Lysinibacter cavernae gen. nov., sp. nov., a new member of the family *Microbacteriaceae* isolated from a karst cave

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A Gram-stain-positive, aerobic, straight or slightly bent rod-shaped, non-motile, non-spore-forming bacterium, designated strain CC5-806^T, was isolated from a soil sample collected from a wild karst cave in the Wulong region, Chongqing, PR China and examined using a polyphasic approach to clarify its taxonomic position. This bacterium did not produce substrate mycelium or aerial hyphae, and no diffusible pigments were observed on the media tested. Strain CC5-806^T grew optimally without NaCl at 20 °C and at pH 7.0. Phylogenetic analysis, based on 16S rRNA gene sequences, indicated that strain CC5-806^T belonged to the family *Microbacteriaceae* and showed the highest levels of 16S rRNA gene sequence similarities with *Frigoribacterium endophyticum* EGI 6500707^T (97.56 %), *Frigoribacterium faeni* 801^T (97.53 %) and *Glaciihabitans tibetensis* MP203^T (97.42 %). Phylogenetic trees revealed that strain CC5-806^T did not show a clear affiliation to any genus within the family *Microbacteriaceae*. The DNA G+C content of strain CC5-806^T was 62.6 mol%. The cell-wall peptidoglycan contained L-lysine as a diagnostic diamino acid. The predominant menaquinones were MK-11, MK-10 and MK-9. Phosphatidylglycerol, diphosphatidylglycerol, an unidentified glycolipid, four unidentified phospholipids and other polar lipids were detected in the polar lipid extracts. The major fatty acids were anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{14:0}$. On the basis of the phylogenetic analysis, and phenotypic and chemotaxonomic characteristics, strain CC5-806^T was distinguishable from phylogenetically related genera in the family *Microbacteriaceae*. It represents a novel species of a novel genus, for which the name *Lysinibacter cavernae* gen. nov., sp. nov. is proposed. The type strain is CC5-806^T (=DSM 27960^T=CGMCC 1.14983^T).

The family *Microbacteriaceae* was first proposed by Park *et al.* (1993) and then emended by Stackebrandt *et al.* (1997) and Zhi *et al.* (2009). At the time of writing, 49 genera with validly published names have been reported within the family *Microbacteriaceae* (http://www.bacterio.net/-classifgenerafamilies.html#Microbacteriaceae; Euzéby, 1997). In the last 3 years 13 genera, isolated from different habitats, have been reported to be novel members of the family *Microbacteriaceae*: *Alpinimonas* (Schumann *et al.*, 2012), *Diaminobutyricimonas* (Jang *et al.*, 2012), *Gryllotalpicola* (Kim *et al.*, 2012a), *Homoserinimonas* (Kim *et al.*, 2012b), *Compostimonas* (Kim *et al.*, 2012c), *Naasia* (Weon *et al.*, 2013), *Pontimonas* (Jang *et al.*, 2013a), *Lysinimonas* (Jang *et al.*, 2013b), *Rudaibacter* (Kim *et al.*, 2013), *Galbitalea* (Kim *et al.*, 2014a), *Conyzicola* (Kim *et al.*, 2014b), *Glaciihabitans* (Li *et al.*, 2014) and *Rhodoluna* (Hahn *et al.*, 2014).

During a study on the cultivable actinobacterial diversity of soil in the Wulong region (29° 29′ 42″ N 107° 54′ 32″ E), Chongqing, PR China, strain CC5-806^T was isolated from a soil sample collected in a wild karst cave. Based on phylogenetic analysis, strain CC5-806^T showed high levels of 16S rRNA gene sequence similarities with members of the family *Microbacteriaceae*. Polyphasic taxonomic studies showed that strain CC5-806^T differed from previously described genera within the family *Microbacteriaceae* and represented a novel genus. The taxonomic position of this strain is reported on here.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC5-806^T is KP411613.

Four supplementary figures and two supplementary tables are available with the online Supplementary Material.

