

Lysobacter xinjiangensis sp. nov., a moderately thermotolerant and alkali-tolerant bacterium isolated from a gamma-irradiated sand soil sample

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A yellow-pigmented bacterial strain, designated RCML-52^T, was isolated from an abandoned gold mine in the desert in Xinjiang, China. Strain RCML-52^T was Gram-negative, aerobic and non-motile. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain RCML-52^T was affiliated with the genus *Lysobacter*. Strain RCML-52^T exhibited <95.6 % 16S rRNA gene sequence similarity to the type strains of all species of the genus *Lysobacter*. The major fatty acids were iso-C_{16:0} (27.6 %), iso-C_{15:0} (19.1 %), iso-C_{17:1ω9c} (16.4 %), iso-C_{11:0} 3-OH (6.5 %) and iso-C_{11:0} (5.3 %). The DNA G + C content was 69.7 mol%. The major isoprenoid quinone was Q-8. On the basis of phylogenetic, phenotypic and chemotaxonomic analysis, strain RCML-52^T should be assigned to a novel species of the genus *Lysobacter*, for which the name *Lysobacter xinjiangensis* sp. nov. is proposed. The type strain is RCML-52^T (=CCTCC AB 208194^T =KCTC 22558^T).

Radiation-resistant bacteria have been assigned to several genera, for example *Deinococcus*, *Rubrobacter*, *Hymenobacter*, *Methylobacterium*, *Kocuria* and *Kineococcus* (Rainey *et al.*, 2005). However, the mechanism underlying their radiation resistance is still not completely understood (Narumi, 2003; Cox & Battista, 2005). It has been suggested that desiccated environments are conducive to the development of radiation resistance in bacteria (Mattimore & Battista, 1996; Rainey *et al.*, 2005; Fredrickson *et al.*, 2008) and it is likely that radiation- and heavy metal-resistant bacteria are to be found in desiccated environments such as abandoned mines. Such resistant bacteria may be beneficial in the bioremediation of complex polluted environments.

The genus *Lysobacter* was established by Christensen & Cook (1978) for non-fruiting, gliding bacteria with high G + C content and 17 species have been described with validly published names (<http://www.bacterio.cict.fr/l/lysobacter.html>). *Lysobacter* species are found in a diverse range of geographical and environmental habitats and, especially, in agricultural soil (Christensen & Cook, 1978; Lee *et al.*, 2006; Weon *et al.*, 2006, 2007; Park *et al.*, 2008).

However, as far as is known, no representatives of the genus *Lysobacter* have been recovered from desiccated environments to date.

To evaluate the diversity of hexavalent chromium- and gamma radiation-resistant bacteria, 52 hexavalent chromium-resistant bacterial strains were recovered after gamma irradiation of a sample of sand soil from an abandoned gold mine in the desert of Xinjiang, China. Of these, 26 representatives were selected for 16S rRNA gene sequencing and phylogenetic analysis. The phylogenetic tree showed that five groups were represented by the isolates: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Deinococcus-Thermus*. As far as is known, chromium resistance has not been described for the genera *Lysobacter*, *Knoellia*, *Nocardioideis*, *Paracoccus*, *Pontibacter* or *Microvirga*. The present study describes one of the isolates, designated RCML-52^T.

Samples (1 g) of sand were exposed to 5 kGy irradiation at a rate of 300 Gy min⁻¹ at room temperature and isolates were obtained by serial dilution plating on modified PTYG medium [containing (w/v): 0.2 % glucose, 0.1 % yeast extract, 0.1 % tryptone, 0.1 % malt extract, 1.5 % agar and 200 µM steam-sterilized K₂CrO₄] and incubation at 30 °C for 20 days. Pure cultures were obtained from single colonies by repeated subcultivation on R2A agar (Difco).

For 16S rRNA gene sequence analysis, genomic DNA was extracted as described by Earl *et al.* (2002). The 16S rRNA gene was amplified by PCR with bacterial universal primers

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RCML-52^T is EU833988.

A supplementary table and a supplementary figure are available with the online version of this paper.

27F and 1492R (Lane, 1991) and the amplification products were sequenced by Invitrogen Biotechnology. Similarity searches with the derived sequence were done in the EzTaxon database (Chun *et al.*, 2007). After multiple alignment of the data using CLUSTAL X (Thompson *et al.*, 1997), phylogenetic analysis was performed using MEGA version 3.1 (Kumar *et al.*, 2004). Distances were calculated according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed using the neighbour-joining algorithm (Saitou & Nei, 1987). The topology of the neighbour-joining tree was evaluated by bootstrap resampling (Felsenstein, 1985) with 1000 replications.

