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Lysobacter thermophilus sp. nov., isolated from a geothermal soil sample in Tengchong, south-west China

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Abstract A Gram-negative and aerobic bacterium, designated YIM 77875^T, was isolated from a geothermal soil sample collected at Rehai National Park, Tengchong, Yunnan Province, south-west China. Bacterial growth occurred from 37 to 65 °C (optimum 50 °C), pH 6.0–8.0 (optimum pH 7.0) and 0–1 % NaCl (w/v). Cells were rod-shaped and colonies were convex, circular, smooth, yellow and non-transparent. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain YIM 77875^T belongs to the genus *Lysobacter*. The 16S rRNA gene sequence similarity values between strain YIM 77875^T and other species of the genus *Lysobacter* were all below 94.7 %. The polar lipids of strain YIM 77875^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and five unknown phospholipids. The predominant respiratory quinone was Q-8 and the G+C content was 68.8 mol%. Major fatty acids were iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{11:0}$. On the basis of the morphological and chemotaxonomic characteristics, as well as genotypic data, strain YIM 77875^T represents a novel species, *Lysobacter thermophilus* sp. nov., in the genus

Da-Qiao Wei and Tian-Tian Yu contributed equally to this study.

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Lysobacter. The type strain is YIM 77875^T (CCTCC AB 2012064^T = KCTC 32020^T).

Keywords Tengchong · *Lysobacter thermophilus* sp. nov. · 16S rRNA gene · Polyphasic taxonomy

Introduction

The genus *Lysobacter* has been grouped within the family *Xanthomonadaceae* (Saddler and Bradbury 2005) and, so far, the genus comprises 23 species with validly published names: *Lysobacter antibioticus*, *L. arseniciresistens*, *L. brunescens*, *L. bugurensis*, *L. capsici*, *L. concretionis*, *L. daejeonensis*, *L. defluvii*, *L. dokdonensis*, *L. enzymogenes*, *L. gummosus*, *L. koreensis*, *L. korlensis*, *L. niabensis*, *L. niastensis*, *L. oryzae*, *L. panaciterrae*, *L. ruishenii*, *L. soli*, *L. spongiicola*, *L. ximonensis*, *L. xinjiangensis* and *L. yangpyeongensis* (Oh et al. 2011; Zhang et al. 2011; Wang et al. 2011; Liu et al. 2011; Luo et al. 2011). Species of the genus *Lysobacte*r were mostly isolated from soil or water and are mostly mesophilic (Romanenko et al. 2008; Yassin et al. 2007; Luo et al. 2011). The members of the genus were found to have great potential for development as biocontrol agents based on their ability to lyse a variety of microorganisms, including some pathogens (Christensen and Cook 1978; Hashizume et al. 2004).

The typical chemotaxonomic characters of the members of the genus *Lysobacte*r include: the predominant quinone is Q-8; major fatty acids are iso-branched fatty acids; high G+C contents (61.7–70.7 mol%); the major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol (Park et al. 2008; Romanenko et al. 2008; Zhang et al. 2011; Wang et al. 2011; Luo et al. 2011). During an investigation of the thermophilic microbial resources at Rehai National Park, a new member of the genus *Lysobacte*r, which is moderately thermophilic, was isolated. In this study, we report the taxonomic characterization of the new strain, YIM 77875^T.

Materials and methods

Strains and culture conditions

Strain YIM 77875^T was isolated from a geothermal soil sample (N 24.95002° W 98.43742°) collected at Rehai National Park, Tengchong, Yunnan Province, south-west China, by the serial dilution technique using R2A medium (BD; Becton, Dickinson and Company). The isolated strain was routinely cultivated on R2A medium at 50 °C and stored as aqueous glycerol suspensions (20 %, v/v) at −80 °C. Biomass for chemical and molecular studies was obtained by cultivation on R2A agar medium for ~3 days.