Strain CC5-806^T was isolated by using the dilution plating technique on FA agar (containing, l^{-1} distilled water: 5.0 g fulvic acid, 6.4 g Na_2HPO_4, 1.5 g KH_2PO_4, 0.5 g NH_4Cl, 0.24 g $MgSO_4$, 4.0 g $CaCO_3$, 20.0 g agar; pH 7.2) plates after 4 weeks of incubation at 15 °C. Isolated colonies were transferred and streaked onto the ISP 2 agar (Shirling & Gottlieb, 1966) until pure cultures were obtained. The strain was cultivated, maintained on ISP 2 agar slants at 4 °C and stored as aqueous glycerol suspensions (20 %, v/v) at −80 °C.

Cultural, physiological and biochemical characteristics of strain CC5-806^T were tested using two reference strains with experiments carried out under the same conditions: *Frigoribacterium faeni* 801^T (=DSM 10309^T) was from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and *Glaciihabitans tibetensis* MP203^T (=CGMCC 1.12484^T) was from the China General Microbiological Culture Collection Center (CGMCC, Beijing, China). Cultural characteristics were determined by observing the growth of the strain at 28 °C for 3–4 weeks on ISP 2, ISP 3, ISP 4, ISP 5, ISP 7 agars (Shirling & Gottlieb, 1966), nutrient agar (Waksman, 1961), R2A agar (Bacto), tryptic soy agar (TSA; Bacto) and Bennett's agar (Gordon & Smith, 1955). ISCC-NBS colour charts (Kelly, 1964) were used to assess the colony colours. The cell morphology and dimensions were observed and recorded by scanning electron microscopy (Quanta 200; FEI) using gold-coated dehydrated specimens and by transmission electron microscopy (JEM-1400; JEOL) after incubation on ISP 2 agar at 28 °C. The Gram-staining test was performed as described by Magee *et al.* (1975). Motility was studied by the hanging drop method (Skerman, 1967). The temperature range for growth was determined by incubation of the strain on R2A agar at 4, 10, 15, 20, 25, 28, 37, 42 and 50 °C for 14 days. The pH range for growth was measured in R2A broth at various pH values (pH 4.0–13.0, at intervals of 1 pH unit) for 4 weeks. For the experiments on pH, different buffers were used, as described by Xu *et al.* (2005). Salt tolerance was tested on R2A agar supplemented with concentrations of 0, 1, 3, 5, 7 and 10 % (w/v) NaCl for 14 days. Catalase activity was determined by bubble production in 3 % (v/v) H_2O_2. Oxidase activity was assessed by using 1 % (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of cellulose, starch, gelatin, and Tweens 20, 40 and 80, production of melanin and H_2S, milk coagulation and peptonization were tested as described by Gonzalez *et al.* (1978). Acid production from carbon sources was examined by using the API 50CH system (bioMérieux). Oxidation of the carbon sources and sensitivity to antimicrobial compounds were both tested by using Biolog GEN III MicroPlates. Other physiological and biochemical characteristics and enzyme activities were tested by using the API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Strain CC5-806^T was Gram-stain-positive, non-spore-forming and aerobic. Colonies of strain CC5-806^T on ISP 2 agar were circular, brilliant yellow, smooth and entire. Neither substrate mycelium nor aerial mycelium were formed and no diffusible pigments were produced on the media tested. Cells were straight or slightly bent rod-shaped (Figs S1 and S2, available in the online Supplementary Material). Strain CC5-806^T grew well on ISP 2 agar, R2A agar, TSA and nutrient agar. Poor growth occurred on Bennett's agar and ISP 3 agar. No growth occurred on ISP 4, ISP 5, ISP 7 agars (Table S1). Strain CC5-806^T grew at 4–37 °C, pH 6.0–12.0 and tolerated 0–5 % (w/v) NaCl. No growth occurred at 42 °C, 50 °C, pH 5.0, pH 13.0 or in the presence of 7 % (w/v) NaCl. The best growth occurred at pH 7.0, 20 °C and without NaCl. The detailed physiological and biochemical characteristics of strain CC5-806^T are given in Table 1 and in the species description.

