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ORIGINAL PAPER

Lysobacter tongrenensis sp. nov., isolated from soil of a manganese factory

Jingxin Li[1] · Yushan Han[1] · Wei Guo[1] · Qian Wang[1,3] · Shuijiao Liao[1,2] · Gejiao Wang[1]

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Abstract

A Gram-staining negative, aerobic, non-motile, rod-shaped bacterial strain, designated YS-37^T, was isolated from soil in a manganese factory, People's Republic of China. Based on16S rRNA gene sequence analysis, strain YS-37^T was most closely related to *Lysobacter pocheonensis* Gsoil 193^T (97.0%), *Lysobacter dokdonensis* DS-58^T (96.0%) and *Lysobacter daecheongensis* Dae08^T (95.8%) and grouped together with *L. pocheonensis* Gsoil 193^T and *Lysobacter dokdonensis* DS-58^T. The DNA–DNA hybridization value between strain YS-37^T and *L. pocheonensis* KCTC 12624^T was 43.3% (± 1). The major respiratory quinone of strain YS-37^T was ubiquinone-8, and the polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phospholipid, phosphatidylmethylethaolamine and two unknown lipids. Its major cellular fatty acids (> 5%) were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{16:0}$, iso-$C_{11:0}$ 3-OH and iso-$C_{11:0}$ and the G + C content of the genomic DNA was 67.1 mol%. Strain YS-37^T also showed some biophysical and biochemical differences with the related strains, especially in hydrolysis of casein. The results demonstrated that strain YS-37^T belongs to genus *Lysobacter* and represents a novel *Lysobacter* species for which the name *Lysobacter tongrenensis* sp. nov. is proposed. The type strain is YS-37^T (= CCTCC AB 2016052^T = KCTC 52206^T).

Keywords *Lysobacter tongrenensis* · Soil · Polyphasic taxonomy · 16S rRNA gene

Introduction

Genus *Lysobacter* was first described by Christensen and Cook in 1978 with *Lysobacter enzymogenes* as the type species (Christensen and Cook 1978). This genus was classified within family *Xanthomonadaceae* of *Gamma-Proteobacteria* (Saddler and Bradbury 2005) and the description was later emended by Park et al. (2008). To data, the genus *Lysobacter* consists of 43 species (http://www.bacterio.net/lysobacter.html). *Lysobacter* members are generally Gram-negative, aerobic, gliding and have a high DNA G + C contents (61.7–70.7%), and the major quinones, fatty acids and polar lipids are of ubiquinone-8 (Q-8), iso-branched fatty acids, and diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), respectively (Christensen and Cook 1978; Christensen 2005; Weon et al. 2006; Ten et al. 2008, 2009; Srinivasan et al. 2010; Liu et al. 2011; Wang et al. 2011; Oh et al. 2011; Luo et al. 2012; Ye et al. 2015; Ngo et al. 2015). Here we describe the polyphasic characteristics of strain YS-37^T that related to *Lysobacter* members.

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Jingxin Li and Yushan Han contributed equally to this work.

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✉ Gejiao Wang
gejiao@mail.hzau.edu.cn

[1] State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, People's Republic of China

[2] College of Basis Science, Huazhong Agricultural University, Wuhan, Hubei 430070, People's Republic of China

[3] Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717, USA

Materials and methods

Bacterial strain and culture conditions

Strain YS-37^T was isolated from soil of a manganese factory named Guizhou Dalong Manganese Industry (109°04′E, 27°28′N) in Tongren city, Guizhou Province, People's Republic of China. The soil sample was suspended in 0.85% NaCl (w/v) and the diluted solutions were spread on R2A agar plates and incubated at 28 °C for 1 week. A total of 32 strains were isolated (data not shown) and a strain named YS-37^T was selected for this study due to the preliminary low 16S rRNA gene sequence similarity. The resistance levels of strain YS-37^T for multi-metal(loids) were tested with the minimal inhibition concentration (MIC) on R2A agar plates using $MnCl_2$, Na_3AsO_3, $K_2Sb_2(C_4H_2O_6)_2$, K_2CrO_4, $CdCl_2$ and $PbCl_2$. Strains *L. pocheonensis* KCTC 12624^T, *L. dokdonensis* KCTC 12822^T and *L. daecheongensis* KCTC 12600^T were purchased from the Korean Centre of Type Cultures and used as reference strains.