Morphological, physiological and biochemical characteristics

For analysis of morphological, physiological and biochemical characteristics, the new isolate YIM 77875^T and one closely related reference type strain (*L. xinjiangensis* CCTCC 208194^T) were both cultured on R2A medium. Gram staining was carried out by using the standard Gram reaction and was confirmed by using the KOH lysis test method (Cerny 1978). Gliding ability was determined as described by Bowman (2000) and motility testing performed using R2A broth with 0.3 % agar. The morphological characteristics of strain YIM 77875^T were observed by light microscopy and scanning electron microscopy (QUANTA200; FEI), after 3 days incubation on R2A medium. Growth at different temperatures (25, 28, 37, 45, 50, 55 and 60 °C) was tested on R2A as the basal medium by incubating cells for 15 days. NaCl-tolerance tests were examined at different NaCl concentrations (0, 0.5, 1, 3, 5, 7, 10, 12 and 15 % w/v) on R2A as basal medium at 50 °C. The pH range (pH 4, 5, 6, 7, 8, 9 and 10, using the buffer system described by Xu et al. 2005) for growth was tested at 50 °C for 15 days by culturing the strain in R2A broth.

Catalase activity was detected by the production of bubbles after the addition of a drop of 3 % (v/v) H_2O_2. Oxidase activity was determined by the oxidation of tetramethyl-*p*-phenylenediamine. Hydrolysis of CM-cellulose (0.1 %, w/v; Sinopharm Chemical Reagent), starch (1 %, w/v; Sinopharm Chemical Reagent), casein (1 %, w/v; Sinopharm Chemical Reagent), Tween 20, 40, 60 and 80 (1 %, w/v; Sigma), chitin (1 %, w/v; Sigma) and tyrosine (0.5 %, w/v; Solarbio Chemical Reagent) was tested on R2A agar medium. Enzyme activities and other biochemical characteristics were determined using API ZYM and API 20 NE kits according to the manufacturer's instructions (bioMérieux, France).

Chemotaxonomy

Quinones of strain YIM 77875^T were extracted by using the method of Collins et al. (1977) and separated by HPLC (Kroppenstedt 1982). Cellular fatty acid analysis of strains YIM 77875^T and *L. xinjiangensis* CCTCC 208194T was performed by using the method as described by Sasser (1990) and identification using the Microbial Identification software package (Sherlock Version 6.1; MIDI database: TSBA6). The G+C DNA content of strain YIM 77875^T was determined by using the HPLC method of Mesbah et al. (1989). Polar lipids were extracted and examined by using published procedures (Minnikin et al. 1979; Collins and Jones 1980).

Molecular analysis

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene from strain YIM 77875^T were performed as described previously (Li et al. 2007). The sequence obtained was compared with available 16S rRNA gene sequences of cultured species from GenBank via the BLAST program and the EzTaxon server (http://www.eztaxon.org, Chun et al. 2007). Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out by using CLUSTAL_X (Thompson et al. 1997). Phylogenetic analyses were performed by using three tree-making algorithms via the software packages MEGA version 4.0 (Tamura et al. 2007) and PHYLIP version 3.6 (Felsenstein 2002). The neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) methods were employed. Neighbour-joining and maximum-parsimony phylogenetic trees were constructed using MEGA version 4.0 (Tamura et al. 2007). The algorithm of Kimura's two parameter model was used to calculate evolutionary distance matrices in the neighbour-joining method (Kimura 1980). Bootstrap analysis (1,000 resamplings) was used to evaluate the topology of phylogenetic trees (Felsenstein 1985).