For chemotaxonomic studies of polar lipids, menaquinones and fatty acids, strain CC5-806^T was analysed together with the reference strains. Analysis of the peptidoglycan structure of strain CC5-806^T was carried out by the Identification Service of the DSMZ. Cell-wall peptidoglycans were isolated and analysed by the methods of Schleifer & Kandler (1972), Schleifer (1985) and Schumann (2011). The polar lipids were extracted and analysed by two-dimensional TLC on a silica gel 60 F_{254} plate (Merck) as described by Minnikin *et al.* (1984). The solvent systems of the first dimension and the second dimension were chloroform/methanol/water (64 : 27 : 5, by vol.) and chloroform/methanol/acetic acid/water (80 : 18 : 12 : 5, by vol.), respectively. Menaquinones were isolated and purified according to the method of Collins *et al.* (1977), then analysed and confirmed by HPLC and a single quadrupole mass spectrometer (Guo *et al.*, 2015). For the analysis of whole-cell fatty acids, cell mass of strain CC5-806^T and reference strains was harvested from R2A medium at 20 °C when the bacterial communities reached the late-exponential stage of growth. The whole-cell fatty acids were saponified, methylated and extracted, according to the standard protocol described by Sasser (1990), and analysed by using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C single quadrupole mass spectrometer equipped with the Nist08 Library software database. A capillary column, HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies), was used for the separation of fatty acid methyl esters. The initial temperature of 90 °C was maintained for 1 min, raised to 180 °C at a rate of 10 °C min^{-1}, then raised to 210 °C at a rate of 2 °C min^{-1} and finally to 270 °C at a rate of 20 °C min^{-1} and maintained for 2 min. Helium was used as a carrier gas with a flow rate of 1.0 ml min^{-1}. Injection (2 μl) was made in the splitless mode at an injector temperature of 270 °C. Mass spectra were obtained using electron impact (EI; 70 eV).

For the determination of G+C content, the genomic DNA of strain CC5-806^T was prepared according to the method described by Marmur (1961). The DNA G+C content was determined by reversed-phase HPLC, as described by Mesbah *et al.* (1989).

Table 1. Comparison of the characteristics of strain CC5-806^T and its phylogenetic neighbours

Strains: 1, CC5-806^T; 2, *Frigoribacterium faeni* 801^T; 3, *Glaciihabitans tibetensis* MP203^T. All data shown were obtained in this study unless indicated otherwise. All strains were Gram-stain-positive, positive for catalase, but negative for the hydrolysis of Tween 80, gelatin and urease and for the production of H_2S and indole and for nitrate reduction. In API 20NE kits, all strains were negative for the assimilation of *N*-acetyl-D-glucosamine, captate, malic acid, trisodium citrate, adipate and phenylacetate. In API ZYM kits, all strains were positive for esterase, esterase lipase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for β-glucuronidase and α-fucosidase. +, Positive; −, negative; W, weakly positive; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, unidentified glycolipid; PL, unidentified phospholipid(s); L, unidentified lipid(s).