The Gram reaction, oxidase and catalase activities and hydrolysis of starch, casein and aesculin were assessed according to methods described by Smibert & Krieg (1994). Hydrolysis of CM-cellulose (0.1 %, w/v; Sinopharm Chemical Reagent), chitin (1 %, w/v; Sigma) and tyrosine (0.5 %, w/v; Shanghai Ruji Bio-Technology Development) was tested on R2A agar. Gliding motility was observed as described by Bowman (2000). Growth at 4, 12, 18, 30, 37, 42 and 45 °C and with 0–3 % (w/v) NaCl (at intervals of 1 % NaCl) was determined on R2A agar (Difco) and growth at pH 5–11 (at intervals of 1 pH unit) was determined in R2A broth. The API 20 NE and API ZYM systems (bioMérieux) were also used, according to the manufacturer's instructions. Fatty acid methyl esters were analysed using the Sherlock Microbial Identification System (MIDI), according to the manufacturer's instructions, after incubation on R2A agar at 30 °C for 48 h. Respiratory quinones were extracted and determined by HPLC as described by Xie & Yokota (2003). The DNA G+C content of strain RCML-52^T was determined using HPLC according to Mesbah *et al.* (1989).

To determine the tolerance of strain RCML-52^T to UV radiation, cells were harvested from TGY broth (0.5 % tryptone, 0.3 % yeast extract, 0.1 % glucose) during

exponential growth (OD₆₀₀ approx. 0.5). by centrifugation and washed and resuspended in 10 mM potassium phosphate buffer (pH 7.0) to a density of 10⁷ cells ml⁻¹. A cell suspension (2 ml) was placed in a 6-cm-diameter plastic dish and exposed to UV-C (254 nm), using a digital radiometer (Spectroline DRC-100H; Bioblock Scientific) to monitor the dose, and then incubated at 30 °C for 3 days. *Lysobacter oryzae* DSM 21044^T (cultured with TGY medium) and *Escherichia coli* K-12 (cultured with LB medium) were also tested at the same time. Relative survival was determined by comparing with non-irradiated cultures.

The 16S rRNA gene sequence of strain RCML-52^T showed <95.6 % similarity to those of the type strains of all recognized species in the genus *Lysobacter*. The most closely related type strains were *Lysobacter niabensis* GH34-4^T (95.5 % 16S rRNA gene sequence similarity) and *Lysobacter niastensis* GH41-7^T (95.0 %). The neighbour-joining phylogenetic tree showed that strain RCML-52^T formed a cluster with *Lysobacter* sp. KO_CM43 and an uncultured bacterium (bootstrap value 100 %; Fig. 1). It has long been recognized that organisms with >3 % 16S rRNA gene sequence dissimilarity belong to different genomic species (Stackebrandt & Goebel, 1994).

Cells of strain RCML-52^T were Gram-negative, non-motile rods. Colonies were yellow, circular and convex with entire edges. Strain RCML-52^T grew at 18–42 °C (optimum 37 °C), at pH 7–11 (optimum pH 8) and with 0–2 % NaCl (optimum 1 % NaCl). The characteristics of strain RCML-52^T are given in the species description and those that differentiate strain RCML-52^T from members of the genus *Lysobacter* are given in Table 1 and Supplementary Table S1 (available in IJSEM Online). Strain RCML-52^T exhibited characteristics that are consistent with those of the genus *Lysobacter*, such as the ability to hydrolyse gelatin, the inability to assimilate potassium gluconate, adipic acid,

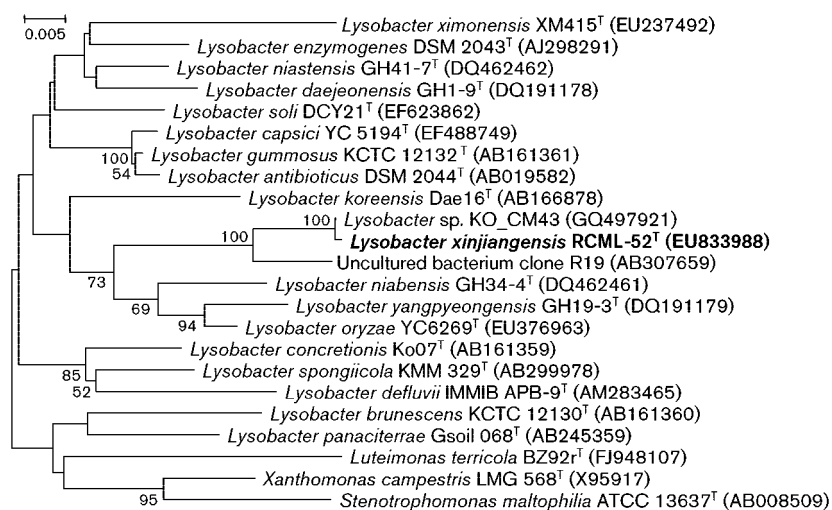


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain RCML-52^T in the genus *Lysobacter*. Bootstrap values (>50 %) are shown at branch nodes. Bar, 0.5 % sequence variation.