Results and discussion

Cells of strain YIM 77875^T were observed to be Gram-negative, non-motile rods. Colonies were yellow and circular on R2A agar medium. A scanning electron micrograph displaying the general morphological characteristics of strain YIM 77875^T is shown in Fig. S1 in Supplementary material. Growth of strain YIM 77875^T occurred in the pH range 6.0–8.0 and 0–1 % NaCl (w/v), with optimum growth at pH 7.0 and 0 % NaCl (w/v). The temperature range for growth was 37–55 °C, with the optimum temperature being 50 °C. Detailed results of morphological, physiological and biochemical characteristics of YIM 77875^T are given in the species description. In these results, strain YIM 77875^T exhibited characteristics that were consistent with those of the genus *Lysobacter*, such as Gram-stain negative, rod-shape, positive for catalase, oxidase and gelatin hydrolysis, and negative for urease activity and indole production. However, there were some traits that were different from other members of the genus *Lysobacter*, such as thermophilic growth

Table 1 Different characteristics between strain YIM 77875^T and selected representatives of the genus *Lysobacter*

Characteristic	1	2	3
Ranges for growth temperature (°C)	37–55	18–42*	10–37
Ranges for growth pH	6–8	7–11*	6–11
Growth with 2 % NaCl	−	+*	+
Hydrolysis of			
Gelatin	+	+	−
Tyrosine	+	−	+
Tween 80	−	+	ND
Assimilation of			
D-Glucose	+	−	−
L-Arabinose	+	−	−
D-Mannose	+	−	−
D-Mannitol	+	−	−
N-Acetylglucosamine	+	−	−
Malic acid	+	−	−
Trisodium citrate	+	−	−
Enzyme activities			
Lipase C14	−	+	−
Valine arylamidase	−	+	−
N-Acetyl-beta-glucosaminidase	+	−	−
Acid production from glucose	+	−	+

Taxa: 1, strain YIM 77875^T; 2, *L. xinjiangensis* CCTCC AB 208194 ^{T}; 3, *L. bugurensis* ZLD-29^T. Data for strains 1–2 are from this study, except *data, which are from Liu et al. (2011), data for strain 3 are from Zhang et al. (2011)

+ Utilized, − not utilized, *ND* no data available

Table 2 Differential phenotypic characteristics of strain YIM 77875^T and the type strains of the genus *Lysobacter*

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Catalase/oxidase	+/+	+/+	W/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/−
Nitrate reduction	−	−	W	−	−	−	−	+	−	−	+	−
Aesculin hydrolysis	+	+	+	−	+	−	−	+	−	−	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	−	−	−	ND	−	−	−	−	−	−	−	ND
Gliding/motility	N	N	G	N	G	M	G	N	M	G	G	G
Salinity range (%)	0–1	0–2	0–3	0–0.5	0–1	0–4	0–2	0–3	0–6	0–6	0–1	0–2
Assimilation of												
D-Glucose	+	−	−	−	−	−	−	+	−	−	−	+
L-Arabinose	+	−	−	−	−	−	−	−	−	−	−	−
D-Mannose	+	−	−	−	−	−	−	−	−	−	−	+
D-Mannitol	+	−	−	−	−	−	−	−	−	+	−	−
N-Acetyl-glucosamine	+	−	−	−	−	−	−	−	−	−	+	−
Maltose	−	−	−	W	−	−	−	+	−	+	+	+
Malic acid	+	−	−	ND	−	−	−	−	−	−	−	−
Trisodium citrate	+	−	−	−	−	−	−	−	−	+	−	−
Enzyme activities												
Trypsin	−	−	−	−	ND	+	+	+	−	+	+	+
α-Galactosidase	−	−	−	−	−	−	−	−	−	−	−	+
α-Glucosidase	−	−	−	ND	+	+	−	+	−	−	−	+
N-Acetyl-β-glucosaminidase	+	−	−	ND	−	−		−	−	−	−	+
β-Glucosidase	−	−	−	ND	+	−	−	−	−	−	−	+
β-Galactosidase	−	−	−	−	−	−	−	−	−	−	+	−
DNA G + C content (mol %)	68.8	69.7	68.2	68.1	67.7	70.7	63.8	61.7	69.0	67.1	69.0	65.4

Characteristic	13	14	15	16	17	18	19	20	21	22	23	24
Catalase/oxidase	+/+	+/+	+/+	+/+	−/+	+/−	+/+	+/+	−/+	+/+	+/+	W/+
Nitrate reduction	−	−	−	−	−	−	+	−	−	+	−	W
Aesculin hydrolysis	−	+	−	+	−	+	+	+	+	ND	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	ND	+	−
Arginine dihydrolase	−	−	−	ND	−	−	−	−	−	ND	+	−
Gliding/motility	N	G	ND	G	M	G	G	G	G	G	G	G
Salinity range (%)	0–2	0–2	0–1	0	0–1	0–1	0–1	0–2	0–3	ND	0–1	0.5–4
Assimilation of												
D-Glucose	−	+	−	−	−	+	+	+	+	+	+	−
L-Arabinose	+	+	−	−	−	−	−	−	−	−	ND	−
D-Mannose	−	+	−	−	−	+	+	+	+	+	−	−
D-Mannitol	+	+	−	−	−	−	−	+	−	−	ND	−
N-Acetyl-glucosamine	−	+	−	−	−	+	+	+	+	+	+	−
Maltose	−	+	−	−	−	+	+	+	+	+	−	−
Malic acid	−	+	−	−	+	−	+	+	+	ND		−
Trisodium citrate	+	+	−	−	−	−	−	−	+	−	ND	−
Enzyme activities												
Trypsin	−	+	+	+	+	−	−	−	ND	−	+	+
α-Galactosidase	−	−	−	−	−	−	−	−	ND	−	−	−

Table 2 continued

Characteristic	13	14	15	16	17	18	19	20	21	22	23	24
α-Glucosidase	−	+	+	+	+	+	−	+	ND	+	−	−
N-Acetyl-β-glucosaminidase		−	+	−	+	+	−	+	ND	+	−	−
β-Glucosidase	−	+	−	−	−	−	+	+	ND	+	−	−
β-Galactosidase	−	+	−	−	−	+	−	+	−	−	−	+
DNA G + C content (mol %)	68.9	66.6	62.5	67.4	67.3	63.5	69.2	65.7	67.0	65.4	67.1	67.9

Taxa: 1, strain YIM 77875^T; 2, *L. xinjiangensis* CCTCC AB 208194^T; 3, *L. bugurensis* ZLD-29^T; 4, *L. dokdonensis* DS-58^T; 5, *L. brunescens* KCTC 12130^T; 6, *L. arseniciresistens* KCTC 23365^T; 7, *L. concretionis* DSM 16239^T; 8, *L. daejeonensis* KACC 11406^T; 9, *L. spongiicola* DSM 18482^T; 10, *L. defluvii* DSM 21749^T; 11, *L. enzymogenes* DSM 2043^T; 12, *L. capsici* KCTC 22007^T; 13, *L. koreensis* KACC 11581^T; 14, *L. niastensis* DSM 18481^T; 15, *L. niabensis* DSM 18244^T; 16, *L. oryzae* YC6269 ^{T}; 17, *L. yangpyeongensis* DSM 17635^T; 18, *L. ximonensis* XM415^T; 19, *L. antibioticus* DSM 2044^T; 20, *L. gummosus* DSM 6980^T; 21, *L. panaciterrae* DSM 17927^T; 22, *L. soli* DCY2^T; 23, *L.ruishenii* CTN-1^T; 24, *L. korlensis* ZLD-17^T. Data for strains 1–2 are from this study, data for strains 3–24 are from Luo et al. (2011)

+ positive, − negative, *W* weakly positive, *ND* no data available, *N* non-motile, *M* motile, *G* gliding

Table 3 Cellular fatty acid contents (%) of strain YIM 77875^T and the type strains of recognized *Lysobacter* species