Characteristic	1	2	3
Cell size(μm)	0.3–0.5 × 0.4–1.5	0.2–0.3 × 1.0–1.5*	0.4–0.5 × 1.0–1.5†
Motility	−	+*	−†
Colony colour	Brilliant yellow	Yellow	Yellow
Growth pH range	6.0–12.0	6.0–10.0	6.0–11.0†
Temperature range for growth (optimum) (°C)	4–37 (20)	4–28 (4–10)	0–25† (15–20)
NaCl tolerance range (optimum) (%, w/v)	0–5 (0)	0–7 (3–5)	0–1 (0)
Oxidase	+	−	+
Milk peptonization and coagulation	−	+	−
Hydrolysis of:			
Cellulose	−	−	−
Starch	+	−	−
Tween 40	+	+	W
Tween 20	−	W	−
Carbon source utilization:			
D-Glucose	+	−	−
L-Arabinose	+	−	−
D-Mannose	+	−	−
D-Mannitol	+	−	−
D-Maltose	+	−	−
Potassium gluconate	+	−	−
API ZYM:			
Alkaline phosphatase	−	+†	−
Valine arylamidase	+	+†	W
Cystine arylamidase	−	+†	W
Trypsin	−	W†	−
β-Galactosidase	−	+†	+
α-Chymotrypsin	−	+†	+
Lipase	W	−†	−
α-Galactosidase	−	+†	+
N-Acetyl-β-glucosaminidase	−	−†	W
α-Mannosidase	−	−†	−
Acid phosphatase	+	+	+
α-Glucosidase	+	+	+
β-Glucosidase	+	+	+
Major fatty acids (>10 %)	anteiso-$C_{15:0}$ (41.23 %), iso-$C_{16:0}$ (34.72 %), iso-$C_{14:0}$ (15.03 %)	anteiso-$C_{15:0}$ (45.30 %), iso-$C_{16:0}$ (25.28 %), $C_{16:0}$ (19.55 %)	anteiso-$C_{15:0}$ (63.40 %), iso-$C_{16:0}$ (22.03 %)

*Data from Kämpfer *et al.* (2000).
†Data from Li *et al.* (2014).

The peptidoglycan of strain CC5-806^T contained lysine, alanine, serine, glycine, threonine, glutamic acid and muramic acid in an approximate molar ratio of 1.0 : 1.5 : 0.1 : 0.6 : 0.6 : 1.3 : 1.1. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of L-Lys, D-Glu, L-Glu, Ser, Gly, L-Thr, D-Thr, L-Ala and D-Ala. The partial hydrolysate contained the peptides Gly-D-Glu, Ala-Ala and two unknown peptides. Dinitrophenylation indicated lysine and alanine as the *N*-terminal amino acids. The peptidoglycan structure of strain CC5-806^T could not be elucidated from the available data, but it represented a novel variation of peptidoglycan, which

was inconsistent with all hitherto published structures of peptidoglycan (www.peptidoglycan-types.info). The acyl type was acetyl.

The predominant menaquinones of strain CC5-806^T were MK-11 (62.2 %), MK-10 (23.3 %) and MK-9 (11.4 %). The polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, an unidentified glycolipid, four unidentified phospholipids and other polar lipids (Fig. S3). The whole-cell fatty acids contained large amounts of anteiso-$C_{15:0}$ (41.23 %), iso-$C_{16:0}$ (34.72 %), iso-$C_{14:0}$ (15.03 %) and small amounts of $C_{16:0}$ (4.06 %), anteiso-$C_{17:0}$ (2.38 %), iso-$C_{15:0}$ (2.08 %) and $C_{14:0}$ (0.50 %). The cellular fatty acid contents of strain CC5-806^T and the reference strains are presented in Table S2. The DNA G+C content of strain CC5-806^T was 62.6 mol%. The major components of the menaquinones, fatty acids and polar lipids of the two reference strains were similar to those previously reported (Kämpfer *et al.*, 2000; Li *et al.*, 2014). The differences in the proportion of fatty acids and slight differences in the types of polar lipids may be due to variation in the experimental conditions used.

The extraction of genomic DNA from strain CC5-806^T and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). The purified PCR products were cloned by using the pEASY-T1 Cloning kit (TransGen Biotech) and sequenced by an ABI PRISM 3730XL DNA Analyser. The 16S rRNA gene sequence similarity values between strain CC5-806^T and related species were obtained from the EzTaxon server (http://eztaxon-e.ezbiocloud.net/; Chun *et al.*, 2007). Multiple alignments were made using CLUSTAL X (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods with MEGA version 5.0 (Tamura *et al.*, 2011). The topologies of the phylogenetic trees were evaluated by using the bootstrap method of Felsenstein (1985) with 1000 repeats.