Table 1. Differential phenotypic characteristics of strain RCML-52^T and selected representatives of the genus *Lysobacter*

Strains: 1, *Lysobacter xinjiangensis* sp. nov. RCML-52^T; 2, *L. niabensis* DSM 18244^T; 3, *L. yangpyeongensis* DSM 17635^T; 4, *L. oryzae* DSM 21044^T. Data were obtained in this study unless indicated. All strains were positive for acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. All strains were negative for assimilation of L-arabinose, D-glucose, maltose, D-mannose, D-mannitol, N-acetylglucosamine, adipic acid, capric acid, potassium gluconate, trisodium citrate and phenylacetic acid and activities of α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase and α -mannosidase.

Characteristic	1	2	3	4
Colony characteristics*				
Texture	S	I	S	S
Colour	Y	Y	Y	PY
Gliding motility	—	—	—	+
Catalase	+	+	—	+
Growth with 2 % NaCl	+	—	—	—
Ranges for growth				
Temperature (°C)	18–42	5–37	15–40	15–42
pH	7–11	5–8 ^{a†}	5–8 ^b	5.5–10 ^c
Hydrolysis of:				
Starch	—	+	+	—
Aesculin	+	—	—	+
Assimilation of:				
Malic acid	—	—	+	—
Enzyme activities				
N-Acetyl- β -glucosaminidase	—	+	+	—
α -Glucosidase	—	—	+	+
Lipase (C14)	+	—	—	—
Trypsin	—	—	+	—
Valine arylamidase	+	—	+	+
DNA G + C content (mol%)	69.7	62.5 ^a	67.3 ^b	67.4 ^c

*I, Irregular; PY, pale yellow; S, smooth; Y, yellow.

[†]Data taken from: a, Weon *et al.* (2007); b, Weon *et al.* (2006); c, Aslam *et al.* (2009).

capric acid and phenylacetic acid and the absence of indole production, glucose acidification, arginine dihydrolase and urease (Wang *et al.*, 2009). However, there were some traits that did not conform to any of the recognized species of the genus *Lysobacter*, such as the temperature, pH and NaCl ranges for growth, the ability to hydrolyse aesculin and the presence and absence of some enzymes.

The predominant quinone of strain RCML-52^T was Q-8, which corresponds to that described for members of the genus *Lysobacter* (Aslam *et al.*, 2009; Bae *et al.*, 2005; Lee *et al.*, 2006; Park *et al.*, 2008; Romanenko *et al.*, 2008; Srinivasan *et al.*, 2010; Wang *et al.*, 2009; Weon *et al.*, 2006, 2007; Yassin *et al.*, 2007). The DNA G + C content of strain

RCML-52^T was 69.7 mol%, which was within the range described for the genus *Lysobacter* (61.7–70.1 mol%; Christensen & Cook, 1978; Weon *et al.*, 2006). In Table 2, the fatty acids of strain RML-52^T are compared with those obtained under the same conditions for type strains of species of the genus *Lysobacter*. The major fatty acids (>5 %) of strain RCML-52^T were iso-C_{16:0}, iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{11:0} 3-OH and iso-C_{11:0}, which were consistent with those described for the genus *Lysobacter*, but the proportions were different from those determined in this study for *L. niabensis* DSM 18244^T, *L. yangpyeongensis* DSM 17635^T and *L. oryzae* DSM 21044^T (Table 2).

Strain RCML-52^T exhibited unusually high resistance to UV light (Supplementary Fig. S1). Survival rates for strain RCML-52^T and *L. oryzae* DSM 21044^T after exposure to 240 J m^{−2} UV radiation were 4.6–8.0 and 1.1–2.4 %, respectively, and after exposure to 360 J m^{−2} were 0.9–1.5 and 0.1 %, respectively. Strain RCML-52^T exhibited no growth with 200 μM K₂CrO₄, but it can tolerate high levels of other heavy metals (unpublished results).

Thus, on the basis of 16S rRNA gene sequence analysis and physiological and chemotaxonomic properties, strain RCML-52^T represents a distinct, previously undescribed species within the genus *Lysobacter*, for which the name *Lysobacter xinjiangensis* sp. nov. is proposed.

Description of *Lysobacter xinjiangensis* sp. nov.

Lysobacter xinjiangensis (xin.jiang.en'sis. N.L. masc. adj. *xinjiangensis* pertaining to Xinjiang, in north-western China, where the type strain was isolated).

