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

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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* IMMB 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\(_{17:1}\)\(\omega 8c\) (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. gumarous*, *L. concretionis*, *L. daejeonensis*, *L. yongyeongensis*, *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. yangyeongensis* 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 and NO\(_3\) concentrations were 14.6, 0.4, 1.6, 0.4 and 0.019 g kg\(^{-1}\), respectively.
respectively. Bacterial isolation was performed using a chemically defined medium (CDM) (per litre: MgSO₄·7H₂O, 2.0 g; NH₄Cl, 1.0 g; Na₂SO₄, 1.0 g; K₂HPO₄, 0.013 g; CaCl₂·2H₂O, 0.067 g; sodium lactate, 5.0 g; Fe₂SO₄·7H₂O, 0.033 g; NaHCO₃, 0.798 g; agar, 15.0 g; pH 7.2; Weeger et al., 1999) containing 0.8 mM NaAsO₂. About 10⁶ cf.u. (g soil)⁻¹ were obtained and a total of 15 different As(III)-resistant bacterial strains were isolated. Strain ZS79ᵀ was chosen for this study due to its high As(III) resistance and potential novelty.

For analysis of morphological, physiological and biochemical characteristics, strain ZS79ᵀ and the four most closely related strains, L. concretionis DSM 16239ᵀ, L. daejeonensis DSM 11406ᵀ, L. defluvii DSM 18482ᵀ and L. spongiicola DSM 21749ᵀ, 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 Lányi (1987). Methyl red and Voges–Proskauer tests, H₂S and indole production were determined as recommended by Smibert & Krieg (1994). Catalase activity was determined by assessing bubble production in 3 % (w/v) H₂O₂, 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), norflaxacin (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 ZS79ᵀ 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 NaAsO₂; growth of each strain was measured at OD₆₀₀ 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’-CCGACACAAG-CGGTGAGATAT-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 PHYLML online web server (Guindon et al., 2005). Phylogenetic trees were reconstructed using the neighbour-joining (p-distance; Saitou & Nei, 1987), maximum-parsimony (Krugu & 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 ZS79ᵀ and the four reference strains (L. concretionis DSM 16239ᵀ, L. daejeonensis KACC 11406ᵀ, L. defluvii DSM 18482ᵀ and L. spongiicola DSM 21749ᵀ) 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 ZS79ᵀ and three reference strains (L. concretionis DSM 16239ᵀ, L. daejeonensis KACC 11406ᵀ and L. defluvii DSM 18482ᵀ) 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 ZS79ᵀ are given in...
Table 1. Differential phenotypic characteristics of strain ZS79\(^T\) and type strains of members of the genus Lysobacter

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*N, Non-motile; M, motile; G, gliding.
the species description. A transmission electron micro-
graph (Fig. S1; available in IJSEM Online) shows the
general morphology of strain ZS79<sup>T</sup>. As observed in other
members of the genus Lysobacter (Ten et al., 2009; Zhang
et al., 2011), strain ZS79<sup>T</sup> was also negative for urease
activity and indole production. The main differential
phenotypic characters of strain ZS79<sup>T</sup> and other species
of the genus Lysobacter are shown in Table 1. In addition,
the MIC for As(III) of strain ZS79<sup>T</sup> 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 <i>L. concretionis</i>
DSM 16239<sup>T</sup>, <i>L. daejeonensis</i> KACC 11406<sup>T</sup>, <i>L. defluvii</i>
DSM 18482<sup>T</sup> and <i>L. spongicola</i> DSM 21749<sup>T</sup>, respectively).

The 1466 bp 16S rRNA gene sequence of strain ZS79<sup>T</sup>
shared sequence similarities in the range 96.9–93.5 % with
other members of the genus Lysobacter. Highest sequence
similarities were found with <i>L. concretionis</i> Ko07<sup>T</sup> (=DSM
16239<sup>T</sup>) (96.9 %), <i>L. daejeonensis</i> GH1-9<sup>T</sup> (=KACC 11406<sup>T</sup>)
(96.1 %), <i>L. defluvii</i> IMIMB APB-9<sup>T</sup> (=DSM 18482<sup>T</sup>)
(96.0 %), <i>L. spongicola</i> KMM 329<sup>T</sup> (=DSM 21749<sup>T</sup>)
(95.8 %) and <i>L. ruishenii</i> CTN-1<sup>T</sup> (95.3 %) using EzTaxon
server 2.1. A phylogenetic tree constructed using the
neighbour-joining algorithm revealed that strain ZS79<sup>T</sup>
was closely related to members of the genus Lysobacter
and grouped in the same cluster with <i>L. concretionis</i> Ko07<sup>T</sup>,
<i>L. daejeonensis</i> GH1-9<sup>T</sup>, <i>L. defluvii</i> IMIMB APB-9<sup>T</sup>, <i>L. spongicola</i>
KMM 329<sup>T</sup> and <i>L. ruishenii</i> CTN-1<sup>T</sup> (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<sup>T</sup> 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<sub>15 : 0</sub> (28.6 %), iso-C<sub>17 : 1</sub>ω9c
(19.9 %), iso-C<sub>16 : 0</sub> (13.6 %), iso-C<sub>11 : 0</sub> (12.6 %) and iso-
C<sub>11 : 0</sub> 3-OH (12.4 %) (Table 2). The major polar lipids
found in strain ZS79<sup>T</sup>, <i>L. concretionis</i> DSM 16239<sup>T</sup>, <i>L. daejeonensis</i>
KACC 11406<sup>T</sup> and <i>L. defluvii</i> DSM 18482<sup>T</sup> were DPG, PE, PG and an unknown phospholipid (Fig.
S4). In addition, an unknown aminophospholipid was
found in <i>L. daejeonensis</i> KACC 11406<sup>T</sup> [Fig. S4(c) I, II and
III]. Strain ZS79<sup>T</sup> 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<sup>T</sup> were very similar to
those of <i>L. enzymogenes</i> DSM 2043<sup>T</sup> (Christensen & Cook,
1978; Ten et al., 2009; Zhang et al., 2011) and other species

![Phylogenetic relatedness of strain ZS79<sup>T</sup> 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.](image-url)
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*iso-C<sub>15:1</sub> F should correspond to either iso-C<sub>15:1</sub> o6c and/or iso-C<sub>15:1</sub> o5c. The double bond position is presumptive (Yassin et al., 2007).
†Summed feature 3 comprises C<sub>16</sub>:0<sup>o7c</sup> and/or iso-C<sub>15:0</sub> 2-OH.
of the genus Lysobacter (Zhang et al., 2011). However, strain ZS79T 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 ZS79T 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. arsenicicum 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 2Si s 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 on R2A, 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-acetylgalactosamine, D-sorbitol, D-mannitol, inositol, 3-hydroxybenzoic acid, propionic acid, malic acid, phenylacetic acid, capric acid, adipic acid, itaconic acid, capric acid, adic 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, z-glucosidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and chymotrypsin, but negative for cystine arylamidase, β-galactosidase, β-glucuronidase, β-glucosidase, β-mannosidase, β-fucosidase or N-acetyl-β-glucosaminidase (API ZYM tests). Acid is produced from mannose, D-galactose, maltose, D-xylose, inositol, L-sorbosel, 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-C15:0, iso-C17:0 3-0-C9c, iso-C16:0, iso-C11:0 and iso-C15:0 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, ZS79T (=CGMCC 1.10752T=KCTC 23365T), 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%.

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**References**