Phylogenetic analysis

Genomic DNA of strain YS-37^T was extracted according to standard procedures (Sambrook and Russell 2001). The primers 27F and 1492R were used for amplification of the 16S rRNA gene fragment as described by Brosius et al. (1978). The PCR product was purified and cloned into pGEM-T vector (Promega), subsequently PCR amplified using primers T7 and SP6 and sequenced in Tsingke Company (Beijing, China). The nearly completed 16S rRNA gene sequence (1506 bp) of strain YS-37^T was aligned with those from EzTaxon database (http://eztaxon-e.ezbiocloud.net) (Chun et al. 2007) using the CLUSTAL X program (Thompson et al. 1997). Phylogenetic analyses were performed with MEGA 6 (Tamura et al. 2013) using neighbor-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) algorithms. For each method, bootstrap values were calculated based on 1000 replications (Felsenstein 1985).

Morphological, physiological, and biochemical characterization

Cellular morphology was observed by scanning electron microscopy (SEM, JSM-6390; JEOL) (Fig. S1). Gram reaction was determined using a Gram-staining kit (Jiancheng Biotech, China) in combination with the 3% KOH method (Smibert and Krieg 1994). Gliding motility was tested on a fresh R2A broth culture using the hanging-drop method (Bernardet et al. 2002). Growth was assessed at various temperatures (0, 4, 16, 20, 25, 28, 32, 37, 42 and 45 °C) on R2A agar, and in various NaCl concentrations (0, 0.5, 1, 2, 3, 5, 6, and 7%, w/v) in R2A liquid medium at 28 °C. The pH range (pH 4–10) was tested in R2A liquid medium with different buffer systems (0.1 M citric acid/0.1 M sodium citrate, for pH 4.0–5.0; 0.1 M KH_2PO_4/0.1 M NaOH, for pH 6.0–8.0; 0.1 M $NaHCO_3$/0.1 M Na_2CO_3, for pH 9–10). Growth under anaerobic condition was determined by incubation in an anaerobic chamber at 28 °C for 2 weeks on R2A agar. Oxidase and catalase activities were determined using oxidase reagent and 3% (v/v) H_2O_2, respectively (Smibert and Krieg 1994). Nitrate and nitrite reduction tests were performed as described by Lányí (1987). Hydrogen sulfide production, indole test and hydrolyses of casein (5%, w/v), gelatin (15%, w/v), Tween 20, 40, 60, 80 and starch (0.2%, w/v) were determined according to Smibert and Krieg (1994). Acid production from carbohydrates were also tested (Dong and Cai 2001). The API 20 NE, API ID 32GN and API ZYM systems (bioMérieux) were used to determine biochemical properties, utilization of carbohydrates and enzyme activities according to the manufacturer's protocols, and if necessary, in combination with traditional methods.

Chemotaxonomic characterization

For whole-cell fatty acid analysis, cells of strain YS-37^T and the three reference strains were inoculated on R2A agar and harvested when the cells growth reached the exponential phase determined by the quadrant streak pattern method according to the protocol of MIDI (Sherlock Microbial Identification System, MIDI) and analyzed by gas chromatography (MIDI Sherlock version 4.5; MIDI database TSBA40 4.10) (Sasser 1990). For the extraction of respiratory quinones and polar lipids, the strains were cultured in R2A medium and freeze-dried after harvesting. Respiratory quinone analysis was performed by HPLC as described by Minnikin et al. (1984). Polar lipid analyses of strain YS-37^T and *L. dokdonensis* KCTC 12822^T were determined by two-dimensional TLC method as described by Tindall (1990). The DNA G + C content was determined by HPLC according to the method of Mesbah et al. (1989). Genomic DNA was extracted as described by Pitcher and Saunders (1989) for DNA–DNA hybridization analysis, which was performed using the thermal denaturation and renaturation method of De Ley et al. (1970).

Results and discussion

The 16S rRNA gene sequence of strain YS-37^T was closely related to *Lysobacter pocheonensis* Gsoil 193^T (97.0%), *Lysobacter dokdonensis* DS-58^T (96.0%) and *Lysobacter daecheongensis* Dae08^T (95.8%), and showed similarities less than 95.4% with the other members of the genus *Lysobacter* including the type species strain *Lysobacter enzymogenes* (93.9%). In the phylogenetic tree based on neighbor-joining algorithm, strain YS-37 ^{T} was closely related to the *Lysobacter* members and formed a branch with *L. pocheonensis* Gsoil 193^T and *L. dokdonensis* DS-58^T (Fig. 1). The phylogenetic trees based on the maximum-parsimony and maximum-likelihood algorithms showed similar relationships to those of the neighbor-joining method (marked with * in Fig. 1).

Cells of strain YS-37^T were Gram-staining-negative, aerobic, non-motile and rod-shaped (for details, see species description). In addition, strain YS-37^T is very resistant to Mn(II), As(III) and Sb(III) with MICs of 50, 2.0 and 3.0 mmol/L, respectively. The MICs for Cr(VI), Cd(II) and Pb(II) are 1.0, 0.1 and 0.8 mmol/L, respectively, indicating a certain degree of resistance. Strain YS-37^T was positive for catalase, oxidase and hydrolysis of gelatin, negative for urease activity, indole production and hydrogen sulfide production. These characteristics are consistent with the strains of the genus *Lysobacter* (Weon et al. 2006; Luo et al. 2012; Ye et al. 2015). The flexirubin-type pigment was absent. Strain YS-37^T showed some biophysical and biochemical differences with the related strains, especially in hydrolysis of casein (Table 1, supplementary material Table S1).

The DNA–DNA hybridization value between strain YS-37^T and the closely related strain *L. pocheonensis* KCTC 12624^T was 43.3% (± 1, $n = 2$), which is below the threshold value of 70% recommended for species delineation (Wayne et al. 1987). The whole-cell fatty acid compositions of strain YS-37^T, *L. pocheonensis* KCTC 12624^T, *L. dokdonensis* KCTC 12822^T, *L. daecheongensis* KCTC 12600^T and *L. enzymogenes* DSM 2043^T are shown in Table 2. The major fatty acids of strain YS-37^T were the branched compounds iso-$C_{15:0}$ (26.9%), iso-$C_{17:1}\omega9c$ (18.1%), iso-$C_{16:0}$ (9.6%), iso-$C_{11:0}$ 3-OH (13.7%) and iso-$C_{11:0}$ (8.4%). The fatty acid profile of strain YS-37^T is similar to the *Lysobacter* type strains (Weon et al. 2007; Bae et al. 2005; Ngo et al. 2015; Siddiqi and Im 2016), but a little different from the most closed *L. pocheonensis* KCTC 12624^T which has summed feature 9 (Siddiqi and Im 2016). The only respiratory quinone of strain YS-37^T was Q-8, which is typical of

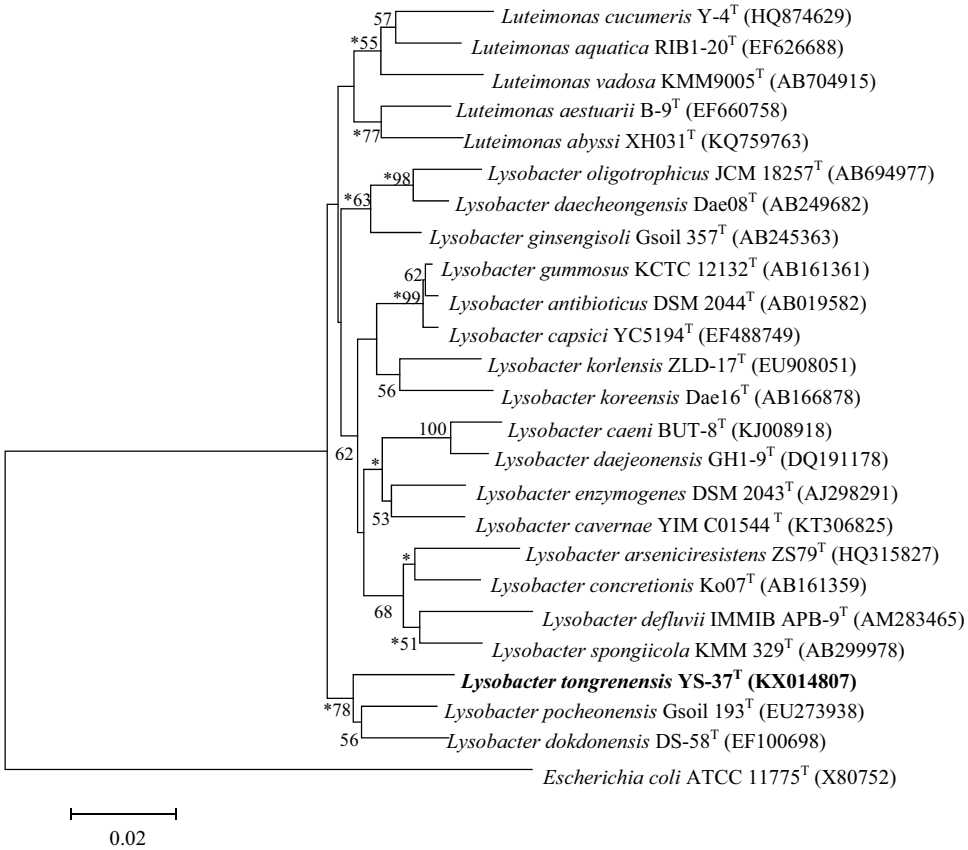

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain YS-37^T and closely related strains. Bootstrap values > 50% (based on 1000 replications) are shown at branching points. *Bar* 0.02 substitutions per nucleotide position. The asterisk (*) indicates identical branch topologies of the maximum-parsimony and maximum-likelihood trees

Table 1 Differential phenotypic characteristics of strain YS-37^T and related *Lysobacter* strains

Characteristic	1	2	3	4	5
Colony color	LY	LY	LY	C	DY-C
NaCl tolerance (%, w/v)	0-0.5	0-0.5	0-0.5	0–7	0–2
pH range	6.5–7.5	5.0–9.0	6.5-8.0	6.0–8.0	6.0–10.0
Catalase	+	−	+	−	+
Hydrolysis of					
Casein	−	+	+	+	+
Gelatin	+	+	+	−	+
Aesculin	−	−	−	+	+
Esculin ferric citrate	−	−	+	+	ND
Urea	−	+	−	−	−
Assimilation of					
L-proline	+	−	−	−	+
L-arginine	−	+	−	−	−
Glycogen	−	−	+	+	+
Caprate	−	−	−	+	ND
N-acetylglucosamine	−	−	−	+	+
Maltose	−	−	+	−	+
Alanine	−	−	+	−	ND
Enzyme activities					
Valine arylamidase	+	+	+	−	ND
Cystine arylamidase	w	+	w	−	ND
α-glucosidase	−	−	w	−	+
Lipaes (C14)	−	−	−	+	ND
α-chymotrypsin	−	+	−	−	ND
DNA G + C content (mol%)	67.1	64.8[a]	68.1[b]	69.3[c]	69.0[d]

Strains: 1, strain YS-37^T; 2, *L. pocheonensis* KCTC 12624^T; 3, *L. dokdonensis* KCTC 12822^T; 4, *L. daecheongensis* KCTC 12600^T; 5, *L. enzymogenes* DSM 2043^T (Christensen and Cook 1978). These data of 1, 2, 3 and 4 are from this study except for the G + C% contents. Data of 5 are from Christensen and Cook 1978; Bae et al. 2005; Ten et al. 2009

+ positive, − negative, *w* weakly positive, *ND* no data available, *LY* light yellow, *DY* deep yellow, *C* cream

[a]Data from Siddiqi and Im 2016

[b]Data from Oh et al. 2011

[c]Data from Ten et al. 2008

[d]Data from Christensen and Cook 1978

the *Lysobacter* strains (Christensen and Cook 1978; Yassin et al. 2007; Choi et al. 2014; Ngo et al. 2015; Siddiqi and Im 2016). The polar lipids of strain YS-37^T were DPG, PE, PG, phosphatidylmethylethaolamine (PME), phospholipid (PL) and two unknown polar lipids (ND) (Fig. S2A). The polar lipids of *L. dokdonensis* KCTC 12822^T were DPG, PE, PG, P, PL, aminolipid (AL) and phosphoaminolipid (PN) (Fig. S2B). The present of PME and the two NDs and the absent of PN and AL could differentiate strain YS-37^T with the related strain *L. dokdonensis* KCTC 12822^T. In addition, the polar lipids of *L. daecheongensis* KCTC 12600^T were DPG, PE, PG, PME and unknown aminolipid (Ten et al. 2008), strain KCTC 12600^T did not contain PL which is different from strain YS-37^T. The DNA G + C content of strain YS-37^T is 67.1 mol%, which is within the range described for the genus *Lysobacter* (61.7–70.7 mol%) (Christensen and Cook 1978; Weon et al. 2006; Park et al. 2008; Luo et al. 2012; Liu et al. 2015).