An almost full-length 16S rRNA gene sequence of strain CC5-806^T (1482 bp) was obtained; a BLAST search showed that strain CC5-806^T exhibited the highest levels of 16S rRNA gene sequence similarities with *Frigoribacterium endophyticum* EGI 6500707^T (97.56 %), *Frigoribacterium faeni* 801^T (97.53 %) and *Glaciihabitans tibetensis* MP203^T (97.42 %). Similarities with other species within the family *Microbacteriaceae* were all less than 97 %. Phylogenetic trees based on 16S rRNA gene sequences generated by using all three tree-making methods showed that strain CC5-806^T was a member of the family *Microbacteriaceae*. According to neighbour-joining and maximum-likelihood tree analysis, strain CC5-806^T clustered with the genera *Frigoribacterium*, *Glaciihabitans*, *Marisediminicola*, *Galbitalea* and *Frondihabitans*, but this was not highly supported as indicated by the low bootstrap values (Figs 1 and S4). The maximum-parsimony tree showed that strain CC5-806^T was grouped with the genera *Glaciihabitans*, *Marisediminicola*, *Galbitalea*, *Subtercola* and *Frondihabitans* (data not shown). These unstable tree topologies revealed that strain CC5-806^T could not be clearly assigned to any genus of the family *Microbacteriaceae* with a validly published name.

Strain CC5-806^T could be clearly differentiated from six phylogenetically related genera (*Frigoribacterium*, *Glaciihabitans*, *Marisediminicola*, *Galbitalea*, *Frondihabitans*, *Subtercola*) within the family *Microbacteriaceae* by comparing some features such as the peptidoglycan type and menaquinone composition. The cell wall of strain CC5-806^T consisted of a novel variation of peptidoglycan with L-lysine as the diagnostic diamino acid, while the cell walls of members of six related genera within the family *Microbacteriaceae* contained 2,4-diaminobutyric acid or ornithine as the diagnostic diamino acid, except for the genus *Frigoribacterium*, which contained lysine (Dastager *et al.*, 2008; Wang *et al.*, 2015) or D-lysine (Kämpfer *et al.*, 2000). The major menaquinones of strain CC5-806^T were MK-11, MK-10 and MK-9, which differentiated strain CC5-806^T from all six related genera within the family *Microbacteriaceae* (Table 2). Strain CC5-806^T could also be clearly differentiated from the genera *Frigoribacterium* and *Glaciihabitans*, its closest phylogenetic neighbours, by differences in other chemotaxonomic properties in addition to peptidoglycan type and menaquinone composition. The dominant fatty acids of strain CC5-806^T were anteiso-$C_{15:0}$ (41.23 %), iso-$C_{16:0}$ (34.72 %) and iso-$C_{14:0}$ (15.03 %), compared with anteiso-$C_{15:0}$ (45.30 %), iso-$C_{16:0}$ (25.28 %) and iso-$C_{14:0}$ (19.55 %) for Frigoribacterium faeni 801T, the type strain of genus *Frigoribacterium* and anteiso-$C_{15:0}$ (63.40 %), iso-$C_{16:0}$ (22.03 %) and iso-$C_{14:0}$ (7.96 %) for Glaciihabitans tibetensis MP203T, the type strain of genus *Glaciihabitans* (Table S2). With respect to polar lipids, strain CC5-806^T shared the presence of phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid with the genera *Frigoribacterium* and *Glaciihabitans*. However, there were some differences in the unidentified phospholipids between CC5-806^T and the genera *Frigoribacterium* and *Glaciihabitans* (Fig. S3). Other chemotaxonomic and phenotypic characteristics differentiating strain CC5-806^T from members of related genera within the family *Microbacteriaceae* are shown in Tables 1 and 2.

