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TAXONOMIC DESCRIPTION
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Lysobacter tabacisoli sp. nov., isolated from rhizosphere soil of *Nicotiana tabacum* L.

Min Xiao,[1]† Xing-Kui Zhou,[2,3]† Xing Chen,[2]† Yan-Qing Duan,[2] Dalal Hussien M. Alkhalifah,[4] Wan-Taek Im,[5] Wael N. Hozzein,[6] Wei Chen[2,*] and Wen-Jun Li[1,*]

Abstract

A Gram-stain-negative, aerobic, rod-shaped bacterium, designated strain C8-1^T, was isolated from the rhizosphere soil of *Nicotiana tabacum* L. collected from Kunming, south-west China. The cells showed oxidase-positive and catalase-positive reactions. Growth was observed at 10–40 °C, at pH 6.0–8.0 and in the presence of up to 1 % (w/v) NaCl, with optimal growth at 30 °C and pH 7.0. The predominant isoprenoid quinone was Q-8. The major fatty acids were identified as iso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{17:1}\omega9c$. The cellular polar lipids contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, five unidentified phospholipids and two unidentified aminophospholipids. The genomic DNA G+C content was 70.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain C8-1^T should be assigned to the genus *Lysobacter*. 16S rRNA gene sequence similarity analysis showed that strain C8-1^T was closely related to *Lysobacter cavernae* YIM C01544^T (98.6 %), *Lysobacter soli* DCY21^T (97.6 %), *Lysobacter panacisoli* CJ29^T (97.3 %), *Lysobacter firmicutimachus* PB-6250^T (97.3 %), *Lysobacter niastensis* GH41-7^T (97.3 %) and *Lysobacter gummosus* KCTC 12132^T (97.1 %). DNA–DNA hybridization data indicated that the isolate may represent a novel genomic species belonging to the genus *Lysobacter*. Polyphasic taxonomic characteristics indicated that strain C8-1^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter tabacisoli* sp. nov. is proposed. The type strain is C8-1^T (=KCTC 62034^T=CGMCC 1.16271^T) .

The genus *Lysobacter* was first proposed by Christensen and Cook and classified within the family *Xanthomonadaceae* belonging to the class *Gammaproteobacteria*. Members of this genus are generally characterized as Gram-negative, aerobic, non-fruiting and gliding bacteria with a high DNA G+C content [1, 2]. The genus *Lysobacter* contains ubiquinone 8 (Q-8) as the major respiratory quinone and shows a predominance of iso-branched fatty acids [3–5]. Species of the genus *Lysobacter* are commonly found in diverse soil and aquatic habitats and in plants [6–11]. Some members of the genus have potential for development of biocontrol agents based on their ability to lyse a variety of microorganisms such as Gram-positive and Gram-negative bacteria, filamentous fungi, green algae and nematodes [12–15]. This paper reports the isolation and characterization of another member of this genus, which was separated from a rhizosphere soil sample of *Nicotiana tabacum* L.

The rhizosphere soil sample for the study was collected from a tobacco field (24° 30′ N 102° 12′ E), located in Kunming, south-west China, in 2015. Following inoculation of the soil suspension on International *Streptomyces* Project (ISP) 2 agar plates [16], colonies of strain C8-1^T were obtained after incubation for 2 weeks at 30 °C. The purified strain was routinely maintained on ISP 2 agar slants and also preserved as glycerol suspensions (20 %, w/v) at −80 °C. Biomass of strain C8-1^T for chemical and molecular studies was obtained from cultures grown on ISP 2 medium at 30 °C for 4 days, unless otherwise mentioned. The

Author affiliations: [1]State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China; [2]China Tobacco Yunnan Industrial Co. Ltd, Kunming, 650231, PR China; [3]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming 650091, PR China; [4]Biology Department, Faculty of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia; [5]Department of Biotechnology, Hankyong National University, Kyonggi-do 456-749, Republic of Korea; [6]Bioproducts Research Chair (BRC), College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia.
***Correspondence:** Wei Chen, kibchenwei@126.com; Wen-Jun Li, liwenjun3@mail.sysu.edu.cn
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Abbreviations: APL, unidentified aminophospholipid; DPG, diphosphatidylglycerol; ISP, International *Streptomyces* Project; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid; PME, phosphatidylglycerol.
†These authors contributed equally to this work.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain C8-1^T is MF153474. The whole genome shotgun project of strain C8-1^T has been deposited at DDBJ/ENA/GenBank under accession QXJI00000000.
One supplementary table and three supplementary figures are available with the online version of this article.

reference strains *Lysobacter cavernae* YIM C0 1544^T, *Lysobacter soli* KCTC 22011^T, *Lysobacter niastensis* DSM 18481^T and *Lysobacter panacisoli* JCM 19212^T were also cultivated under similar conditions for comparative analyses.

Colony morphology of strain C8-1^T was observed from cultures grown on ISP 2 medium. Cell morphology was observed by transmission electron microscopy (JEM-2100; JEOL) with 3-day-old growth on ISP 2 medium. Motility was determined on the basis of development/absence of turbidity by an inoculum of the strain in a tube containing semi-solid ISP 2 medium as described by Skerman *et al.* [17]. