Lysobacter mobilis sp. nov., isolated from abandoned lead-zinc ore

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An aerobic and Gram-stain-negative bacterial strain, designated 9NM-14^T, was isolated from abandoned lead-zinc ore from Meizhou, Guangdong Province, south China. Strain 9NM-14^T was motile by means of a single polar flagellum. Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain 9NM-14^T was affiliated with the genus *Lysobacter* and was most closely related to *Lysobacter xinjiangensis* RCML-52^T and *Lysobacter bugurensis* ZLD-29^T (97.4 % and 96.3 % 16S rRNA gene sequence similarity, respectively). The DNA–DNA relatedness value between strain 9NM-14^T and *L. xinjiangensis* RCML-52^T was 30.1 ± 7.6 %. The major respiratory quinone was unbiquinone 8 (Q-8) and the major cellular fatty acids consisted of iso-$C_{17:1}\omega 9c$ (29.1 %), iso-$C_{15:0}$ (28.9 %), iso-$C_{17:0}$ (9.4 %), iso-$C_{16:0}$ (8.6 %), iso-$C_{11:0}$ 3-OH (6.9 %) and iso-$C_{11:0}$ (5.8 %). The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, an unidentified aminolipid and five unidentified phospholipids. The genomic DNA G+C content of strain 9NM-14^T was 70.7 ± 0.1 mol%. On the basis of the data from this polyphasic taxonomic study, strain 9NM-14^T should be assigned to a novel species of the genus *Lysobacter*, for which the name *Lysobacter mobilis* sp. nov. is proposed. The type strain is 9NM-14^T (=GIMCC 1.659^T=CCTCC AB 2014273^T=DSM 27574^T).

The genus *Lysobacter* was established by Christensen & Cook (1978) and subsequently assigned to the family *Xanthomonadaceae* (Saddler & Bradbury, 2005). Recently, a member of the genus *Lysobacter*, *Lysobacter thermophilus* (Wei *et al.*, 2012), has been reclassified to a new genus as *Vulcaniibacterium thermophilum* (Yu *et al.*, 2013). At the time of writing, the genus *Lysobacter* comprised 26 species with validly published names (http://www.bacterio.net/lysobacter.html), including the recently described species *Lysobacter panacisoli* (Choi *et al.*, 2014). Most members of the genus are able to glide, but are non-motile. Here, we report the polyphasic characterization of a motile bacterial strain, designated 9NM-14^T, isolated from an abandoned lead-zinc ore; we propose it to be a novel member of the genus *Lysobacter*.

Strain 9NM-14^T was isolated from an abandoned lead-zinc ore sample collected from Meizhou, Guangdong Province, south China (24° 21′ 44″ N 116° 16′ 34″ E). Bacterial isolation was performed using R2A agar (Qingdao Hope, China), as described previously (Feng *et al.*, 2014). Strain 9NM-14^T was stored at –80 °C in liquid R2A broth (Qingdao Hope, China) supplemented with 30 % (v/v) glycerol. For phylogenetic analysis, the genomic DNA was extracted using a bacterial genomic DNA isolation kit (Sangon). The 16S rRNA gene was amplified by PCR with the bacterial universal primers fD1 and rP1 (Weisburg *et al.*, 1991). The PCR products were cloned to the vector pCR 2.1 and sequenced by Invitrogen Biotechnology. Similarity searches were carried out using the EzTaxon-e server (Kim *et al.*, 2012). Distances were calculated according to Kimura's two-parameter model (Kimura, 1980). Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood methods (Felsenstein, 1981) with MEGA 5.0 (Tamura *et al.*, 2011). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method (Felsenstein, 1985) with 1000 replications.

Growth was tested for 7 days at 30 °C on different media, including R2A agar, tryptic soy agar (TSA, Huankai), nutrient agar (NA, Huankai) and half-strength MB agar (Zhang *et al.*, 2011). The morphology of cells grown for 4 days at 30 °C on R2A agar was observed under a

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 9NM-14^T is JQ608331.

Three supplementary figures are available with the online Supplementary Material.

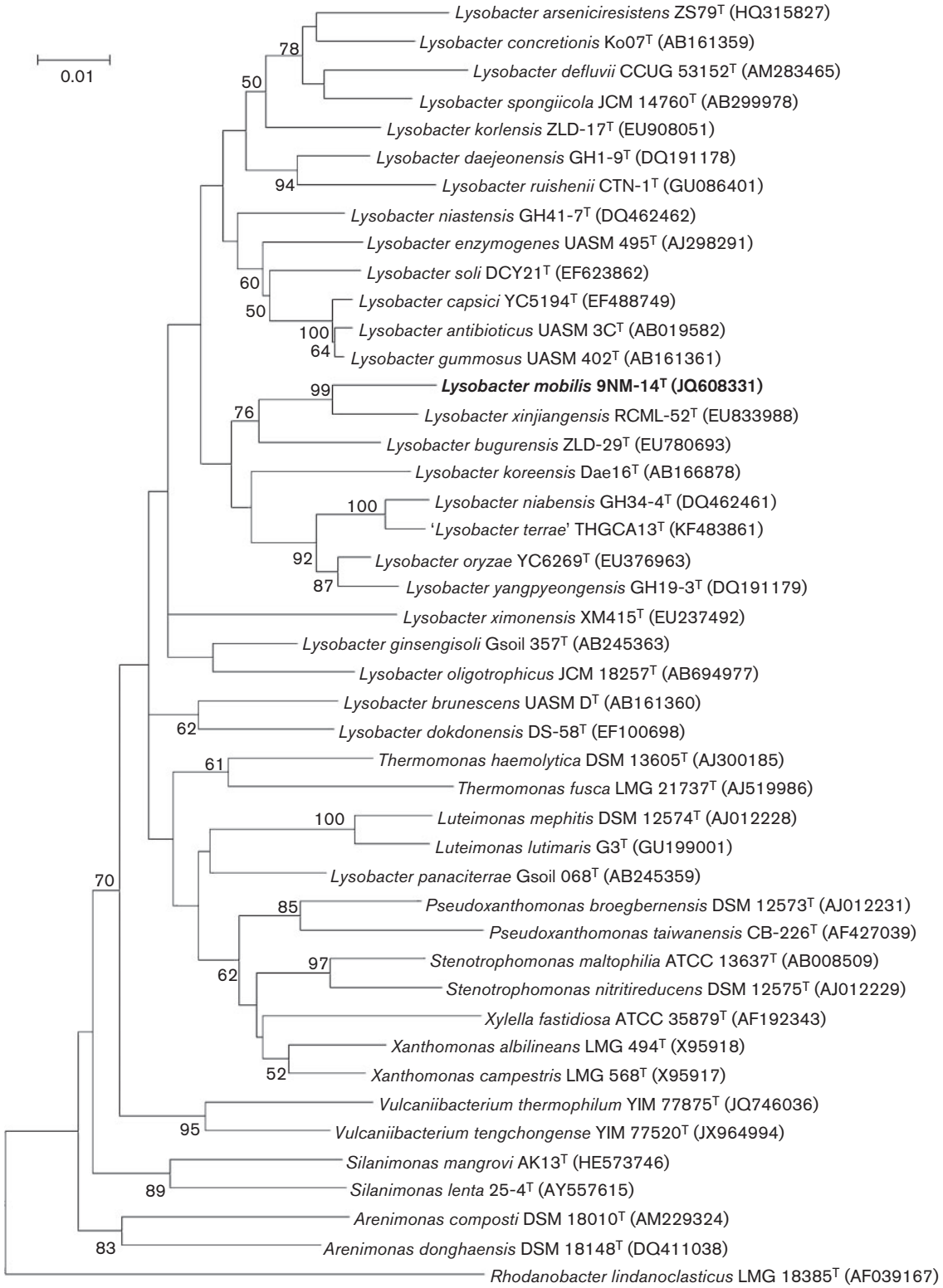

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences of strain 9NM-14^T and other members of the genus *Lysobacter*. Bootstrap values are expressed as a percentages of 1000 replications. Only bootstrap values of more than 50 % are shown. '*Lysobacter terrae*' represents the species which has not been validly published. Bar, 0.01 substitutions per nucleotide position.

transmission electron microscope (Hitachi, H7650). Motility testing was performed using R2A broth supplemented with 0.3 % agar. Gliding motility was determined as described by Bowman (2000). Anaerobic growth was observed in an anaerobic pouch (MGC) for 7 days at 30 °C on R2A agar. The pH range for growth (pH 4–10, with a pH interval of 1) and tolerance to NaCl (0–6 %, w/v, at intervals of 0.5 % NaCl) were tested in R2A broth for 7 days at 30 °C. Growth at different temperatures (4, 10, 15, 20, 25, 30, 32, 35, 37 and 40 °C) was determined on R2A agar slants for 7 days. The Gram reaction, catalase activity, methyl-red test and Voges–Proskauer test were conducted as described by Tindall *et al.* (2007). Hydrolysis of starch (1 %, w/v), CM-cellulose (0.1 %, w/v), casein (1 %, w/v), chitin (1 %, w/v), tyrosine (0.5 %, w/v) and Tweens 20, 40 and 80 (1 %, w/v) were tested on R2A agar. Oxidase, arginine dihydrolase, β-galactosidase, urease, nitrate reduction, gelatin and aesculin hydrolysis, the utilization of citrate and substrate, and indole production were determined using API 20NE (bioMérieux) and GN3 MicroPlates (Biolog) at 30 °C, according to the manufacturers' instructions.

The DNA G+C content of strain 9NM-14^T was tested using HPLC, as described by Mesbah *et al.* (1989). DNA–DNA hybridization experiments were conducted in triplicate using a Lambda 35 UV/VIS spectrometer equipped with a temperature program controller (PerkinElmer), as described by De Ley *et al.* (1970). For whole-cell fatty acid analysis, strain 9NM-14^T and closely related type strains were incubated in R2A broth at 30 °C for 5 days (stationary phase) under the same conditions. Fatty acids were determined according to the protocol of the Sherlock Microbial Identification System (MIDI) (Sasser, 1990). Respiratory quinones were extracted and analysed by HPLC (UltiMate 3000; Dionex) according to the methods described by Collins *et al.* (1977) and Hiraishi *et al.* (1996). Polar lipids were determined as described by Tindall *et al.* (2007).

The 16S rRNA gene sequence of strain 9NM-14^T showed the highest similarity to *Lysobacter xinjiangensis* RCML-52^T (97.4 %) followed by *Lysobacter bugurensis* ZLD-29^T (96.3 %) and other recognized species of the genus *Lysobacter* (92.7–95.4 %). The neighbour-joining phylogenetic tree showed that strain 9NM-14^T fell in the genus of *Lysobacter* and formed a cluster with *L. xinjiangensis* RCML-52^T and *L. bugurensis* ZLD-29^T (Fig. 1). The maximum-parsimony and maximum-likelihood trees (Figs S1 and S2, available in the online Supplementary Material) also supported strain 9NM-14^T being closely related to *L. xinjiangensis* RCML-52^T and *L. bugurensis* ZLD-29^T, and belonging to the genus *Lysobacter*. The DNA–DNA relatedness value between strain 9NM-14^T and *L. xinjiangensis* RCML-52^T was determined to be 30.1 ± 7.6 %, which is significantly below the threshold of 70 % proposed for species discrimination by Wayne *et al.* (1987). Results of the phylogenetic analysis indicated that strain 9NM-14^T represents a novel species of the genus *Lysobacter*.

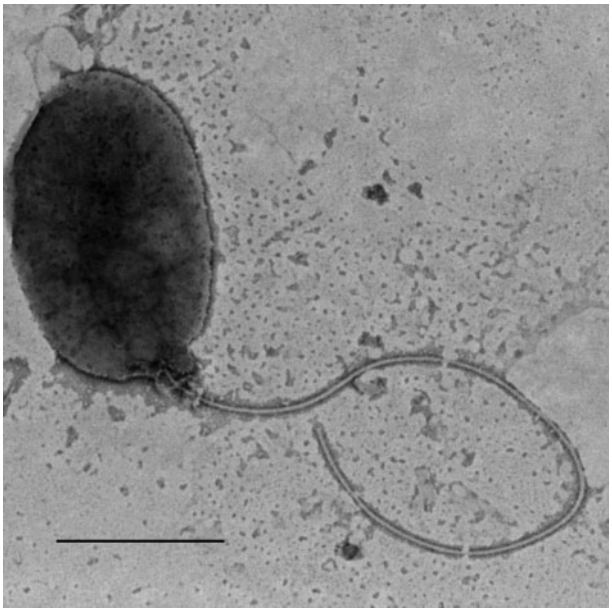

Fig. 2. Transmission electron micrograph of a cell of strain 9NM-14^T. Bar, 500 nm.