In conclusion, based on a phylogenetic analysis, and the phenotypic and chemotaxonomic characteristics, strain CC5-806^T represents a novel species of a novel genus within the family *Microbacteriaceae*, for which the name *Lysinibacter cavernae* gen. nov., sp. nov. is proposed.

Description of *Lysinibacter* gen. nov.

Lysinibacter (Ly.si.ni.bac′ter. N.L. neut. n. *lysinum* lysine, Lys; N.L. masc. n. *bacter* a rod. N.L. masc. n. *Lysinibacter* a rod with lysine in the cell wall).

Cells are Gram-stain-positive, aerobic, straight or slightly bent rod-shaped, non-motile, with neither substrate nor primary mycelium formed. No diffusible pigments are produced on any media tested. Positive for oxidase and

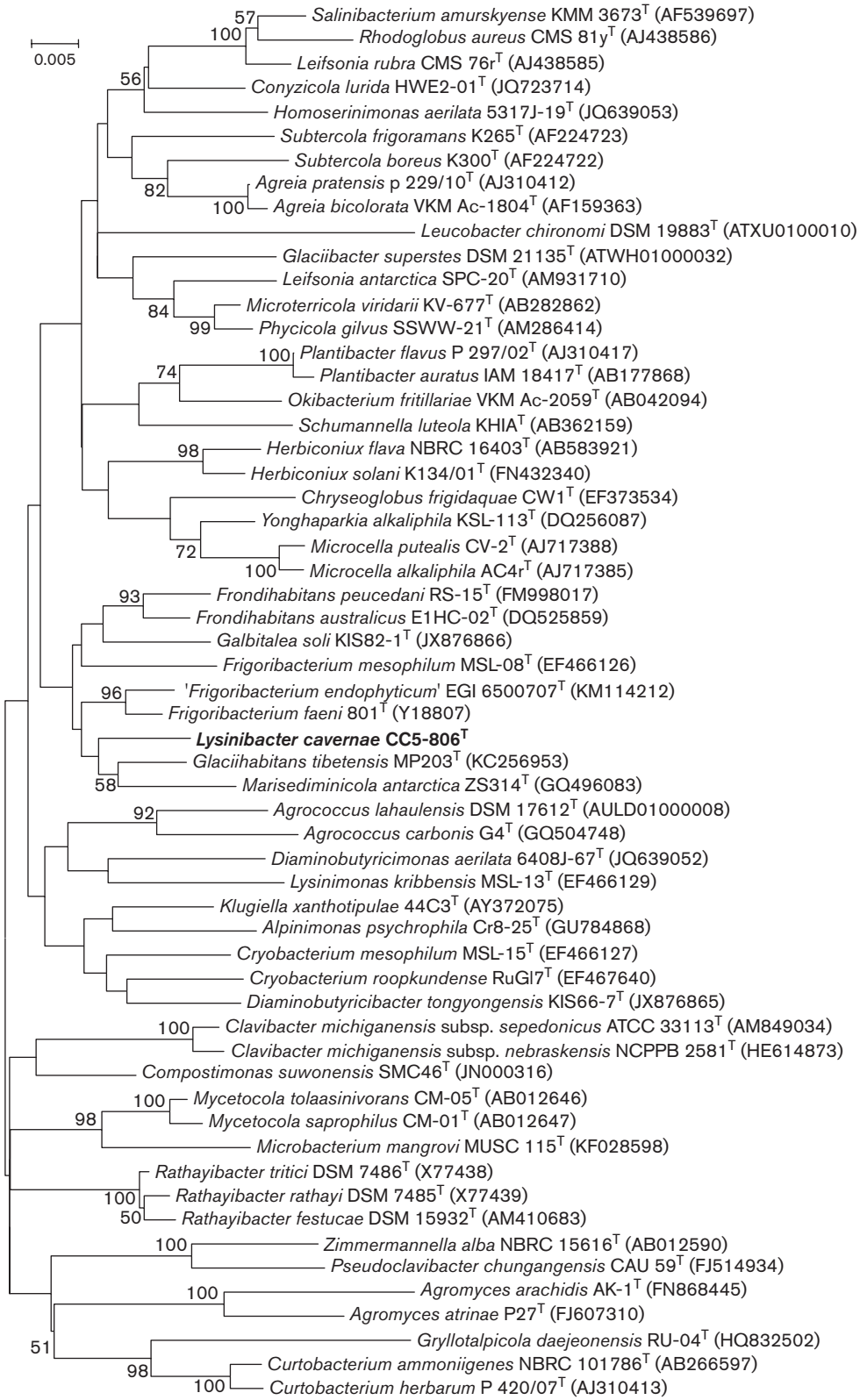

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain CC5-806^T and related species of related genera within the family *Microbacteriaceae*. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values >50 % are shown). Bar, 5 nucleotide substitutions per 1000 nt.

Table 2. Differential characteristics between strain CC5-806^T and related genera in the family *Microbacteriaceae*

Taxa: 1, strain CC5-806^T (data from this study); 2, *Frigoribacterium* [data from this study and Kämpfer *et al.* (2000)]; 3, *Glaciihabitans* [data from this study and Li *et al.* (2014); 4, *Marisediminicola* (Li *et al.*, 2010); 5, *Frondihabitans* (Greene *et al.*, 2009; Lee, 2010; Cardinale *et al.*, 2011); 6, *Galbitalea* (Kim *et al.*, 2014a); 7, *Subtercola* (Männistö *et al.*, 2000). DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, unidentified glycolipid; PL, unidentified phospholipid(s); L, unidentified lipid(s); Lys, lysine; Orn, ornithine; DAB, 2,4-diaminobutyric acid; i, iso; ai, anteiso.

