**Lysobacter korlensis** sp. nov. and **Lysobacter bugurensis** sp. nov., isolated from soil

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Two Gram-negative, rod-shaped, gliding, yellow-pigmented bacterial strains, designated ZLD-17T and ZLD-29T, were isolated from arid soil samples collected from Xinjiang Province, north-west China, and subjected to analysis using a polyphasic taxonomic approach. Both novel strains required 1.0–2.0 % (w/v) sea salts for optimal growth. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these two strains belong to the genus *Lysobacter* within the class *Gammaproteobacteria*. Strain ZLD-17T showed highest 16S rRNA gene sequence similarities to *Lysobacter capsici* CCTCC AB 207175T (96.1 %), *Lysobacter spongicola* DSM 21749T (96.8 %) and *Lysobacter korlensis* KCTC 12204T (96.8 %), whereas strain ZLD-29T showed highest sequence similarity to *Lysobacter niastensis* DSM 18481T (96.0 %) and *Lysobacter enzymogenes* DSM 2043T (95.9 %). 16S rRNA gene sequence similarity between strains ZLD-17T and ZLD-29T was 96.1 %. The DNA G+C contents of strains ZLD-17T and ZLD-29T were 67.9 and 68.2 mol%, respectively. The major cellular fatty acids of both strains were summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c), iso-C17:1ω9c, iso-C16:0, C16:0 and iso-C11:0 3-OH; their predominant isoprenoid quinone was Q-8 and their major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol. Based on their phenotypic characteristics, phylogenetic position as determined by 16S rRNA gene sequence analysis and chemotaxonomic data, strains ZLD-17T (=CCTCC AB 207174T) and ZLD-29T (=CCTCC AB 207175T) represent two novel species of the genus *Lysobacter*, for which the names *Lysobacter korlensis* sp. nov. and *Lysobacter bugurensis* sp. nov. are proposed, respectively.

The genus *Lysobacter* was first described by Christensen & Cook (1978) and the description was emended by Park et al. (2008). Members of the genus *Lysobacter*, in the family *Xanthomonadaceae*, contain ubiquinone Q-8 as the major respiratory quinone and have a high DNA G+C content (Park et al., 2008; Wang et al., 2009). Members of the genus are strongly proteolytic and characteristically lyse a variety of micro-organisms, including Gram-negative bacteria, Gram-positive bacteria, cyanobacteria, filamentous fungi, yeasts, algae and nematodes (Christensen & Cook, 1978). At the time of writing, the genus comprised 17 species with validly published names, including the recently described species *Lysobacter soli* (Srinivasan et al., 2010) and *Lysobacter panaciterrae* (Ten et al., 2009). During the course of an investigation of the culturable bacterial community in soil from an arid area in Xinjiang Province, north-west China, 180 bacterial strains were isolated; 60 pure cultures were randomly selected for 16S rRNA gene sequencing and phylogenetic analysis. These isolates included members of the phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Deinococcus-Thermus*. In this study, a polyphasic taxonomic approach was used to characterize two isolates, designated strains ZLD-17T and ZLD-29T, belonging to the *Gammaproteobacteria*. On the basis of phenotypic characteristics, chemotaxonomic data and phylogenetic analysis of 16S rRNA gene sequences, the two isolates were found to represent two novel species of the genus *Lysobacter*.

Strains ZLD-17T and ZLD-29T were isolated from soil taken from an arid area (42° 78'–43° 15' N 88° 65'–88° 83' E)
in Xinjiang Province, north-west China. Soil samples were suspended in sterilized water and diluted solutions were spread on agar plates containing half-strength marine broth 2216 (MB; Difco) supplemented with 1.5% agar. Isolation was achieved after incubation at 28 °C for 1 week. Unlike other Lysobacter species with validly published names, strains ZLD-17T and ZLD-29T showed poor growth on R2A agar (Difco); the former did not grow and the latter formed several small colonies after incubation at 28 °C for 1 week. Thus, the isolates were routinely cultivated on half-strength MB agar at 28 °C and stored as glycerol suspensions (20%, v/v) at −80 °C.

For 16S rRNA gene sequencing and phylogenetic analysis, DNA was extracted using a commercial genomic DNA extraction kit (ChaoShi-Bio). The primer pair 27f (5′-GAGTTTGATCCTGGCTCAG-3′) and 1527r (5′-AGAAAGGAGGTGATCCAGCC-3′) was used for amplification of the 16S rRNA gene (Lane, 1991). PCR and 16S rRNA gene sequencing were carried out as described by Lin et al. (2004). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Phylogenetic analysis was performed by using MEGA version 4.1 (Tamura et al., 2007), after multiple alignment of the data via CLUSTAL_X (Thompson et al., 1997). A distance matrix method (distance options according to the Kimura two-parameter model), including clustering by using the neighbour-joining method, and a discrete character-based maximum-parsimony method were used. In each case, bootstrap values were calculated based on 1000 replications.

