Lysobacter ximonensis sp. nov., isolated from soil

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A bacterial isolate, strain XM415^T, obtained from soil of Tibet in China was characterized using a polyphasic taxonomic approach. Strain XM415^T was aerobic, Gram-negative, gliding, rod-shaped, oxidase-negative and catalase-positive. Strain XM415^T showed the highest 16S rRNA gene sequence similarity with *Lysobacter niastensis* GH41-7^T (96.0 %). Ubiquinone Q-8 and branched fatty acids, such as iso-$C_{16:0}$ (24.0 %), iso-$C_{15:0}$ (22.6 %), iso-$C_{17:1}\omega9c$ (6.7 %), iso-$C_{14:0}$ (6.1 %) and iso-$C_{11:0}$ 3-OH (5.2 %), were predominant in strain XM415^T as well as in all type strains of recognized *Lysobacter* species. The DNA G+C content of XM415^T was 63.5 mol%. The genotypic and phenotypic data show that strain XM415^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter ximonensis* sp. nov. is proposed. The type strain is XM415^T (=CCTCC AB 207091^T =NRRL B-51263^T).

The genus *Lysobacter* was first proposed by Christensen & Cook (1978), and the type species is *Lysobacter enzymogenes*. Although the description of an antibiotic-producing species, '*Lysobacter lactamgenus*', has been published (Ono *et al.*, 1984; Kimura *et al.*, 1996), its taxonomic position has not yet been confirmed and its name has not been validly published. The species of the genus *Lysobacter* are related to members of the genera *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Thermomonas* and *Xylella*, containing ubiquinone Q-8 as the major respiratory quinone and having fatty acid profiles with a predominance of iso-branched fatty acids. At the time of writing, the genus *Lysobacter* encompasses 13 species with validly published names: *Lysobacter antibioticus*, *L. brunescens*, *L. enzymogenes*, *L. gummosus* (Christensen & Cook, 1978), *L. concretionis* (Bae *et al.*, 2005), *L. daejeonensis*, *L. yangpyeongensis* (Weon *et al.*, 2006), *L. koreensis* (Lee *et al.*, 2006), *L. niabensis*, *L. niastensis* (Weon *et al.*, 2007), *L. defluvii* (Yassin *et al.*, 2007), *L. capsici* (Park *et al.*, 2008) and *L. spongiicola* (Romanenko *et al.*, 2008). Members of the genus are strongly proteolytic and characteristically lyse a variety of micro-organisms (both Gram-negative and Gram-positive bacteria), as well as nematodes (Christensen, 2005; Yassin *et al.*, 2007), suggesting that they have a particular biological function in microbial ecosystems.

In this study, strain XM415^T was isolated from a soil sample presented by Chaohua Liao (a Chinese geologist) from Ximo, at an altitude of 750 m in Tibet (most areas of Tibet are above 2000 m). The soil sample was suspended in sterilized water and diluted solutions were spread on 0.1 × tryptic soy broth (TSB; Difco) agar. The plates were incubated at 30 °C for 4 days, and strain XM415^T was subsequently isolated. Cellular morphology was determined by using light microscopy with 2-day-old cells. The gliding motility test was performed as described by Bowman (2000). Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by observation of bubble production after the application of 3 % (v/v) hydrogen peroxide solution. Tests were also made for hydrolysis of starch, CM-cellulose (0.1 %, w/v), chitin from crab shells (1 %, w/v), casein (5 %, w/v) and tyrosine (0.5 %, w/v) (Smibert & Krieg, 1994). The temperature range (5, 10, 15, 20, 28, 30 and 37 °C) and pH range (pH 4–11 at intervals of 1 pH unit) and the requirement for 0, 0.5, 1, 2, 3 and 5 % NaCl (w/v) for growth were determined by using 0.1 × TSB medium.

The API ZYM, API 20E and API 20NE systems (bioMérieux) were used to determine enzyme activities, fermentation tests and biochemical properties of strain XM415^T following the manufacturer's instructions. Utilization of some carbohydrates and organic acids by strain XM415^T was tested by using the Biolog Microbial Identification System according to the protocol provided by the manufacturer.

Genomic DNA was extracted with a Bacteria Genomic DNA Isolation kit (Shanghai Chaoshi bio Technologies Co. Ltd). The 16S rRNA gene was amplified by PCR with bacterial universal primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1527R (5′-AGAAAGGAGGTGATCCAGCC-3′) (Rainey *et al.*, 1996), and the PCR products were sequenced by Invitrogen. Similarity searches with the derived sequence were done in the EzTaxon database (Chun *et al.*, 2007). Phylogenetic analysis was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain XM415^T is EU237492.

performed by using MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data via CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained using options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values from 1000 replications were used to determine the confidence level of the branches (Felsenstein, 1985).

Analysis of the 16S rRNA gene sequence of strain XM415^T resulted in a sequence of 1458 bp. 16S rRNA gene sequence similarities to recognized members of the genus *Lysobacter* were 96.0 % (*L. niastensis* GH41-7^T), 95.8 % (*L. enzymogenes* DSM 2043^T), 95.5 % (*L. brunescens* KCTC 12130^T), 95.4 % (*L. antibioticus* DSM 2044^T), 95.4 % (*L. gummosus* KCTC 12132^T) and 95.2 % (*L. capsici* YC5194^T), i.e. below the threshold for demarcating bacterial species (Stackebrandt & Goebel, 1994). No other recognized bacterial species showed more than 96.0 % 16S rRNA gene sequence similarity to the new isolate. As shown in the phylogenetic tree (Fig. 1), the novel isolate XM415^T forms a distinct subline within the genus *Lysobacter*, branching together with the type strain of *L. brunescens* by the

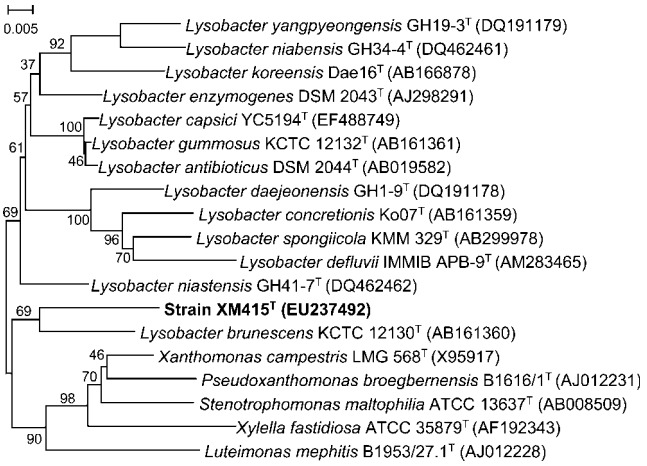

Fig. 1. Neighbour-joining phylogenetic tree, showing the position of strain XM415^T within the radiation of the order *Xanthomonadales*. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete. Numbers at nodes are bootstrap percentages, based on 1000 resamplings. Bar, 0.005 sequence divergence.