Characteristic	1	2	3	4	5	6	7
Isolation source	Soil	Air, dust, animal shed	Glacier water	Sediment	Soil, leaf litter	Soil	Ground water
Peptidoglycan type	Unknown peptidoglycan structure	B2β	B10	B2β	B2β	B1β	B2γ
Diamino acid (s)	L-Lys	D-Lys	DAB	Orn	Orn	DAB	DAB
Major menaquinones	11, 10, 9	9*	10, 11, 9†	10	8, 7, 9	11, 10, 12	9, 10
Polar lipids	DPG, PG, GL, PL, L	DPG, PG, GL, L*	DPG, PG, GL, L†	DPG, PG, GL	DPG, PG, GL, PL, L	DPG, PG, GL	PG, DPG, PL, GL
Fatty acids	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$*	ai-$C_{15:0}$, i-$C_{16:0}$†	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	$C_{18:1}\omega7c$, ai-$C_{15:0}$, i-$C_{16:0}$, i- $C_{15:1}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$
DNA G+C content (mol%)	62.6	71	64	67	68-71	63.4	64-68

*Data from this study for *Frigoribacterium faeni* 801^T.
†Data from this study for *Glaciihabitans tibetensis* MP203^T.

catalase. The cell-wall peptidoglycan contains L-lysine as the diagnostic diamino acid. The predominant menaquinones are MK-11, MK-10 and MK-9. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid. The whole-cell fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{14:0}$. The DNA G+C content of the type strain of the type species is 62.6 mol%. The type species is *Lysinibacter cavernae*.

Description of *Lysinibacter cavernae* sp. nov.

Lysinibacter cavernae (ca.ver′nae. L. gen. n. *cavernae* of a cavern, the site from which the type strain was isolated).