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
$C_{10:0}$	–	–	–	–	–	–	–	–	–	–	–	1.0
$C_{10:0}$ 3-OH	–	–	–	–	–	–	–	–	–	–	–	1.0
Iso-$C_{10:0}$	1.6	–	–	–	–	–	–	–	–	–	–	–
Iso-$C_{11:0}$	11.8	9.8	4.9	5.9	5.9	12.6	10.8	5.0	9.1	10.9	3.4	4.9
Iso-$C_{11:0}$ 3-OH	8.3	11.4	6.1	7.9	7.2	12.4	9.8	5.8	10.8	8.5	6.2	8.8
Iso-$C_{12:0}$	1.2	1.2	–	–	–	–	–	1.4	–	–	–	–
$C_{14:0}$			1.0	–	–	–	–	–	–	–	1.6	2.8
Iso-$C_{14:0}$	–	–	–	4.6	3.7	–	1.4	11.7	2.1	–	–	–
Iso-$C_{15:0}$	15.6	19.0	3.6	18.7	19.6	28.6	30.6	18.4	36.0	28.8	35.9	30.3
Iso-$C_{15:1}$ at 5	–	–	–	–	–	–	–	–	–	–	–	1.8
Iso-$C_{15:1}$ F$^{\#}$	–	–	–	–	1.7	1.7	3.4	5.3	4.1	1.3	–	–
Anteiso-$C_{15:0}$	–	–	–	4.9	2.6	–	1.0	1.3	1.3	–	1.7	1.4
$C_{16:0}$	7.8	1.1	13.2	1.9	1.5	1.5	1.9	1.3	–	2.4	4.6	7.6
$C_{16:1}\omega7c$ alcohol	–	–	–	–	–	–	–	–	–	–	–	–
$C_{16:1}\omega11c$	–		–	–	–	–	–	–	–	–	–	3.1
Iso-$C_{16:0}$	32.0	28.0	7.3	30.5	23.5	13.6	15.5	29.5	16.4	27.6	5.5	2.1
Iso-$C_{16:1}$ H	–	1.5	–	1.1	1.5	–	–	1.9	–	–	–	–
$C_{17:0}$ cyclo	–	–	–	–	–	–	1.6	–	–	1.0	1.5	–
Iso-$C_{17:0}$	5.7	3.9	3.7	2.5	2.3	4.9	2.7	–	2.8	5.6	2.6	4.8
Iso-$C_{17:1}\omega9c$	–	–	18.8	14.0	15.5	19.9	15.4	10.7	12.9	9.0	5.7	6.6
Anteiso-$C_{17:0}$	–	–	–	0.6	–	–	–	–	–	–	–	–
$C_{18:1}\omega7c$	–	–	3.2	–	–	–	–	–	–	–	3.0	2.1
Summed feature 3[a]	2.2	1.6	28.4	2.8	9.5	1.0	1.5	2.9	–	1.8	15.9	14.0

Fatty acid	13	14	15	16	17	18	19	20	21	22	23	24
$C_{10:0}$	–	–	–	–	–	–	–	–	–	1.1	–	1.1
$C_{10:0}$ 3-OH	–	–	–	–	–	–	–	–	–	–	–	–
Iso-$C_{10:0}$	–	–	–	–	–	–	–	–	–	–		

Table 3 continued

Fatty acid	13	14	15	16	17	18	19	20	21	22	23	24
Iso-$C_{11:0}$	5.2	3.8	6.5	3.9	4.3	3.7	3.1	3.8	3.8	4.1	3.9	4.5
Iso-$C_{11:0}$ 3-OH	2.8	6.4	7.3	3.2	5.5	5.2	8.0	9.7	6.9	5.8	3.9	6.8
Iso-$C_{12:0}$	–	–	–	–	1.1	–	–	–	–	–	–	1.4
$C_{14:0}$	–	–	1.0	2.8	–	1.7	1.1	–	–	–	–	1.2
Iso-$C_{14:0}$	1.5	1.8	1.8	–	4.5	6.1	1.3	–	–	–	3.6	–
Iso-$C_{15:0}$	20.0	42.1	15.7	12.5	14.5	22.6	249	25.2	29.5	34.3	21.4	5.3
Iso-$C_{15:1}$ at 5	4.1	1.2	2.0	3.9	3.1	–	1.0	1.7	–	–	–	–
Iso-$C_{15:1}$ F[#]	–	–	–	–	–	1.8	–	–	–	–	4.4	1.4
Anteiso-$C_{15:0}$	–	–	1.6	2.2	5.1	6.3	3.8	5.5	4.5	1.8	1.9	4.6
$C_{16:0}$	2.9	2.5	2.3	2.7	3.1	7.0	8.0	6.0	5.2	1.4	6.9	9.3
$C_{16:1}\omega$7c alcohol	6.1	–	4.5	3.5	8.8	–	1.6	1.7	–	–	–	–
$C_{16:1}\omega$11c	1.0	–	–	2.1	2.2	–	4.1	4.5	–	–	–	–
Iso-$C_{16:0}$	18.6	9.8	20.8	8.5	27.6	24.0	10.3	5.7	3.4	7.5	23.0	14.2
Iso-$C_{16:1}$ H	–	–	–	–	1.1	–	–	–	–	–	–	–
$C_{17:0}$ cyclo	–	–	–	–	–	–	7.3	1.0	–	–	–	–
Iso-$C_{17:0}$	10.1	5.7	7.7	12.3	1.9	1.5	3.4	7.8	16.0	17.2	3.5	2.1
Iso-$C_{17:1}\omega$9c	20.5	14.0	17.6	21.5	6.7	6.7	6.4	12.2	23.1	19.5	15.3	16.0
Anteiso-$C_{17:0}$	–	–	–	–	1.1	–	–	1.4	1.4	–	–	2.4
$C_{18:1}\omega$7c	–	–	–	–	–	1.2	1.7	2.5	–	1.1	–	1.9
Summed feature 3[a]	–	4.7	1.0	1.1	3.3	2.9	8.3	6.4	4.8	3.4	4.5	21.2

Taxa: 1, strain YIM 77875^T; 2, *L. xinjiangensis* CCTCC AB 208194 ^{T}; 3, *L. bugurensis* ZLD-29^T; 4, *L. dokdonensis* DS-58 ^{T}; 5, *L. brunescens* KCTC 12130 ^{T}; 6, *L. arseniciresistens* KCTC 23365 ^{T}; 7, *L. concretionis* DSM 16239 ^{T}; 8, *L. daejeonensis* KACC 11406 ^{T}; 9, *L. spongiicola* DSM 18482^T; 10, *L. defluvii* DSM 21749^T; 11, *L. enzymogenes* DSM 2043^T; 12, *L. capsici* KCTC 22007^T; 13, *L. koreensis* KACC 11581^T; 14, *L. niastensis* DSM 18481^T; 15, *L. niabensis* DSM 18244^T; 16, *L. oryzae* YC6269^T; 17, *L. yangpyeongensis* DSM 17635^T; 18, *L. ximonensis* XM415^T; 19, *L. antibioticus* DSM 2044^T; 20, *L. gummosus* DSM 6980^T; 21,*L. panaciterrae* DSM 17927^T; 22, *L. soli* DCY2^T; 23, *L. ruishenii* CTN-1^T; 24, *L. korlensis* ZLD-17^T. Data for strains 1–2 are from this study, data for strains 3–24 are from Luo et al. (2011)

[a] Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega$7c # iso-C15:1 F should correspond to either iso-$C_{15:1}$ ω6c and/or iso-$C_{15:1}$ ω5c. The double bond position is presumptive (Yassin et al. 2007)

– Less than 1 % or not detected

temperature differentiated strain YIM 77875^T from other members of the genus *Lysobacter*, and other differential characteristics are given in Table 1. The growth pH range, the tolerance of NaCl concentration range, the ability to assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, malic acid and trisodium citrate and the presence of the activity of *N*-acetyl-beta-glucosaminidase differentiated strain YIM 77875^T from the reference strains *L. xinjiangensis* CCTCC 208194^T and *L. bugurensis* ZLD-29^T, as shown in Table 2.

The predominant quinone of strain YIM 77875^T was ubiquinone-8 (Q-8), which is a characteristic feature of the genus *Lysobacter* (Bae et al. 2005). The polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and five unknown phospholipids (Fig. S2 in Supplementary material), which is consistent with the characteristic polar lipid compositions of members of the genus *Lysobacter* (Park et al. 2008; Romanenko et al. 2008). Major fatty acids (>5 %) of strain 77875^T were found to be iso-$C_{16:0}$ (32.0 %), iso-$C_{15:0}$ (15.6 %), iso-$C_{11:0}$ (11.9 %), iso-$C_{11:0}$ 3-OH (8.3 %), $C_{16:0}$ (7.8 %), iso-$C_{17:0}$ (5.7 %) and iso-$C_{17:1}$ ω9c (5.1 %), which were also consistent with those described for the genus *Lysobacter*, although the proportions of these fatty acids were different from those determined in this study for *L. xinjiangensis* CCTCC 208194^T and by others for other species of the genus *Lysobacter* (Table 3). The G+C content of strain YIM 77875^T

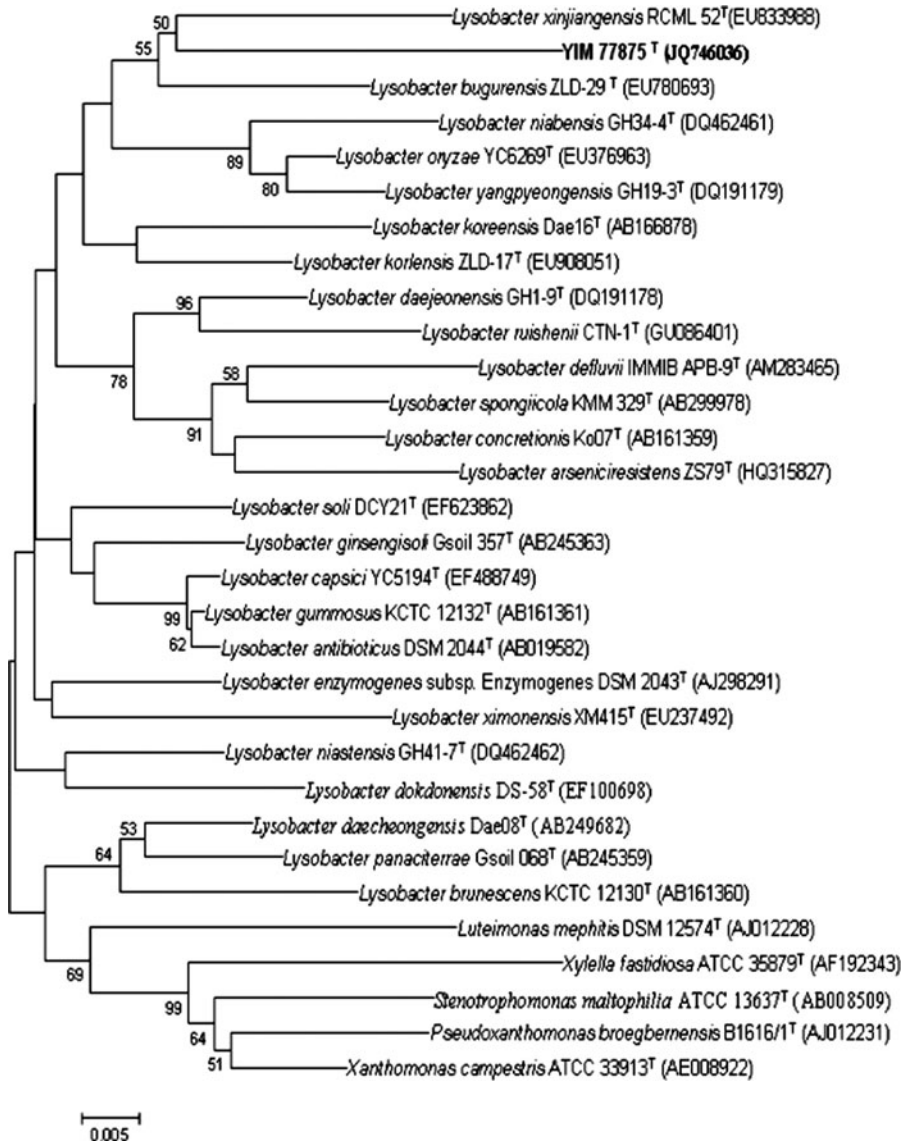

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1410 bp) of strain YIM 77875^T and members of the genus *Lysobacter*. Bootstrap values (expressed as percentages of 1,000 replications) of above 50 % are shown at the branch points. *Bar* 0.005 substitutions per nucleotide position