Strain 9NM-14^T was Gram-stain-negative, aerobic, rod-shaped, and motile by means of a single polar flagellum (Fig. 2). To our knowledge, most members of the genus *Lysobacter* are non-motile and this is only the third report, following *Lysobacter spongiicola* KMM 329^T (Romanenko *et al.*, 2008) and *Lysobacter arseniciresistens* ZS79^T (Luo *et al.*, 2012), of the occurrence of a member of the genus *Lysobacter* having a flagellum. On R2A agar colonies appeared yellow, circular, transparent and convex with entire edges. The characteristics of strain 9NM-14^T are presented in Table 1 and in the species description. The characteristics of strain 9NM-14^T (such as the ability to hydrolyse gelatin, and the absence of indole production, urease, glucose acidification and arginine dihydrolase) are consistent with other species assigned to the genus *Lysobacter* (Wang *et al.*, 2009). However, strain 9NM-14^T could be clearly distinguished from closely related species of the genus *Lysobacter* and other species recognized to belong to the genus due to the flagellum, conditions for growth, oxidase activity, nitrate reducing ability, starch and Tween 80 hydrolysis, and tyrosine and substrate utilization.

As we found that strain 9NM-14^T and *L. bugurensis* ZLD-29^T hardly grew on the same medium, the fatty acid profile of strain 9NM-14^T was only compared, under the same conditions, with that of *L. xinjiangensis* RCML-52^T. The major cellular fatty acids of strain 9NM-14^T (>5 % of the total) comprised iso-$C_{17:1}\omega9c$ (29.1 %), iso-$C_{15:0}$ (28.9 %), iso-C17 : 0 (9.4 %), iso-$C_{16:0}$ (8.6 %), iso-$C_{11:0}$3-OH (6.9 %) and iso-$C_{11:0}$ (5.8 %), while the fatty acids of *L. xinjiangensis* RCML-52^T were mainly composed of iso-$C_{16:0}$ (32.3 %), iso-$C_{15:0}$ (20.8 %), iso-$C_{17:1}\omega9c$ (20.7 %) and iso-$C_{17:0}$ (7.0 %) (Table 2). The major

Table 1. Differential phenotypic characteristics of strain 9NM-14^T and closely related species of the genus *Lysobacter*

Strains: 1, 9NM-14^T; 2, *L.r xinjiangensis* RCML-52^T; 3, *L. bugurensis* ZLD-29^T. All data were obtained from this study under the same conditions, unless indicated otherwise. +, Positive; −, negative; W, weakly positive; M, motile; G, gliding; N, non-motile.

Characteristic	1	2	3
Growth occurred on			
TSA	−	+	−
Half-strength MB agar	−	+	+
NA	−	+	−
R2A agar	+	+	W
Gliding/motility	M	N*	G†
Ranges for growth			
Temperature (°C)	15−37	18−42	10−37
NaCl (%, w/v)	0−0.5	0−2	0−3
Oxidase	−	+	+
Nitrate reduction	+	−	+
Hydrolysis of:			
Starch	−	−	+
Tween 80	−	+	−
Tyrosine	+	+	−
Assimilation of:			
D-Lactic acid methyl ester	+	−	−
L-Histidine	+	−	+
Pectin	+	−	−
Glycyl L-proline	−	+	−
L-Glutamic acid	−	+	−
L-Aspartic acid	−	+	−
DNA G+C content (mol%)	70.7	69.7*	68.2†

*Data from Liu *et al.* (2011).
†Data from Zhang *et al.* (2011).