The almost complete 16S rRNA gene sequences for strains ZLD-17T and ZLD-29T (1448 and 1453 bp, respectively) were determined. The 16S rRNA gene sequence similarity between strains ZLD-17T and ZLD-29T was 96.1%. In phylogenetic trees based on neighbour-joining and maximum-parsimony algorithms (Fig. 1; Supplementary Fig. S1, available in IJSEM Online), strains ZLD-17T and ZLD-29T fell within the radiation of the cluster comprising species of the genus Lysobacter. However, the topology of the phylogenetic tree generated using the maximum-parsimony algorithm (Supplementary Fig. S1) differed somewhat from that of the tree reconstructed using the neighbour-joining method (Fig. 1). Strain ZLD-17T formed a clade with Lysobacter koreensis KCTC 12204T in the neighbour-joining tree but formed a distinct monophyletic group in the maximum-parsimony tree. Strain ZLD-29T formed a clade with Lysobacter xinjiangensis RCML-52T in both phylogenetic trees. Strain ZLD-17T exhibited highest 16S rRNA gene sequence similarities to Lysobacter capsici KCTC 22007T (96.9%), Lysobacter spongicola DSM 21749T (96.8%) and L. koreensis KCTC 12204T (96.8%), whereas strain ZLD-29T showed highest sequence similarities to Lysobacter niastensis DSM 18481T (96.0%) and Lysobacter enzymogenes DSM 2043T (95.9%). The low levels of 16S rRNA gene sequence similarity (<97%) among Lysobacter species with validly published names and the novel isolates indicated that the two isolates represented two novel genomic species of the genus Lysobacter (Stackebrandt & Goebel, 1994).

For strains ZLD-17T and ZLD-29T, cellular morphology was determined by using phase-contrast microscopy and transmission electron microscopy with 2-day-old cells grown on half-strength MB agar. The Gram reaction was carried out according to the classical Gram procedure described by Doetsch (1981). Gliding motility was determined as described by Bowman (2000). Growth was assessed on half-strength MB medium at 4, 7, 10, 15, 20, 28, 33, 37, 42 and 45 °C and at pH 4–11 at intervals of one pH unit to determine the temperature and pH ranges for growth.

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains ZLD-17T and ZLD-29T and closely related species. Bootstrap values >50% (based on 1000 replications) are shown at branching points. Bar, 0.005 substitutions per nucleotide position.](image-url)
growth. Growth in the absence of NaCl and in the presence of 0.5–5.0 % (w/v) NaCl (in increments of 0.5 %) was investigated in peptone–yeast extract (PY) medium (Bacto peptone, 2.5 g; yeast extract, 0.5 g; distilled water, 1 l). Growth was also determined in PY medium containing 0–5 % (w/v) sea salts (increments of 0.5 %; Sigma). Growth under anaerobic conditions was determined by incubation for 1 week in an anaerobic chamber (Oxoid AnaeroJar System) on half-strength MB agar. Oxidase activity was tested using a 1 % solution of tetramethyl-p-phenylenediamine (Kovacs, 1956). Catalase activity was detected by assessing the production of bubbles after the addition of a drop of 3 % H2O2. Tests to determine decomposition of hippurate was determined as described by Gordon & Smith (1955). Hydrolysis of starch and casein was tested as described by Smibert & Krieg (1994). Hydrolysis of hippurate was determined as described by Kinyon & Harris (1979). Haemolytic activity was tested on half-strength MB agar supplemented with 5 % (v/v) defibrinated sheep blood. Hydrolysis of CM-cellulose (0.1 %, w/v) and chitin from crab shells (1 %, w/v) was also tested. Growth was evaluated on trypticase soy agar (TSA; Difco), MacConkey agar (Difco) and brain–heart infusion (BHI) agar (Becton Dickinson) at 28 °C. Acid production from carbohydrates was determined as described by Leifson (1963). The commercially available API 20 E, API 20 NE, API ID 32GN and API ZYM systems (bioMérieux) were used to determine biochemical properties, utilization of carbohydrates and enzyme activities according to the manufacturer’s instructions except that all suspension media for strains ZLD-17T and ZLD-29T were supplemented with 1 % (w/v) sea salts.