was found to be 68.8 mol %, which is in accordance with the range (61.7–70.7 mol %) for the genus *Lysobacter* (Christensen and Cook. 1978; Weon et al. 2006).

The complete 16S rRNA gene sequence of strain YIM 77875^T (1,542 bp) was determined in this study (GenBank accession number JQ746036). The 16S rRNA gene sequence similarity values between strain YIM 77875^T and other validly named species of the genus *Lysobacter* were calculated by using EzTaxon (Chun et al. 2007) and they were all below 94.7 %. The most closely related type strains were *L. bugurensis* ZLD-29^T (94.7 %), *L. dokdonensis* DS-58^T (94.6 %), *L. brunescens* KCTC 12130^T (94.5 %) and *L. xinjiangensis* CCTCC 208194^T (94.4 %). The neighbour-joining phylogenetic tree showed that strain YIM 77875^T formed a cluster with *L. xinjiangensis* CCTCC 208194^T with a 50 % bootstrap value (Fig. 1). The topology of the phylogenetic tree generated using the maximum-parsimony and maximum-likelihood algorithm was somewhat different from that of the tree constructed using the neighbour-joining method. However, all the trees constructed by these three methods showed that strain YIM 77875^T formed a cluster with *L. xinjiangensis* CCTCC 208194^T and *L. bugurensis* ZLD-29^T (Fig. S3 in

Supplementary material). The low level of 16S rRNA gene sequence similarity (<95 %) between *Lysobacter* species with validly published names and the novel isolate indicated that the isolate represents a novel genomic species of the genus *Lysobacter* (Stackebrandt and Goebel 1994).

Therefore, on the basis of phylogenetic analysis and phenotypic differences compared with the closely related type strains shown in Tables 1 and 2, YIM 77875^T is considered as a member of a novel species in the genus *Lysobacter*, for which the name *L. thermophilus* sp. nov. is proposed.

Description of *L. thermophilus* sp. nov.

L. thermophilus (ther.mo'phi.lus. Gr.n. therme heat; Gr.adj. philus loving; M.L. masc.adj. thermophilus heat loving)

The strain is Gram-negative and aerobic. Cells are rod-shaped and colonies are convex, circular, smooth, non-transparent and yellow. Oxidase and catalase are positive. Growth occurs from 37 to 55 °C, pH 6.0–8.0 and 0–1 % NaCl (w/v). The optimal temperature and pH value for growth are 50 °C and pH 7.0, respectively. Strain YIM 77875^T can hydrolyze tyrosine and Tween 40 and 60 but not CM-cellulose, starch, casein, chitin and Tween 20 and 80. It shows positive reactions for acid production from glucose, aesculin hydrolysis and gelatin hydrolysis but negative reactions for reduction nitrate, production indole, arginine dihydrolase, urease. Positive for assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, malic acid and trisodium citrate but negative for assimilation of phenylacetic acid, adipic acid, capric acid, potassium gluconate and maltose (as determined by API 20 NE test strips). It is positive for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-beta-glucosaminidase but negative for lipase C14, valine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase (as determined by API ZYM tests). The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and five unknown phospholipids. The predominant ubiquinone is Q-8. Major fatty acids (>10 %) are iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{11:0}$. The G+C content is 68.8 mol %.

The GenBank accession number for the 16S rRNA gene sequence of strain YIM 77875^T is JQ746036. The type strain YIM 77875^T (CCTCC AB 2012064^T = KCTC 32020^T) was isolated from a geothermal soil sample collected at Rehai National Park, Tengchong, Yunnan Province, south-west China.

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