Table 2. Cellular fatty acid contents of strain 9NM-14^T and the related species of the genus *Lysobacter*, *L. xinjiangensis* RCML-52^T

Strains: 1, 9NM-14^T; 2, *L. xinjiangensis* RCML-52^T. All the data were obtained from the present study under identical conditions. Data for fatty acids that represented <1 % of the total in both strains are not shown. −, <1 % or not detected.

Fatty acid	1	2
iso-$C_{11:0}$	5.8	4.2
iso-$C_{11:0}$ 3-OH	6.9	4.9
iso-$C_{15:1}$ F	1.3	−
iso-$C_{15:0}$	28.9	20.8
iso-$C_{16:0}$	8.6	32.3
$C_{16:0}$	3.7	1.9
iso-$C_{17:0}$	9.4	7.0
iso-$C_{17:1}\omega 9c$	29.1	20.7
*Summed feature 3	3.3	1.9

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$.

respiratory quinone of strain 9NM-14^T was ubquinone 8 (Q-8), which was consistent with other members of the genus *Lysobacter*. The polar lipids of strain 9NM-14^T consisted of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid and five unidentified phospholipids (Fig. S3). The DNA G+C content of strain 9NM-14^T was 70.7 ± 0.1 mol%, which was different to that of *L. xinjiangensis* RCML-52^T (69.7 mol%) and *L. bugurensis* ZLD-29^T (68.2 mol%) (Table 2).

Therefore, on the basis of the data presented, we propose that strain 9NM-14^T should be considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter mobilis* sp. nov. is proposed.

Description of *Lysobacter mobilis* sp. nov.

Lysobacter mobilis (mo'bi.lis. L. masc. adj. *mobilis* motile).

Cells are Gram-stain-negative, aerobic, oxidase-negative, catalase-positive, motile by means of a single polar flagellum, and rod-shaped (0.4–0.6 × 0.8–1.0 μm). After 4 days at 30 °C on R2A agar, colonies are yellow, convex, smooth, circular, transparent and 1–2 mm in diameter. Growth occurs on R2A agar, but not on TSA, NA, or half-strength MB agar. Growth occurs at 15–37 °C (optimum, 28–30 °C), pH 6.0–8.0 (optimum, pH 7.0) and 0–0.5 % NaCl (w/v) (optimum growth occurs in the absence of NaCl). Negative for urease, β-galactosidase, arginine dihydrolase and indole production. The methyl-red and Voges–Proskauer tests are negative. Tween 40, tyrosine and gelatin are hydrolysed, but Tweens 20 and 80, aesculin, starch, chitin and CM-cellulose are not. Nitrate reduction is positive. Acid is not produced from glucose. Assimilates D-lactic acid methyl ester, L-histidine, pectin, glucuronamide, β-hydroxy-DL-butyric acid and acetoacetic acid. Does not assimilate the following compounds: dextrin, maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, lactose, melibiose, L-arabinose, methyl β-D-glucoside, D-salicin, *N*-acetylglucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetylneuraminic acid, D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D- and L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, *myo*-inositol, glycerol, potassium gluconate, D-glucose 6-phosphate, D-fructose 6-phosphate, L- and D-aspartic acid, D- and L-serine, glycyl L-proline, L-alanine, L-arginine, L-glutamic acid, L-pyroglutamic acid, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxyphenylacetic acid, capric acid, adipic acid, methyl pyruvate, L-lactic acid, citric acid, citrate, α-ketoglutaric acid, malic acid, bromosuccinic acid, γ-aminobutyric acid, α-hydroxybutyric acid, α-ketobutyric acid, propionic acid, acetic acid and formic acid. Major cellular fatty acids are iso-$C_{17:1}\omega 9c$, iso-$C_{15:0}$, iso-C17 : 0, iso-$C_{16:0}$, iso-$C_{11:0}$3-OH

and iso-$C_{11:0}$. The predominant respiratory quinone is Q-8 and the polar lipids contain phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid and five unidentified phospholipids.

The type strain, 9NM-14^T (=GIMCC 1.659^T=CCTCC AB 2014273^T=DSM 27574^T) was isolated from an abandoned lead-zinc ore from Meizhou, Guangdong Province, south China. The genomic DNA G+C content of the type strain is 70.7±0.1 mol%.

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