These data indicate that strain YS-37^T belongs to genus *Lysobacter*, but can be differentiated from the related *Lysobacter* members by DNA–DNA hybridization, fatty acids and biochemical and physiological characteristics. Based on the polyphasic taxonomic results described in this study, strain YS-37^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter tongrenensis* sp. nov. is proposed.

Description of *Lysobacter tongrenensis* sp. nov

Lysobacter tongrenensis (tong.ren.en'sis. N.L. masc. adj. *tongrenensis* pertaining to Tongren, a city of Guizhou

Table 2 Cellular fatty acid composition of strain YS-37^T and related *Lysobacter* strains

Fatty acid	1	2	3	4	5
$C_{14:0}$	1.0	–	1.1	0.8	1.2
$C_{16:0}$	2.1	0.7	4.5	1.7	5.1
iso-$C_{11:0}$	8.4	5.8	6.2	11.2	4.3
iso-$C_{11:0}$ 3OH	13.7	8.3	8.2	11.2	6.0
iso-$C_{13:0}$	1.1	–	–	–	–
iso-$C_{14:0}$	2.7	6.6	2.9	4.9	1.4
iso-$C_{15:0}$	26.9	30.4	28.5	43.2	43.0
iso-$C_{15:1}$ F	4.8	1.8	0.9	2.0	–
anteiso-$C_{15:0}$	0.8	3.8	3.4	0.8	3.8
iso-$C_{16:0}$	9.6	20.6	15.1	7.2	3.0
$C_{16:0}$ N alcohol	–	–	3.3	–	–
$C_{17:0}$ cyclo	–	–	–	–	10.6
iso-$C_{16:1}$ H	–	1.6	–	0.6	–
iso-$C_{17:0}$	1.6	0.9	2.3	0.7	4.4
$C_{16:1}\omega7c$ alcohol	1.5	–	–	–	–
$C_{16:1}\omega11c$	1.5	–	–	–	–
iso-$C_{17:1}\omega9c$	18.1	–	15.4	8.5	8.8
Summed features*					
1	–	1.2	0.7	1.2	–
3	2.5	2.1	4.0	4.4	–
4	–	–	–	–	8.3
9	–	15.3	–	–	–

Strains: 1, strain YS-37^T; 2, *L. pocheonensis* KCTC 12624^T; 3, *L. dokdonensis* KCTC 12822^T; 4, *L. daecheongensis* KCTC 12600^T; 5, *L. enzymogenes* DSM 2043^T (Christensen and Cook 1978). Data of 1, 2, 3 and 4 are from this study. Data of 5 are from Christensen and Cook 1978; Bae et al. 2005; Ten et al. 2009

– less than 0.5% or not detected

*Summed features combine groups of two or more fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 comprised iso-$C_{15:1}$ H and/or $C_{13:0}$ 3OH; Summed feature 3 comprised $C_{16:1}\omega7c$ and/or $C_{15:0}$ 2OH; Summed feature 4 comprised iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; Summed feature 9 comprised iso-$C_{17:1}$ $\omega9c$/$C_{16:0}$ 10-methyl

Province in Southwest People's Republic of China, from where the type strain was isolated).

Cells are Gram-staining-negative, strictly aerobic, non-motile and rod-shaped (0.3–0.4 × 0.6–1.5 μm). Colonies grown on R2A plates are 0.5–1.0 mm in diameter, circular, smooth, convex and light yellow-colored. Growth occurs at 4–32 °C (optimum 28 °C), at pH 6.5–7.5 (optimum pH 7.0) and NaCl concentrations in the range 0–0.5% (optimum, 0%). Positive for oxidase, catalase, hydrolysis of gelatin and assimilation of L-proline. The leucine arylamidase, valine arylamidase and trypsin are positive, and alkaline phosphatase, esterase (C4), esterase lipase (C8), cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are weakly positive. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{16:0}$, iso-$C_{11:0}$, and iso-$C_{11:0}$ 3-OH. The only respiratory quinone is ubiquinone Q-8, and the polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethaolamine, phospholipid and two unknown lipids. The G + C content of the genomic DNA of the type strain is 67.1 mol%.

The type strain is YS-37^T (= CCTCC AB 2016052^T = KCTC 52206^T), was isolated from soil of a manganese factory in Tongren city, Guizhou Province, People's Republic of China. The Digital Protologue database TaxonNumber for strain YS-37^T is TA00250. The GenBank accession number for the 16S rRNA gene sequence of strain YS-37^T is KX014807.

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