Table 1. Cellular fatty acid profiles of members of the genus *Lysobacter*

Strains: 1, strain XM415^T; 2, *L. antibioticus* DSM 2044^T; 3, *L. brunescens* DSM 6979^T; 4, *L. capsici* KCTC 22007^T; 5, *L. concretionis* KCTC 12205^T; 6, *L. daejeonensis* DSM 17634^T; 7, *L. enzymogenes* DSM 2043^T; 8, *L. gummosus* DSM 6980^T; 9, *L. koreensis* KCTC 12204^T; 10, *L. niabensis* DSM 18244^T; 11, *L. niastensis* DSM 18481^T; 12, *L. spongiicola* JCM 14760^T; 13, *L. yangpyeongensis* DSM 17635^T. Data are expressed as percentages of total fatty acids and were taken from Weon *et al.* (2006), Park *et al.* (2008), Romanenko *et al.* (2008) and this study; −, <1 % or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
$C_{10:0}$ 3-OH	−	−	−	−	−	−	1.1	−	−	−	−	−	−
iso-$C_{11:0}$	3.7	3.1	5.9	2.3	5.7	3.7	3.4	3.8	5.3	6.4	4.1	9.5	4.3
iso-$C_{11:0}$ 3-OH	5.2	8.0	7.2	3.8	6.9	6.0	6.6	9.7	9.0	9.3	8.0	15.5	5.5
$C_{14:0}$	1.7	1.1	−	1.9	−	−	1.0	−	−	−	−	−	−
iso-$C_{14:0}$	6.1	1.3	3.7	−	2.3	11.2	1.4	−	4.0	8.7	4.2	3.3	4.5
iso-$C_{15:0}$	22.6	24.9	19.6	23.3	33.6	13.1	20.5	25.2	12.5	12.7	21.9	23.0	14.5
iso-$C_{15:1}$ at 5	−	1.0	−	−	−	−	−	1.7	4.4	2.4	1.6	−	3.1
iso-$C_{15:1}$ F	1.8	−	1.7	−	3.2	3.2	−	−	−	−	−	−	−
anteiso-$C_{15:0}$	6.3	3.8	2.6	−	1.2	3.2	3.8	5.5	−	5.9	3.8	−	5.1
$C_{16:0}$	7.0	8.0	1.5	10.8	1.5	1.4	8.6	6.0	−	1.1	−	−	3.1
$C_{16:1}\omega7c$ alcohol	−	1.6	−	−	−	−	−	1.7	10.8	7.8	4.5	−	8.8
$C_{16:1}\omega11c$	−	4.1	−	2.2	−	−	−	4.5	−	1.0	−	−	2.2
iso-$C_{16:0}$	24.0	10.3	23.5	−	20.4	33.7	13.8	5.7	26.3	23.7	23.3	32.5	27.5
iso-$C_{16:1}$ H	−	−	1.5	−	−	2.6	−	−	2.1	1.0	1.3	−	1.1
$C_{17:0}$ cyclo	−	7.24	−	−	1.9	−	6.2	1.0	−	−	−	−	−
iso-$C_{17:0}$	1.5	3.4	2.3	3.7	4.1	−	2.9	7.8	1.8	1.6	1.3	2.8	1.9
iso-$C_{17:1}\omega9c$	6.7	6.4	15.5	9.3	15.1	6.7	4.7	12.2	16.7	10.0	10.9	−	6.7
anteiso-$C_{17:0}$	−	−	−	−	−	−	−	1.4	−	−	−	−	1.1
$C_{18:1}\omega7c$	1.2	1.7	−	6.5	−	−	3.3	2.5	−	−	−	−	−
Unknown 11.799	−	2.0	−	−	−	−	1.5	1.8	−	−	1.4	−	−
Summed feature 3*	2.9	8.3	9.5	20.4	−	6.1	15.8	6.4	1.4	2.0	6.5	−	3.3

*Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

Table 2. Differential phenotypic characteristics of strain XM415^T and type strains of species of the genus *Lysobacter*

Strains: 1, strain XM415^T; 2, *L. antibioticus* DSM 2044^T; 3, *L. brunescens* DSM 6979^T; 4, *L. capsici* KCTC 22007^T; 5, *L. concretionis* KCTC 12205^T; 6, *L. daejeonensis* DSM 17634^T; 7, *L. defluvii* DSM 18482^T; 8, *L. enzymogenes* DSM 2043^T; 9, *L. gummosus* DSM 6980^T; 10, *L. koreensis* KCTC 12204^T; 11, *L. niabensis* DSM 18244^T; 12, *L. niastensis* DSM 18481^T; 13, *L. spongiicola* JCM 14760^T; 14, *L. yangpyeongensis* DSM 17635^T. All data are from the present study. All strains are positive in API 20NE tests for gelatin hydrolysis and negative for indole production, glucose acidification, arginine dihydrolase, urease and assimilation of potassium gluconate, capric acid, adipic acid and phenylacetic acid.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Catalase/oxidase	+/−	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/−	+/+	+/+	+/+	−/+
Nitrate reduction	−	+	−	−	−	+	−	−	−	−	−	+	−	−
Aesculin hydrolysis	+	+	+	+	−	+	−	+	+	−	−	+	−	−
Assimilation of:														
D-Glucose	+	+	−	+	−	+	−	+	+	−	−	−	−	−
L-Arabinose	−	−	−	−	−	−	−	+	−	+	−	−	−	−
D-Mannose	+	+	−	+	−	−	−	+	+	−	−	−	−	−
D-Mannitol	−	−	−	−	−	−	+	+	−	+	−	−	−	−
N-Acetylglucosamine	+	+	−	−	−	−	−	+	+	−	−	+	−	−
Maltose	+	+	−	+	−	+	+	+	+	−	−	+	−	−
Malic acid	−	+	−	−	−	−	−	+	+	−	−	−	−	+
Trisodium citrate	−	−	−	−	−	−	−	+	−	+	−	−	−	−
Enzyme activities														
α-Galactosidase	−	−	−	+	−	−	−	−	−	−	−	−	−	−
α-Glucosidase	+	−	+	+	+	+	+	+	+	−	+	−	−	+
N-Acetyl-β-glucosaminidase	+	−	−	−	−	−	−	−	+	−	+	−	−	+
β-Glucosidase	−	+	+	+	−	−	−	+	+	−	−	−	−	−
β-Galactosidase	+	+	−	−	−	−	−	+	+	−	−	+	−	−

