Lysobacter arseniciresistens sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil

Article *in* International Journal of Systematic and Evolutionary Microbiology · September 2011

DOI: 10.1099/ijs.0.034405-0 · Source: PubMed

International Journal of Systematic and Evolutionary Microbiology (2012), **62**, 1659–1665 **DOI** 10.1099/ijs.0.034405-0

Lysobacter arseniciresistens sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil

Guosheng Luo, Zunji Shi and Gejiao Wang

National Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China

Correspondence
Gejiao Wang
gejiaow@yahoo.com.cn *or*
gejiao@mail.hzau.edu.cn

A Gram-negative, aerobic, motile, rod-shaped, arsenite [As(III)]-resistant bacterium, designated strain ZS79^T, was isolated from subsurface soil of an iron mine in China. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain ZS79^T clustered closely with strains of five *Lysobacter* species, with 96.9, 96.1, 96.0, 95.8 and 95.3 % sequence similarities to *Lysobacter concretionis* Ko07^T, *L. daejeonensis* GH1-9^T, *L. defluvii* IMMIB APB-9^T, *L. spongiicola* KMM 329^T and *L. ruishenii* CTN-1^T, respectively. The major cellular fatty acids were iso-$C_{15:0}$ (28.6 %), iso-$C_{17:1}\omega9c$ (19.9 %), iso-$C_{16:0}$ (13.6 %), iso-$C_{11:0}$ (12.6 %) and iso-$C_{11:0}$ 3-OH (12.4 %). The genomic DNA G+C content was 70.7 mol% and the major respiratory quinone was Q-8. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid. On the basis of morphological and physiological/biochemical characteristics, phylogenetic position and chemotaxonomic data, this strain is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter arseniciresistens* sp. nov. is proposed; the type strain is ZS79^T (=CGMCC 1.10752^T=KCTC 23365^T).

The genus *Lysobacter* was first proposed in 1978 and assigned to the family *Lysobacteraceae* (Christensen & Cook, 1978). Later, it was reclassified within the family *Xanthomonadaceae* (Saddler & Bradbury, 2005). The description of *Lysobacter* was emended by Park *et al.* (2008). At the time of writing, *Lysobacter* contained 22 species with validly published names: *Lysobacter enzymogenes* (type species), *L. antibioticus*, *L. brunescens*, *L. gummosus*, *L. concretionis*, *L. daejeonensis*, *L. yangpyeongensis*, *L. koreensis*, *L. niabensis*, *L. niastensis*, *L. defluvii*, *L. capsici*, *L. spongiicola*, *L. panaciterrae*, *L. ximonensis*, *L. oryzae*, *L. soli*, *L. ruishenii*, *L. xinjiangensis*, *L. dokdonensis*, *L. korlensis* and *L. bugurensis* (Oh *et al.*, 2011; Zhang *et al.*, 2011; Wang *et al.*, 2011; Liu *et al.*, 2011). These *Lysobacter* species were mostly isolated from soil except for *L. spongiicola* and *L. concretionis*, which were isolated from deep sea sponge (Romanenko *et al.*, 2008) and sludge (Bae *et al.*, 2005), respectively. The typical characters of members of the genus *Lysobacter* are Q-8 as the major respiratory quinone, a predominance of iso-branched fatty acids, high DNA G+C contents (61.7–70.1 mol%), and diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) as the major polar lipids (Park *et al.* 2008; Romanenko *et al.*, 2008; Zhang *et al.*, 2011; Wang *et al.*, 2011). Some strains of this genus showed strong proteolytic abilities and were able to lyse a variety of bacteria, fungi, yeasts, algae and nematodes (Christensen & Cook, 1978). Moreover, most members of the genus were able to glide, but two members (*L. yangpyeongensis* DSM 17635^T and *L. spongiicola* DSM 21749^T; Weon *et al.*, 2006; Romanenko *et al.*, 2008) were mobile, and four members were non-mobile (*L. daejeonensis* KACC 11406^T, *L. koreensis* KACC 11581^T, *L. dokdonensis* DS-58^T and *L. xinjiangensis* RCML-52^T; Weon *et al.*, 2006; Lee *et al.*, 2006; Oh *et al.*, 2011; Liu *et al.*, 2011). All published species of *Lysobacter* showed negative results for urease activity and indole production (Ten *et al.*, 2009; Zhang *et al.*, 2011).

Arsenic (As) is an extraordinarily toxic metalloid. However, after a long time of evolution in soil contaminated with As, micro-organisms have developed different As-resistant mechanisms to survive in this environment. Highly As-resistant bacteria show variable mechanisms of As resistance (Cai *et al.*, 2009), gaining energy for their growth (Stolz *et al.*, 2010), and even using arsenic instead of phosphorus to grow (Wolfe-Simon *et al.*, 2011). In this study, As-resistant bacteria were isolated from subsurface soil collected from Tieshan iron mine (30° 12′ 25.84″ N 114° 54′ 03.93″ E) of Daye City, Hubei Province, central China. The soil texture was sandy type with a pH of 7.4. The total soil As, Fe and Cu concentrations were 0.029, 282.5 and 5.9 g kg^{-1}, respectively. The total C, N, P, S and NO_3^- concentrations were 14.6, 0.4, 1.6, 0.4 and 0.019 g kg^{-1},

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ZS79^T is HQ315827.

Four supplementary figures are available with the online version of this paper.

respectively. Bacterial isolation was performed using a chemically defined medium (CDM) (per litre: $MgSO_4 . 7H_2O$, 2.0 g; NH_4Cl, 1.0 g; Na_2SO_4, 1.0 g; K_2HPO_4, 0.013 g; $CaCl_2 . 2H_2O$, 0.067 g; sodium lactate, 5.0 g; $Fe_2SO_4 . 7H_2O$, 0.033 g; $NaHCO_3$, 0.798 g; agar, 15.0 g; pH 7.2; Weeger *et al.*, 1999) containing 0.8 mM $NaAsO_2$. About 10^4 c.f.u. (g soil)$^{-1}$ were obtained and a total of 15 different As(III)-resistant bacterial strains were isolated. Strain ZS79^T was chosen for this study due to its high As(III) resistance and potential novelty.

For analysis of morphological, physiological and biochemical characteristics, strain ZS79^T and the four most closely related strains, *L. concretionis* DSM 16239^T, *L. daejeonensis* KACC 11406^T, *L. defluvii* DSM 18482^T and *L. spongiicola* DSM 21749^T, were cultured on R2A medium unless otherwise stated. Gliding ability was determined as described by Bowman (2000). Motility test was performed using R2A broth with 0.3 % agar. Cell morphology was observed by light microscopy (× 1000; Olympus) and a transmission electron microscope (H-7650; Hitachi) (Grossart *et al.*, 2000) with 24 h growth in R2A broth. Gram staining was determined using the method described by Dussault (1955). Growth at different temperatures (4, 20, 28, 32, 37 and 42 °C) and various pH ranges (4–11) was assessed after 7 days incubation in R2A broth (Difco). Salt tolerance was tested in R2A broth without sodium pyruvate, or in LB broth (per litre: tryptone, 10 g; NaCl, 10 g; yeast extract, 5 g), supplemented with 0–7 % (w/v) NaCl after 7 days incubation. Growth under anaerobic conditions was determined by incubation in an anaerobic chamber (Mitsubishi Gas Chemical) at 28 °C for 15 days on the R2A agar.

Hydrolysis of casein, gelatin, starch, Tween 40, Tween 80, DNA, tyrosine, carboxymethylcellulose and urea was performed as described by Cowan & Steel (1965). Tests to determine decomposition of adenine, guanine, hypoxanthine, xanthine and testosterone were performed by the method of Gordon & Smith (1955). Hydrolysis of hippurate was determined as described by Kinyon & Harris (1979). Nitrate reduction was tested by the method described by Lányí (1987). Methyl red and Voges–Proskauer tests, H_2S and indole production were determined as recommended by Smibert & Krieg (1994). Catalase activity was determined by assessing bubble production in 3 % (v/v) H_2O_2, and oxidase activity was determined using 1 % (w/v) tetramethyl-β-phenylenediamine. Acid production from carbohydrates was determined using phenol red broth (Rhoades *et al.*, 1989) and as described by Hinz *et al.* (1998). Enzyme activities, other biochemical characteristics and utilization of carbohydrates were determined using API ZYM, API 20 NE and API ID 32 GN kits, respectively, according to the manufacturer's instructions (bioMérieux).

Antibiotic-susceptibility tests were performed by spreading bacterial suspensions on R2A plates and applying filter-paper discs containing the following (mg ml^{-1}): polymyxin B (25), novobiocin (5), trimethoprim (5), teicoplanin (30), cefalotin (30), chloramphenicol (30), rifampicin (5), amoxicillin (10), penicillin (10 IU), ampicillin (10), carbenicillin (100), cephalosporin V (30), cephalosporin IV (30), cefoxitin (30), kalafungin (30), streptomycin (10), tobramycin (10), vancomycin (30), lincomycin (2), ofloxacin (5), norfloxacin (10), nalidixic acid (30), erythromycin (15), minocin (30), tetracycline (30), neomycin (30) and nitrofurantoin (300) (Hangzhou Microbial Reagent). The strain was incubated at 28 °C for 3 days.

The MIC, defined as the lowest As(III) concentration that completely inhibits the growth of each strain, was determined for ZS79^T and the four reference strains as described by Lim & Cooksey (1993). Triplicate samples of each single bacterial colony were grown overnight at 28 °C with shaking at 160 r.p.m. Then, 2 % original culture was inoculated into 5 ml R2A broth, and with serial concentrations of $NaAsO_2$; growth of each strain was measured at OD_{600} after incubation at 28 °C with shaking at 160 r.p.m. for 7 days.