Cells are Gram-negative, non-motile, non-spore-forming rods. Colonies are yellow on R2A agar. Catalase- and oxidase-positive. Grows at 18–42 °C (optimum 37 °C), at pH 7–11 (optimum pH 8) and with 0–2 % NaCl (optimum 1 % NaCl). Hydrolyses aesculin, casein, gelatin and tyrosine, but not chitin, CM-cellulose or starch. Does not reduce nitrate, ferment glucose or produce indole. With API 20 NE, does not assimilate L-arabinose, D-glucose, maltose, D-mannose, D-mannitol, N-acetylglucosamine, trisodium citrate, potassium gluconate, adipic acid, capric acid, malic acid or phenylacetic acid. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, cystine arylamidase (weak) and α -chymotrypsin (weak), but negative for arginine dihydrolase, trypsin, urease, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and N-acetyl- β -glucosaminidase. The major fatty acids (>5 %) are iso-C_{16:0}, iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{11:0} 3-OH and iso-C_{11:0}. The major respiratory quinone is ubiquinone Q-8. The DNA G + C content of the type strain is 69.7 mol%.

The type strain is RCML-52^T (=CCTCC AB 208194^T =KCTC 22558^T), isolated from an abandoned gold mine in the desert in Xinjiang, China.

Table 2. Cellular fatty acid contents of strain RCML-52^T and type strains of species in the genus *Lysobacter*

Strains: 1, *L. xinjiangensis* sp. nov. RCML-52^T; 2, *L. niabensis* DSM 18244^T; 3, *L. yangpyeongensis* DSM 17635^T; 4, *L. oryzae* DSM 21044^T (data in columns 1–4 from this study); 5, *L. antibioticus* DSM 2044^T; 6, *L. brunescens* DSM 6979^T; 7, *L. concretionis* KCTC 12205^T; 8, *L. daejeonensis* DSM 17634^T; 9, *L. enzymogenes* DSM 2043^T; 10, *L. gummosus* DSM 6980^T; 11, *L. koreensis* KCTC 12204^T; 12 *L. niastensis* DSM 18481^T (data in columns 5–12 from Wang *et al.*, 2009); 13, *L. capsici* KCTC 22007^T (Park *et al.*, 2008); 14, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007); 15, *L. spongiicola* JCM 14760^T (Romanenko *et al.*, 2008); 16, *L. ximonensis* CCTCC AB 207091^T (Wang *et al.*, 2009); 17, *L. soli* KCTC 22011^T (Srinivasan *et al.*, 2010). Values are percentages of total fatty acids; fatty acids that represented <1 % of the total in all strains are not shown. –, Not detected/not reported or <1.0 %.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
iso-C _{11:0}	5.3	4.9	4.3	6.3	3.1	5.9	5.7	3.7	3.4	3.8	5.3	4.1	2.3	1.8	9.5	3.7	4.1
iso-C _{11:0} 3-OH	6.5	5.1	5.6	8.0	8.0	7.2	6.9	6.0	6.6	9.7	9.0	8.0	3.8	7.2	15.5	5.2	5.8
C _{14:0}	–	–	1.6	–	1.1	–	–	–	1.0	–	–	–	1.9	–	–	1.7	–
iso-C _{14:0}	–	7.7	1.4	–	1.3	3.7	2.3	11.2	1.4	–	4.0	4.2	–	–	3.3	6.1	–
iso-C _{15:0}	19.1	15.1	27.5	25.3	24.9	19.6	33.6	13.1	20.5	25.2	12.5	21.9	23.3	40.9	23.0	22.6	34.3
iso-C _{15:1} F	2.1	2.4	–	–	–	1.7	3.2	3.2	–	–	–	–	–	–	–	1.8	–
anteiso-C _{15:0}	1.0	5.6	2.7	–	3.8	2.6	1.2	3.2	3.8	5.5	–	3.8	–	–	–	6.3	1.8
C _{16:0}	3.5	3.4	13.1	6.2	8.0	1.5	1.5	1.4	8.6	6.0	–	–	10.8	2.9	–	7.0	1.4
C _{16:1} ω7c alcohol	1.7	1.4	–	–	1.6	–	–	–	–	1.7	10.8	4.5	–	–	–	–	–
iso-C _{16:0}	27.6	29.8	9.8	15.4	10.3	23.5	20.4	33.7	13.8	5.7	26.3	23.3	–	19.2	32.5	24.0	7.5
iso-C _{16:1} H	–	1.0	–	–	–	1.5	–	2.6	–	–	2.1	1.3	–	–	–	–	–
iso-C _{17:0}	3.9	1.3	8.1	7.5	3.4	2.3	4.1	–	2.9	7.8	1.8	1.3	3.7	11.1	2.8	1.5	17.2
iso-C _{17:1} ω9c	16.4	9.5	8.0	14.9	6.4	15.5	15.1	6.7	4.7	12.2	16.7	10.9	–	5.8	–	6.7	19.5
Summed feature 3*	1.7	3.2	1.4	1.1	8.3	9.5	–	6.1	15.8	6.4	1.4	6.5	20.4	–	–	2.9	–

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

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