neighbour-joining method. The DNA G+C content of XM415^T was determined by HPLC (UltiMate 3000; Dionex) (Mesbah *et al.*, 1989) as 63.5 mol%.

The respiratory quinone system was extracted and determined by HPLC as described by Xie & Yokota (2003). Analysis of the respiratory lipoquinones indicated that the isolate contained ubiquinone-8 (Q-8), corresponding to the characteristic feature of members of the genus *Lysobacter* (Bae *et al.*, 2005). Strain XM415^T was grown on R2A medium (Difco) for 48 h at 30 °C and cellular fatty acids were then analysed as methyl esters by GC (Agilent 6890N) according to the instructions of the Sherlock Microbial Identification System (MIDI). The major fatty acids (>5 %) detected (percentages of the total cellular fatty acids) in strain XM415^T were iso-$C_{16:0}$ (24.0 %), iso-$C_{15:0}$ (22.6 %), $C_{16:0}$ (7.0 %), iso-$C_{17:1}\omega9c$ (6.7 %), iso-$C_{14:0}$ (6.1 %) and iso-$C_{11:0}$ 3-OH (5.2 %). The presence of iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:1}\omega9c$ as the major fatty acids is a characteristic of genera in the *Xanthomonadaceae*, including the genera *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Xylella* and *Luteimonas* (Assih *et al.*, 2002; Roumagnac *et al.*, 2004; Yang *et al.*, 2005). A comparison of fatty acid profiles among related *Lysobacter* species is shown in Table 1.

The new isolate XM415^T exhibits characteristics consistent with those of the genus *Lysobacter*, but appears not to conform to any recognized species. The low level of 16S rRNA gene sequence similarity (<97 %) among *Lysobacter* species with validly published names indicates that their genomic DNA relatedness is less than 70 % (Stackebrandt & Goebel, 1994). In addition, some biochemical and physiological differences confirm that isolate XM415^T is different from recognized members of the genus *Lysobacter* (Table 2).

Based on the polyphasic taxonomic approach described here, we propose that strain XM415^T should be classified within a novel species of the genus *Lysobacter*, with the name *Lysobacter ximonensis* sp. nov.

Description of *Lysobacter ximonensis* sp. nov.

Lysobacter ximonensis (xi.mo.nen′sis. N.L. masc. adj. *ximonensis* pertaining to Ximo, a village in China, from where the type strain was isolated).

Cells are aerobic, Gram-negative, gliding and rod-shaped (0.5 × 1–3 μm). Colonies on 0.1 × TSB agar are mucoid and yellow in colour. Growth occurs at 10–37 °C, 0–1 % (w/v) NaCl and pH 5–10. Oxidase-negative and catalase-positive. Hydrolyses starch, casein, tyrosine and *o*-nitrophenyl β-D-galactopyranoside, but not chitin or CM-cellulose. Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and *N*-acetyl-β-glucosaminidase and weakly positive for esterase C4, esterase lipase C8, cystine arylamidase, α-chymotrypsin and β-galactosidase (API ZYM). Utilizes α-cyclodextrin, dextrin, glycogen, D-fructose,

α-D-glucose, maltose, D-mannose, L-alaninamide, L-alanyl glycine, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, D-glucose 6-phosphate, cellobiose, pyruvic acid methyl ester, β-hydroxybutyric acid, L-alanine and L-aspartic acid, but not Tween 80 or other substances contained in the Biolog GN2 microplate. Other phenotypic characteristics are summarized in Table 2. Contains iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{14:0}$ and iso-$C_{11:0}$ 3-OH as major fatty acids and ubiquinone Q-8 as the respiratory quinone. The G+C content of the genomic DNA of the type strain is 63.5 mol%.

The type strain, XM415^T (=CCTCC AB 207091^T =NRRL B-51263^T), was isolated from a soil sample taken from Ximo, Tibet, China.

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