The 16S rRNA gene was amplified by PCR with universal primers 27F and 1492R (Lane, 1991) and cloned into pGEM-T easy vector (Promega). DNA sequencing was performed using primers T7 and SP6 (Promega) and an internal 16S rRNA gene primer 931R (5′-CCGCACAAG-CGGTGGAGTAT-3′). The sequence was compared with those available in NCBI database and EzTaxon Server version 2.1 (Chun *et al.*, 2007). Multiple alignments were performed with the program CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analysis was carried out using MEGA 4.0 (Tamura *et al.*, 2007) and the PHYML online web server (Guindon *et al.*, 2005). Phylogenetic trees were reconstructed using the neighbour-joining (*p*-distance; Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods with bootstrap analyses based on 1000 replications and viewed with MEGA 4.0. All of the species in the genus *Lysobacter* were included in the phylogenetic trees.

The DNA G+C content was determined by HPLC according to the method of Tamaoka & Komagata (1984). Respiratory quinone analysis was performed by HPLC as described by Minnikin *et al.* (1984). For whole-cell fatty acid analysis, strain ZS79^T and the four reference strains (*L. concretionis* DSM 16239^T, *L. daejeonensis* KACC 11406^T, *L. defluvii* DSM 18482^T and *L. spongiicola* DSM 21749^T) were analysed by GC (Hewlett Packard 6890) according to the instructions of the Sherlock Microbial Identification System (MIDI Sherlock version 4.5, MIDI database TSBA40 4.10). Polar lipids analysis of strain ZS79^T and three reference strains (*L. concretionis* DSM 16239^T, *L. daejeonensis* KACC 11406^T and *L. defluvii* DSM 18482^T) were determined by two-dimensional TLC method as described by Tindall (1990). All strains used for the above tests were cultured in R2A broth at 28 °C and collected in the exponential phase.

Detailed results of morphological, physiological and biochemical characteristics of strain ZS79^T are given in

Table 1. Differential phenotypic characteristics of strain ZS79^T and type strains of members of the genus *Lysobacter*

Taxa: 1, strain ZS79^T; 2, *L. concretionis* DSM 16239^T; 3, *L. daejeonensis* KACC 11406^T; 4, *L. spongiicola* DSM 21749^T; 5, *L. defluvii* DSM 18482^T; 6, *L. enzymogenes* DSM 2043^T (Zhang *et al.*, 2011); 7, *L. capsici* KCTC 22007^T (Zhang *et al.*, 2011); 8, *L. koreensis* KACC 11581^T (Zhang *et al.*, 2011); 9, *L. niastensis* DSM 18481^T (Zhang *et al.*, 2011); 10, *L. niabensis* DSM 18244^T (Zhang *et al.*, 2011); 11, *L. xinjiangensis* RCML-52^T (Liu *et al.*, 2011); 12, *L. oryzae* YC6269^T (Aslam *et al.*, 2009); 13, *L. yangpyeongensis* DSM 17635^T (Weon *et al.*, 2007); 14, *L. ximonensis* XM415^T (Wang *et al.*, 2009); 15, *L. antibioticus* DSM 2044^T (Weon *et al.*, 2007); 16, *L. brunescens* DSM 6979^T (Weon *et al.*, 2007); 17, *L. gummosus* DSM 6980^T (Weon *et al.*, 2007); 18, *L. panaciterrae* DSM 17927^T (Ten *et al.*, 2009); 19, *L. soli* DCY21^T (Srinivasan *et al.*, 2010); 20, *L. dokdonensis* DS-58^T (Oh *et al.*, 2011); 21, *L. ruishenii* CTN-1^T (Wang *et al.*, 2011); 22, *L. korlensis* ZLD-17^T (Zhang *et al.*, 2011); 23, *L. bugurensis* ZLD-29^T (Zhang *et al.*, 2011). Data for strains 1–5 are from the present study. +, Positive; −, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Catalase/oxidase	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/−	+/+	+/+	+/+	+/+	−/+	+/−	+/+	+/+	+/+	−/+	+/+	+/+	+/+	W/+	W/+
Nitrate reduction	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	W	W
Aesculin hydrolysis	−	−	+	−	−	+	+	−	+	−	+	+	−	+	+	+	+	+	ND	−	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	−	+
Arginine dihydrolase	−	−	−	−	−	−	ND	−	−	−	−	ND	−	−	−	−	−	−	ND	ND	+	−	−
Gliding/motility*	M	G	N	M	G	G	G	N	G	ND	N	G	M	G	G	G	G	G	G	N	G	G	G
Salinity range (%)	0–4	0–2	0–3	0–6	0–6	0–1	0–2	0–2	0–2	0–1	0–2	0	0–1	0–1	0–1	0–1	0–2	0–3	ND	0–0.5	0–1	0.5–4	0–3
Assimilation of:																							
D-Glucose	−	−	+	−	−	−	+	−	+	−	−	−	−	+	+	−	+	+	+	−	+	−	−
L-Arabinose	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	ND	−	−
D-Mannose	−	−	−	−	−	−	+	−	+	−	−	−	−	+	+	−	+	+	+	−	−	−	−
D-Mannitol	−	−	−	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	ND	−	−
N-Acetylglucosamine	−	−	−	−	−	+	−	−	+	−	−	−	−	+	+	−	+	+	+	−	+	−	−
Maltose	−	−	+	−	+	+	+	−	+	−	−	−	−	+	+	−	+	+	+	W	−	−	−
Malic acid	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	+	+	ND	ND	ND	−	−
Trisodium citrate	−	−	−	−	+	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−	ND	−	−
Enzyme activities:																							
Trypsin	+	+	+	−	+	+	+	−	+	+	−	+	+	−	−	ND	−	ND	−	−	+	+	−
α-Galactosidase	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	ND	−	−	−	−	−
α-Glucosidase	+	−	+	−	−	−	+	−	+	+	−	+	+	+	−	+	+	ND	+	ND	−	−	−
N-Acetyl-β-glucosaminidase	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	+	ND	+	ND	−	−	−
β-Glucosidase	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	+	+	ND	+	ND	−	−	−
β-Galactosidase	−	−	−	−	−	+	−	−	+	−	−	−	−	+	+	−	+	−	−	−	−	+	−
DNA G+C content (mol%)	70.7	63.8	61.7	69.0	67.1	69.0	65.4	68.9	66.6	62.5	69.7	67.4	67.3	63.5	69.2	67.7	65.7	67.0	65.4	68.1	67.1	67.9	68.2