Cells of strains ZLD-17T and ZLD-29T were aerobic, Gram-negative, rod-shaped (Supplementary Fig. S2). Colonies of both strains were yellow, circular and convex with clear margins after incubation at 28 °C for 2 days on half-strength MB agar (Difco). Sea salts were favourable for growth of both novel strains. Strain ZLD-17T did not grow in PY medium containing NaCl (0–5 %) as the only salt but grew in PY medium supplemented with 0.5–4.0 % sea salts (optimum 1–2 %). Strain ZLD-29T grew very weakly in PY medium but grew much better in PY medium supplemented with 0.5–3.0 % NaCl. However, optimum growth of strain ZLD-29T occurred in PY medium supplemented with 1–2 % sea salts. Both strains were unable to grow on TSA (Difco), MacConkey agar (Difco) or BHI agar (Becton Dickinson). With a longer incubation time (>1 week), the centres of colonies of strain ZLD-17T developed a brown colour. Strain ZLD-17T differed from strain ZLD-29T in its ability to hydrolyse tyrosine, ONPG and p-nitrophenyl-β-D-galactopyranoside (PNPG) but not gelatin. Furthermore, strain ZLD-17T produced enzymes such as trypsin and β-galactosidase but strain ZLD-29T did not. The phenotypic characteristics of strains ZLD-17T and ZLD-29T are summarized in the species description and a comparison of selective characteristics with recognized members of the genus Lysobacter is given in Table 1.

The DNA G+C content was determined by HPLC according to the method of Mesbah et al. (1989). Respiratory quinones were extracted and determined by HPLC as described by Xie & Yokota (2003). Polar lipids were extracted and analysed as described by Tindall (1990). For polar lipid analysis, a 6.75 ml portion of chloroform/methanol/0.3 % aqueous NaCl (1 : 2 : 0.8, v/v/v) was added to 100 mg freeze-dried cell material. The preparation was stirred overnight and cell debris was pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0.3 % aqueous NaCl mixture to a ratio of 1 : 1 : 0.9 (v/v/v) and then dried under nitrogen. The dried polar lipids were resuspended in chloroform/methanol (2 : 1, v/v) and separated by two-dimensional TLC. Total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate Schiff (α-glycols), Dragendorff reagent (quaternary nitrogen) and anisaldehyde-sulfuric acid (glycolipids).

To determine whole-cell fatty acid profiles, strains ZLD-17T and ZLD-29T were grown at 28 °C for 48 h on half-strength MB agar. Analysis of the fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (MIDI, 1999).

The DNA G+C contents of strains ZLD-17T and ZLD-29T were 67.9 and 68.2 mol%, respectively. Both of them had ubiquinone-8 (Q-8) as the major isoprenoid quinone, which is a characteristic feature of members of the genus Lysobacter (Bae et al., 2005). The fatty acid compositions of the novel strains and type strains of recognized Lysobacter species are given in Table 2. The major fatty acids detected (percentages of the total cellular fatty acids) from strains ZLD-17T and ZLD-29T were summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c, 21.2 and 28.4 %, respectively), iso-C15:0 3-OH (16.0 and 18.8 %), iso-C16:0 (14.2 and 7.3 %), C16:0 (9.3 and 13.2 %) and iso-C15:0 3-OH (6.8 and 6.1 %). Strains ZLD-17T and ZLD-29T also differed from each other in the relative amounts of anteiso-C15:0. Compared with all recognized members of the genus Lysobacter, both novel strains contained larger amounts of summed feature 3 but significantly smaller amounts of iso-C15:0. The polar lipid profiles of strains ZLD-17T and ZLD-29T (Fig. 2) were similar in terms of their major components, including phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, which are characteristic polar lipids of the genus Lysobacter (Park et al., 2008; Romanenko et al., 2008). In addition, both strains contained minor amounts of an unknown amino-lipid (AL1) and three unknown phospholipids (PL1–3). Nevertheless, some small differences could be observed in their polar lipid profiles. Strain ZLD-17T differed from strain ZLD-29T by the presence of unknown phospholipids PL4 and PL5 and the absence of unknown phospholipid PL6.
Table 1. Phenotypic characteristics of strains ZLD-17T and ZLD-29T and type strains of the genus Lysobacter

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<td>β-Galactosidase</td>
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*Strain ZLD-17T did not grow with NaCl as the only salt. The value given refers to growth in sea salts.

Therefore, on the basis of the data presented, strains ZLD-17T and ZLD-29T represent two novel species of the genus Lysobacter, for which the names Lysobacter korlensis sp. nov. and Lysobacter bugurensis sp. nov. are proposed, respectively.

**Description of Lysobacter korlensis sp. nov.**

Lysobacter korlensis (kor.len’sis. N.L. masc. adj. korlensis pertaining to Korla, a city of Xinjiang Province in northwest China, from where the type strain was isolated).

Cells are aerobic, Gram-reaction-negative, gliding and rod-shaped (0.3–0.4 × 1.0–2.5 μm). Colonies are yellow, circular, convex and 1–2 mm in diameter with clear margins after incubation at 28 °C for 2 days on half-strength MB agar (Difco). Growth occurs at 10–37 °C (optimum 28 °C), at pH 6–11 (optimum pH 7–8) and in 0.5–4.0 % (w/v) sea salts (optimum 1.0–2.0 %) but no growth occurs in media containing NaCl as the only salt. Weakly positive for catalase; oxidase-positive. Exhibits x-haemolysis. Hydrolyses aesculin, casein, starch, tyrosine, ONPG and PNPG but not gelatin, adenine, guanine, hypoxanthine, xanthine, elastin, keratin, testosterone, hippurate, CM-cellulose or chitin. Nitrate reduction and acetoin production (Voges–Proskauer reaction) are weakly positive. Hydrogen sulfide production, citrate utilization, indole production and activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and trypthphan deaminase are negative (API 20 E). Negative for assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, ribose, sucrose, suberic acid, lactic acid, alanine, glycogen, melibiose, 3-hydroxybutyric acid, proline, inositol, itaconic acid, sodium malonate, sodium acetate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, l-serine, salicin, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate and 4-hydroxybenzoic acid (API 20 NE and API ID 32GN). Acid is produced from D-glucose, but not from D-mannitol, glyceral, maltose, D-arabinose,
L-arabinose, D-ribose, D-xylene, D-galactose, D-fructose, D-mannose, L-rhamnose, lactose, sucrose or trehalose. According to the API ZYM gallery (bioMérieux), positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-galactosidase activities but negative for lipase (C14), valine arylamidase, cystine arylamidase, x-galactosidase, β-glucuronidase, x-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-fucosidase activities. The predominant respiratory quinone is Q-8. The polar lipids consist of diphasitidyglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown aminolipid and five unknown phospholipids. The major fatty acids are summed feature 3 (iso-C15:0 2-OH and/or C16:1ω9c), iso-C17:0 ω9c, iso-C16:0, C16:0 and iso-C14:1ω6c 3-OH; detailed fatty acid compositions are given in Table 2.

The type strain, ZLD-17T (≡ CCTCC AB 207174T = KCTC 23076T), was isolated from an arid soil sample collected from Korla, Xinjiang Province, north-west China. The DNA G+C content of the type strain is 67.9 mol%.

**Description of *Lysobacter bugurensis* sp. nov.**

*Lysobacter bugurensis* (bu.gur.en’sis. N.L. masc. adj. bugurensis pertaining to Bugur, a county of Xinjiang Province in north-west China, from where the type strain was isolated).

Cells are aerobic, Gram-reaction-negative, gliding and rod-shaped (0.6–0.7 × 1.1–1.5 μm). Colonies are yellow, circular, convex and 1–2 mm in diameter with clear margins after incubation at 28 °C for 2 days on half-strength MB agar (Difco). Growth occurs at 10–37 °C (optimum 28 °C), at pH 6–11 (optimum pH 7–8) and in the presence of 0–3% (w/v) NaCl; optimal growth requires the presence of 1–2% (w/v) sea salts. Weakly positive for catalase; oxidase-positive. Exhibits α-haemolysis. Hydrolyses ascusolin, gelatin, casein and starch but not tyrosine.
ONPG, PNPG, adenine, guanine, hypoxanthine, xanthine, elastin, keratin, testosterone, hippurate, CM-cellulose or chitin. Nitrate reduction is weakly positive. Hydrogen sulfide production, acetoin production (Voges–Proskauer reaction), citrate utilization, indole production and oxidase reaction). Nitrite reduction is weakly positive. Hydrogen sulfide production, acetoin production (Voges–Proskauer reaction), citrate utilization, indole production and oxidase reaction). Nitrite reduction is weakly positive. Hydrogen sulfide production, acetoin production (Voges–Proskauer reaction), citrate utilization, indole production and oxidase reaction).

**Fig. 2.** Two-dimensional TLC of polar lipids of strains ZLD-17T (a) and ZLD-29T (b) stained with 5% ethanolic molybdophosphate acid. PE, Phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; AL1, unknown aminolipid; PL1–PL6, unknown phospholipids; PIG, pigment.

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naphthol-AS-BI-phosphohydrolase activities but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. The predominant respiratory quinone is Q-8. The polar lipids consist of diposphatidylglycerol, phosphatidylglycerol, phosphatidyethanolamine, an unknown aminolipid and four unknown phospholipids. The major fatty acids are summed feature 3, iso-C17:1ω9c, C16:1ω9c and iso-C15:0 3-OH; detailed fatty acid compositions are given in Table 2.

The type strain, ZLD-29T (= CCTCC AB 207175T = KCTC 23077T), was isolated from an arid soil sample collected from Bugur, Xinjiang Province, north-west China. The DNA G+C content of the type strain is 68.2 mol%.

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**References**