In addition to those given in the genus description, the following properties are displayed. Cells are approximately 0.4–1.5 μm long and 0.3–0.5 μm wide after incubation for 9 days at 28 °C on ISP 2 agar. Colonies on ISP 2 agar are circular, brilliant yellow, smooth and entire. Strain CC5-806^T grows well on ISP 2 agar, R2A agar, TSA and nutrient agar. Poor growth occurrs on Bennett's agar and ISP 3 agar. No growth occurs on ISP 4, ISP 5 or ISP 7 agars. Growth occurs at 4–37 °C (optimum, 20 °C), pH 6.0–12.0 (optimum, pH 7.0) and with NaCl concentrations of 0–5 % (w/v) (optimum, 0 %). No growth occurs at temperatures of 42 °C and 50 °C, at pH 5 and pH 13.0, or in the presence of 7 % (w/v) NaCl. Cells are positive for the hydrolysis of starch and Tween 40. Hydrolysis of Tweens 20 and 80 and cellulose, nitrate reduction, urease production, and milk peptonization and coagulation are negative. In API ZYM systems, positive for acid phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase. Weakly positive for lipase (C14). Negative for *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and trypsin. In the API 50CH system, produces acid from aesculin, arbutin, D-arabinose, erythritol, D-fructose, L-fucose, glycol, D-lyxose, maltose, mannitol, methyl α-D-glucopyranoside, D-tagatose, D-ribose, L-rhamnose, salicin, xylitol, D-xylose and L-xylose, but not from *N*-acetylglucosamine, D-adonitol, amygdalin, L-arabinose, D-arabitol, L-arabitol, cellobiose, dulcitol, D-fucose, D-galactose, D-gentiobiose, gluconate, D-glucose, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-mannose, melezitose, melibiose, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, raffinose, sucrose, sorbitol, L-sorbose, starch, trehalose, turanose. In the Biolog system, strain CC5-806^T is positive for the oxidation of acetic acid, acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetylneuraminic acid, γ-amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, cellobiose, dextrin, D-fructose, D-fucose, L-fucose, D-fructose 6-phosphate, D-galactonic acid lactone, galacturonic acid, gelatin, gentiobiose, D-gluconic acid, α-D-glucose, D-glucose 6-phosphate, D-glucuronic acid, glucuronamide, L-glutamic acid, glycerol,

glycyl-L-proline, β-hydroxy-DL-butyric acid, *p*-hydroxyphenylacetic acid, inosine, α-ketoglutaric acid, L-lactic acid, D-lactic acid methyl ester, α-lactose, D-malic acid, L-malic acid, maltose, D-mannitol, D-mannose, melibiose, 3-methyl-D-glucose, β-methyl D-glucoside, mucic acid, *myo*-inositol, pectin, L-pyroglutamic acid, quinic acid, raffinose, L-rhamnose, D-saccharic acid, salicin, D-sorbitol, stachyose, sucrose, trehalose, Tween 40 and turanose. Negative for D-alanine, L-aspartic acid, bromosuccinic acid, citric acid, formic acid, D-galactose, L-histidine, α-hydroxybutyric acid, α-ketobutyric acid, methyl pyruvate, propionic acid, D-serine and L-serine. Sensitive to fusidic acid, guanidine hydrochloride, lincomycin, lithium chloride, minocycline, niaproof 4, rifamycin SV, sodium bromate, sodium butyrate, 1 % (w/v) sodium lactate, tetrazolium blue, tetrazolium violet, troleandomycin, vancomycin, pH 5, pH 6, 4 % (w/v) NaCl and 8 % NaCl and resistant to aztreonam, nalidixic acid, potassium tellurite and 1 % (w/v) NaCl. The cell-wall peptidoglycan contains L-lysine as the diagnostic diamino acid. The predominant menaquinones are MK-11, MK-10 and MK-9. The polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, an unidentified glycolipid, four unidentified phospholipids and other polar lipids. The whole-cell fatty acids contain large amounts of anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$ and small amounts of $C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and $C_{14:0}$.

The type strain, CC5-806^T (=DSM 27960^T=CGMCC 1.14983^T), was isolated from a soil sample collected from a wild karst cave in the Wulong region of Chongqing, PR China. The DNA G+C content of the type strain is 62.6 mol%.

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