*N, Non-motile; M, motile; G, gliding.

the species description. A transmission electron micrograph (Fig. S1; available in IJSEM Online) shows the general morphology of strain ZS79^T. As observed in other members of the genus *Lysobacter* (Ten *et al.*, 2009; Zhang *et al.*, 2011), strain ZS79^T was also negative for urease activity and indole production. The main differential phenotypic characters of strain ZS79^T and other species of the genus *Lysobacter* are shown in Table 1. In addition, the MIC for As(III) of strain ZS79^T was 14.0 mM, which was much higher than the MICs of the four closely related strains (2.0, 0.5, 2.0 and 0.5 mM for *L. concretionis* DSM 16239^T, *L. daejeonensis* KACC 11406^T, *L. defluvii* DSM 18482^T and *L. spongiicola* DSM 21749^T, respectively).

The 1466 bp 16S rRNA gene sequence of strain ZS79^T shared sequence similarities in the range 96.9–93.5 % with other members of the genus *Lysobacter*. Highest sequence similarities were found with *L. concretionis* Ko07^T (=DSM 16239^T) (96.9 %), *L. daejeonensis* GH1-9^T (=KACC 11406^T) (96.1 %), *L. defluvii* IMMIB APB-9^T (=DSM 18482^T) (96.0 %), *L. spongiicola* KMM 329^T (=DSM 21749^T) (95.8 %) and *L. ruishenii* CTN-1^T (95.3 %) using EzTaxon server 2.1. A phylogenetic tree constructed using the neighbour-joining algorithm revealed that strain ZS79^T was closely related to members of the genus *Lysobacter* and grouped in the same cluster with *L. concretionis* Ko07^T, *L. daejeonensis* GH1-9^T, *L. defluvii* IMMIB APB-9^T, *L. spongiicola* KMM 329^T and *L. ruishenii* CTN-1^T (Fig. 1). The maximum-parsimony and maximum-likelihood trees (Figs S2 and S3) also supported the phylogenetic position obtained with the neighbour-joining tree.

The DNA G+C content of strain ZS79^T was 70.7 mol%. Q-8 (98.5 %) was the predominant respiratory quinone. The major cellular fatty acids (>10 %) were iso-branched fatty acids including iso-$C_{15:0}$ (28.6 %), iso-$C_{17:1}\omega9c$ (19.9 %), iso-$C_{16:0}$ (13.6 %), iso-$C_{11:0}$ (12.6 %) and iso-$C_{11:0}$ 3-OH (12.4 %) (Table 2). The major polar lipids found in strain ZS79^T, *L. concretionis* DSM 16239^T, *L. daejeonensis* KACC 11406^T and *L. defluvii* DSM 18482^T were DPG, PE, PG and an unknown phospholipid (Fig. S4). In addition, an unknown aminophospholipid was found in *L. daejeonensis* KACC 11406^T [Fig. S4(c) I, II and III]. Strain ZS79^T was Gram-negative, rod-shaped, positive for catalase and oxidase, and negative for urease activity, indole production, and methyl red and Voges–Proskauer tests. It contained Q-8 and iso-branched fatty acids as the predominant respiratory quinone and major cellular fatty acids, respectively. Results of all these phenotypic and chemotaxonomic tests of strain ZS79^T were very similar to those of *L. enzymogenes* DSM 2043^T (Christensen & Cook, 1978; Ten *et al.*, 2009; Zhang *et al.*, 2011) and other species

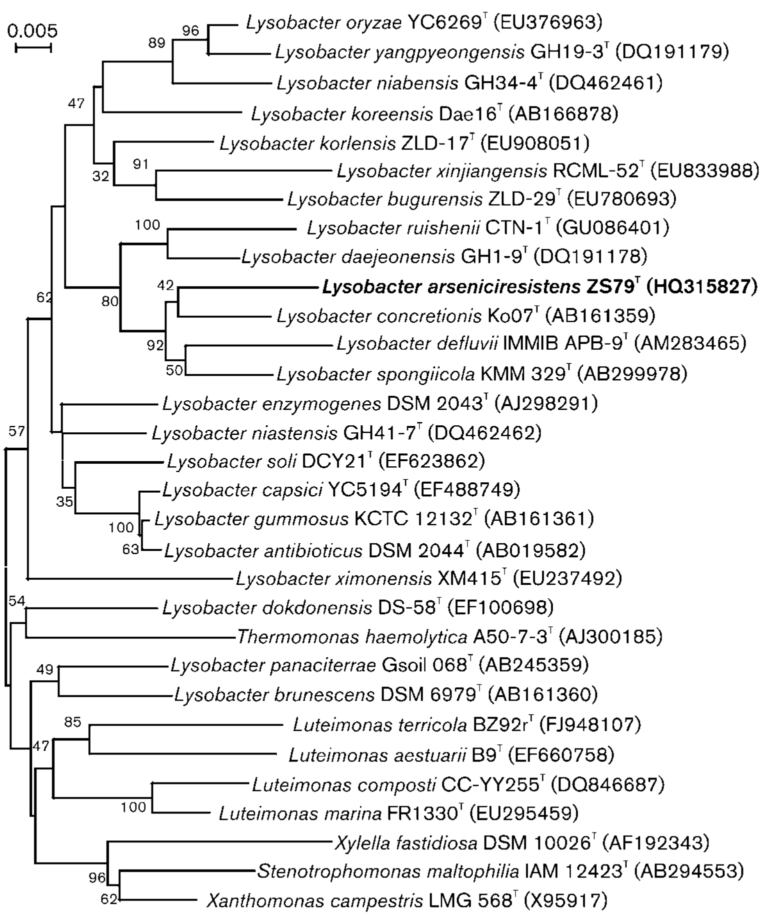

Fig. 1. Phylogenetic relatedness of strain ZS79^T and strains of related species based on 16S rRNA gene sequence comparisons. The dendrogram was generated using neighbour-joining analysis (Saitou & Nei, 1987). Numbers indicate percentages of occurrence of the branching order in 1000 bootstrapped trees. Bar, 5 substitutions per 1000 nt.

Table 2. Cellular fatty acid contents (%) of strain ZS79^T and the type strains of recognized *Lysobacter* species

Taxa: 1, strain ZS79^T; 2, *L. concretionis* DSM 16239^T; 3, *L. daejeonensis* KACC 11406^T; 4, *L. spongiicola* DSM 21749^T; 5, *L. defluvii* DSM 18482^T; 6, *L. enzymogenes* DSM 2043^T (Zhang *et al.*, 2011); 7, *L. capsici* KCTC 22007^T (Zhang *et al.*, 2011); 8, *L. koreensis* KACC 11581^T (Zhang *et al.*, 2011); 9, *L. niastensis* DSM 18481^T (Zhang *et al.*, 2011); 10, *L. niabensis* DSM 18244^T (Zhang *et al.*, 2011); 11, *L. xinjiangensis* RCML-52^T (Liu *et al.*, 2011); 12, *L. oryzae* YC6269^T (Aslam *et al.*, 2009); 13, *L. yangpyeongensis* DSM 17635^T (Weon *et al.*, 2007); 14, *L. ximonensis* XM415^T (Wang *et al.*, 2009); 15, *L. antibioticus* DSM 2044^T (Weon *et al.*, 2007); 16, *L. brunescens* DSM 6979^T (Weon *et al.*, 2007); 17, *L. gummosus* DSM 6980^T (Weon *et al.*, 2007); 18, *L. panaciterrae* DSM 17927^T (Ten *et al.*, 2009); 19, *L. soli* DCY21^T (Srinivasan *et al.*, 2010); 20, *L. dokdonensis* DS-58^T (Oh *et al.*, 2011); 21, *L. ruishenii* CTN-1^T (Wang *et al.*, 2011); 22, *L. korlensis* ZLD-17^T (Zhang *et al.*, 2011); 23, *L. bugurensis* ZLD-29^T (Zhang *et al.*, 2011). Data for strains 1–5 are from this study. −, <1 % or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
$C_{10:0}$	−	−	−	−	−	−	1.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1.1	1.2
$C_{10:0}$ 3-OH	−	−	−	−	−	−	1.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
iso-$C_{11:0}$	12.6	10.8	5.0	9.1	10.9	3.4	4.9	5.2	3.8	6.5	5.3	3.9	4.3	3.7	3.1	5.9	3.8	3.8	4.1	5.9	3.9	4.5	4.9
iso-$C_{11:0}$ 3-OH	12.4	9.8	5.8	10.8	8.5	6.2	8.8	2.8	6.4	7.3	6.5	3.2	5.5	5.2	8.0	7.2	9.7	6.9	5.8	7.8	3.9	6.8	6.1
iso-$C_{12:0}$	−	−	1.4	−	−	−	−	−	−	−	−	−	1.1	−	−	−	−	−	−	−	−	1.4	−
$C_{14:0}$	−	−	−	−	−	1.6	2.8	−	−	1.0	−	2.8	−	1.7	1.1	−	−	−	−	−	−	1.2	1.0
iso-$C_{14:0}$	−	1.4	11.7	2.1	−	−	−	1.5	1.8	1.8	−	−	4.5	6.1	1.3	3.7	−	−	−	4.6	3.6	−	−
iso-$C_{15:0}$	28.6	30.6	18.4	36.0	28.8	35.9	30.3	20.0	42.1	15.7	19.1	12.5	14.5	22.6	24.9	19.6	25.2	29.5	34.3	18.7	21.4	5.3	3.6
iso-$C_{15:1}$ AT 5	−	−	−	−	−	−	1.8	4.1	1.2	2.0	−	3.9	3.1	−	1.0	−	1.7	−	−	−	−	−	−
iso-$C_{15:1}$ F*	1.7	3.4	5.3	4.1	1.3	−	−	−	−	−	2.1	−	−	1.8	−	1.7	−	−	−	−	4.4	1.4	−
anteiso-$C_{15:0}$	−	1.0	1.3	1.3	−	1.7	1.4	−	−	1.6	1.0	2.2	5.1	6.3	3.8	2.6	5.5	4.5	1.8	4.9	1.9	4.6	−
$C_{16:0}$	1.5	1.9	1.3	−	2.4	4.6	7.6	2.9	2.5	2.3	3.5	2.7	3.1	7.0	8.0	1.5	6.0	5.2	1.4	1.9	6.9	9.3	13.2
$C_{16:1}\omega 7c$ alcohol	−	−	−	−	−	−	−	6.1	−	4.5	1.7	3.5	8.8	−	1.6	−	1.7	−	−	−	−	−	−
$C_{16:1}\omega 11c$	−	−	−	−	−	−	3.1	1.0	−	−	−	2.1	2.2	−	4.1	−	4.5	−	−	−	−	−	−
iso-$C_{16:0}$	13.6	15.5	29.5	16.4	27.6	5.5	2.1	18.6	9.8	20.8	27.6	8.5	27.5	24.0	10.3	23.5	5.7	3.4	7.5	30.5	23.0	14.2	7.3
iso-$C_{16:1}$ H	−	−	1.9	−	−	−	−	−	−	−	−	−	1.1	−	−	1.5	−	−	−	1.1	−	−	−
$C_{17:0}$ cyclo	−	1.6	−	−	1.0	1.5	−	−	−	−	−	−	−	−	7.3	−	1.0	−	−	−	−	−	−
iso-$C_{17:0}$	4.9	2.7	−	2.8	5.6	2.6	4.8	10.1	5.7	7.7	3.9	12.3	1.9	1.5	3.4	2.3	7.8	16.0	17.2	2.5	3.5	2.1	3.7
iso-$C_{17:1}\omega 9c$	19.9	15.4	10.7	12.9	9.0	5.7	6.6	20.5	14.0	17.6	16.4	21.5	6.7	6.7	6.4	15.5	12.2	23.1	19.5	14.0	15.3	16.0	18.8
anteiso-$C_{17:0}$	−	−	−	−	−	−	−	−	−	−	−	−	1.1	−	−	−	1.4	1.4	−	0.6	−	2.4	−
$C_{18:1}\omega 7c$	−	−	−	−	−	3.0	2.1	−	−	−	−	−	−	1.2	1.7	−	2.5	−	1.1	−	−	1.9	3.2
Summed feature 3†	1.0	1.5	2.9	−	1.8	15.9	14.0	−	4.7	1.0	1.7	1.1	3.3	2.9	8.3	9.5	6.4	4.8	3.4	2.8	4.5	21.2	28.4

*iso-$C_{15:1}$ F should correspond to either iso-$C_{15:1}$ $\omega 6c$ and/or iso-$C_{15:1}\omega 5c$. The double bond position is presumptive (Yassin *et al.*, 2007).
†Summed feature 3 comprises $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

of the genus *Lysobacter* (Zhang *et al.*, 2011). However, strain ZS79^T showed some clear differences in both the 16S rRNA gene sequence, and physiological and biochemical characteristics (Table 1).

Therefore, on the basis of the close relationship and the distinctive phenotypic and phylogenetic traits, strain ZS79^T is considered to represent a novel species in the genus *Lysobacter*, for which the name of *Lysobacter arseniciresistens* sp. nov. is proposed.

Description of *Lysobacter arseniciresistens* sp. nov.

Lysobacter arseniciresistens (ar.se.ni.ci.re.sis′tens. L. n. *arsenicum* arsenic; L. part. adj. *resistens* resisting; N.L. part. adj. *arseniciresistens* arsenic resisting, referring to the arsenic resistance of the bacterium).

Gram-negative and strictly aerobic. Cells are motile and rod-shaped (0.3–0.5 μm wide and 1.5–2.2 μm long, with one flagellum). Colonies are convex, circular, smooth, non-transparent and yellow after 3 days incubation on R2A agar at 28 °C. Grows on LB agar, R2A and trypticase soy agar (Difco), but does not grow on MacConkey agar (Difco). Temperature range for growth is 4–37 °C (optimum at 28 °C). Growth occurs with NaCl concentrations in the range 0–4 % (optimum, 0 %) and pH 5–9 (optimum, pH 7). Oxidase and catalase are positive. Hydrolyses gelatin, DNA, tyrosine, hippurate and Tween 40, but not starch, casein, carboxymethylcellulose, adenine, guanine, hypoxanthine, xanthine, testosterone or Tween 80. H_2S is produced. Methyl red and Voges–Proskauer tests are negative. Positive for gelatin hydrolysis, but negative for nitrate reduction, arginine dihydrolase, aesculin hydrolysis, indole production, glucose fermentation, urease and β-galactosidase (API 20NE test strips). Negative for assimilation of D-glucose, D-mannose, melibiose, sucrose, maltose, D-fucose, D-ribose, L-arabinose, L-rhamnose, glycogen, *N*-acetylglucosamine, D-sorbitol, D-mannitol, inositol, 3-hydroxybenzoic acid, propionic acid, malic acid, phenylacetic acid, capric acid, adipinic acid, itaconic acid, capric acid, adipic acid, valeric acid, salicin, L-proline, L-histidine, L-serine, trisodium citrate, sodium acetate, potassium 5-ketogluconate, propionate, 2-ketogluconate, 4-hydroxybenzoate, suberate, malonate and lactate, but positive for 3-hydroxybutyric acid (API 20NE and API ID 32GN). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin, leucine arylamidase, valine arylamidase, α-glucosidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and chymotrypsin, but negative for cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, β-fucosidase or *N*-acetyl-β-glucosaminidase (API ZYM tests). Acid is produced from mannose, D-galactose, maltose, D-xylose, inositol, L-sorbose, D-sorbitol, cellobiose and D-mannitol, but not from D-glucose, sucrose, lactose, D-arabinose, D-fructose, trehalose, D-ribose, turanose, melezitose, L-rhamnose, raffinose or propylene glycol. Sensitive to polymyxin B, novobiocin, teicoplanin, cefalotin, chloramphenicol, rifampicin, amoxicillin, penicillin, ampicillin, carbenicillin, cephalosporin V, cephalosporin IV, kalamycin, streptomycin, tobramycin, vancomycin, ofloxacin, norfloxacin, nalidixic acid, erythromycin, minocin, tetracycline and neomycin. Polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid. The major ubiquinone is Q-8. The major cellular fatty acids (>10 %) are iso-$C_{15:0}$, iso-$C_{17:1}\omega 9c$, iso-$C_{16:0}$, iso-$C_{11:0}$ and iso-$C_{11:0}$ 3-OH. This bacterium is highly arsenite resistant and is able to grow at 14.0 mM As(III) in R2A broth.

The type strain, ZS79^T (=CGMCC 1.10752^T=KCTC 23365^T), was isolated from subsurface soil of Tieshan iron mine of Daye City, Hubei Province, Central China. The DNA G+C content of the type strain is 70.7 mol%.

Acknowledgements

We are grateful to the DSMZ for providing *L. concretionis* DSM 16239^T, *L. spongiicola* DSM 21749^T and *L. defluvii* DSM 18482^T and KACC for providing *L. daejeonensis* KACC 11406^T. We are grateful to Dr Jean Euzéby (École National Vétérinaire) for the etymology of the strain's name and Dr Chengxiang Fang (CCTCC, Wuhan University) for respiratory quinone and polar lipid analyses. This work was supported by the National Natural Science Foundation of China (31010103903 and 30970075).

References

Aslam, Z., Yasir, M., Jeon, C. O. & Chung, Y. R. (2009). *Lysobacter oryzae* sp. nov., isolated from the rhizosphere of rice (*Oryza sativa* L.). *Int J Syst Evol Microbiol* **59**, 675–680.

Bae, H. S., Im, W. T. & Lee, S. T. (2005). *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* **55**, 1155–1161.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Cai, L., Liu, G. H., Rensing, C. & Wang, G. J. (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. *BMC Microbiol* **9**, 4.

Christensen, P. & Cook, F. D. (1978). *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* **28**, 367–393.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London, UK: Cambridge University Press.

Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Gordon, R. E. & Smith, M. M. (1955). Proposed group of characters for the separation of *Streptomyces* and *Nocardia*. *J Bacteriol* **69**, 147–150.

Grossart, H. P., Steward, G. F., Martinez, J. & Azam, F. (2000). A simple, rapid method for demonstrating bacterial flagella. *Appl Environ Microbiol* **66**, 3632–3636.

Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. (2005). PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33** (Web Server issue), W557–W559.

