C_{16:0}$	3.6	3.4
Iso-$C_{17:0}$	7.0	5.5
Anteiso-$C_{17:0}$	0.1	–
Unsaturated		
$C_{15:1}\omega6c$	0.1	0.1
Iso-$C_{17:1}\omega9c$	27.4	26.4
Iso-$C_{15:1}$F	2.3	1.7
Hydroxy		
Iso-$C_{11:0}$3-OH	8.1	12.0
Summed features[a]		
Summed feature 1	0.1	0.3
Summed feature 3	1.0	1.2

Strains: 1, ZGLJ7-1^T; 2, *Lysobacter arseniciresistens* CGMCC 1.10752^T (all data from this study). All strains were incubated on TSA at 30 °C for 2 days prior to fatty acid analysis. Values are percentages of total fatty acids

– not detected

[a] Summed features contained fatty acids, which could not be separated by GLC with the Microbial Identification System (MIDI). Summed feature 1 consists of $C_{13:0}$3-OH and/or iso-$C_{15:1}$; Summed feature 3 comprises iso-$C_{15:0}$2-OH and/or $C_{16:1}\omega7c$. Equivalent chain length

Chemotaxonomic Characteristics

The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{11:0}$ and iso-$C_{11:0}$3-OH. Thus, the fatty acid profiles of strain ZGLJ7-1^T resembled those of other *Lysobacter* species [6]. Details of the fatty acid profiles of strain ZGLJ7-1^T and the reference strain are available in Table 2.

The quinone system of strain ZGLJ7-1^T consisted predominantly of ubiquinone-8 (98.7%), which was consistent with members of the genus *Lysobacter* [3], and also traces of ubiquinone-7 (1.3%).

The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid, two unidentified aminolipids and two unidentified lipids (Fig. 3).

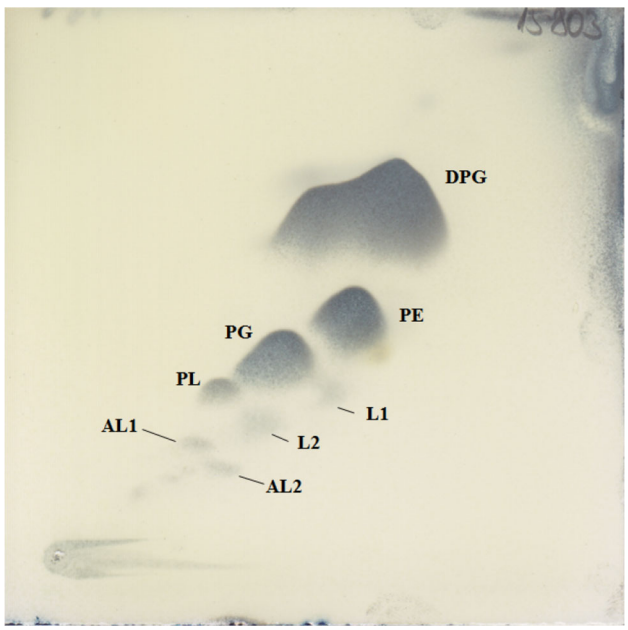

Fig. 3 Two-dimensional thin-layer chromatogram showing the polar lipid of strain ZGLJ7-1^T which was stained with 5% ethanolic molybdatophosphoric acid. Separation was performed using Merck silica gel 60 F254 aluminium-backed thin-layer plates, chloroform/methanol/water (65:25:4) in the first direction and chloroform/acetic acid/methanol/water (80:15:12:4) in the second direction. *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PL* unidentified phospholipid, *AL1-2* unidentified aminolipids, *L1-2* unidentified polar lipids

Description of *Lysobacter zhanggongensis* sp. nov.

Lysobacter zhanggongensis (N. L. fem. adj. Zhanggongensis, pertaining to Zhanggong, a White Wine enterprise, from where the type strain was isolated). Cells are Gram-stain-negative, non-motile, rod-shaped cells, 1.2–1.7 μm long and 0.2–0.3 μm wide. Growth occurs in media with 0–4% (w/v) NaCl, with the optimum at 1%. Grows at 5–40 °C but not at 45 °C on LB (optimum growth at 30 °C). The pH for growth is 5.5–10.0, with the optimum at pH 8.0. Hydrolyses urea and gelatin, but not esculin. Nitrate reduction and Voges–Proskauer activity are not present. In the API ZYM strip, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-Bi-phosphohydrolase, but other enzyme activities in API ZYM systems are negative (Supplementary Table S1). In API 20NE and 20E strips, positive for urease, gelatinase, trisodium citrate and tryptophan deaminase. The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{11:0}$ and iso-$C_{11:0}$3-OH. Strain ZGLJ7-1^T had Q-8 as the predominant ubiquinone. The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid, two unidentified aminolipids and two unidentified lipids. The genomic DNA G+C content of strain ZGLJ7-1^T was 69.5 mol%.

The type strain is ZGLJ7-1^T (=KACC 18547^T = CGMCC 1.15404^T) and was isolated from a pit mud of Zhanggong white wine enterprise in Henan Province of China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Lysobacter zhanggongensis* ZGLJ7-1^T is KT900253. The respective DPD TaxonNumber is TA00131.

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Compliance with Ethical Standards

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

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