Hinz, K. H., Ryll, M. & Köhler, B. (1998). Detection of acid production from carbohydrates by *Riemerella anatipestifer* and related organisms using the buffered single substrate test. *Vet Microbiol* **60**, 277–284.

Kinyon, J. M. & Harris, D. L. (1979). *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae* Harris *et al. Int J Syst Bacteriol* **29**, 102–109.

Kluge, A. G. & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–176. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lányí, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Lee, J. W., Im, W. T., Kim, M. K. & Yang, D. C. (2006). *Lysobacter koreensis* sp. nov., isolated from a ginseng field. *Int J Syst Evol Microbiol* **56**, 231–235.

Lim, C. K. & Cooksey, D. A. (1993). Characterization of chromosomal homologs of the plasmid-borne copper resistance operon of *Pseudomonas syringae*. *J Bacteriol* **175**, 4492–4498.

Liu, M., Liu, Y., Wang, Y., Luo, X., Dai, J. & Fang, C. (2011). *Lysobacter xinjiangensis* sp. nov., a moderately thermotolerant and alkalitolerant bacterium isolated from a gamma-irradiated sand soil sample. *Int J Syst Evol Microbiol* **61**, 433–437.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Oh, K. H., Kang, S. J., Jung, Y. T., Oh, T. K. & Yoon, J. H. (2011). *Lysobacter dokdonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **61**, 1089–1093.

Park, J. H., Kim, R., Aslam, Z., Jeon, C. O. & Chung, Y. R. (2008). *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Microbiol* **58**, 387–392.

Rhoades, K. R., Rimler, R. B. & Sandhu, T. S. (1989). Pasteurellosis and pseudotuberculosis. In *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*, 3rd edn, pp. 14–21. Edited by H. G. Purchase, L. H. Arp, C. H. Domermuth & J. E. Pearson. Kennet Square, PA: American Association of Avian Pathologists.

Romanenko, L. A., Uchino, M., Tanaka, N., Frolova, G. M. & Mikhailov, V. V. (2008). *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* **58**, 370–374.

Saddler, G. S. & Bradbury, J. F. (2005). Family I. *Xanthomonadaceae* fam. nov. In Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 2, pp. 63 (*The Proteobacteria*), part B (*The Gammaproteobacteria*). Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Srinivasan, S., Kim, M. K., Sathiyaraj, G., Kim, H. B., Kim, Y. J. & Yang, D. C. (2010). *Lysobacter soli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **60**, 1543–1547.

Stolz, J. F., Basu, P. & Oremland, R. S. (2010). Microbial arsenic metabolism: new twists on an old poison. *Microbe Magazine* **5**, 53–59.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Ten, L. N., Jung, H. M., Im, W. T., Yoo, S. A., Oh, H. M. & Lee, S. T. (2009). *Lysobacter panaciterrae* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **59**, 958–963.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Wang, Y., Dai, J., Zhang, L., Luo, X., Li, Y., Chen, G., Tang, Y., Meng, Y. & Fang, C. (2009). *Lysobacter ximonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **59**, 786–789.

Wang, G. L., Wang, L., Chen, H. H., Shen, B., Li, S. P. & Jiang, J. D. (2011). *Lysobacter ruishenii* sp. nov., a chlorothalonil-degrading bacterium isolated from a long-term chlorothalonil-contaminated soil. *Int J Syst Evol Microbiol* **61**, 674–679.

Weeger, W., Lièvremont, D., Perret, M., Lagarde, F., Hubert, J. C., Leroy, M. & Lett, M. C. (1999). Oxidation of arsenite to arsenate by a bacterium isolated from an aquatic environment. *Biometals* **12**, 141–149.

Weon, H. Y., Kim, B. Y., Baek, Y. K., Yoo, S. H., Kwon, S. W., Stackebrandt, E. & Go, S. J. (2006). Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. *Int J Syst Evol Microbiol* **56**, 947–951.

Weon, H. Y., Kim, B. Y., Kim, M. K., Yoo, S. H., Kwon, S. W., Go, S. J. & Stackebrandt, E. (2007). *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. *Int J Syst Evol Microbiol* **57**, 548–551.

Wolfe-Simon, F., Blum, J. S., Kulp, T. R., Gordon, G. W., Hoeft, S. E., Pett-Ridge, J., Stolz, J. F., Webb, S. M., Weber, P. K. & other authors (2011). A bacterium that can grow by using arsenic instead of phosphorus. *Science* **332**, 1163–1166.

Yassin, A. F., Chen, W. M., Hupfer, H., Siering, C., Kroppenstedt, R. M., Arun, A. B., Lai, W. A., Shen, F. T., Rekha, P. D. & Young, C. C. (2007). *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Microbiol* **57**, 1131–1136.

Zhang, L., Bai, J., Wang, Y., Wu, G. L., Dai, J. & Fang, C. (2011). *Lysobacter korlensis* sp. nov. and *Lysobacter bugurensis* sp. nov., isolated from soil in north-west China. *Int J Syst Evol Microbiol* **61**, 2259–2265.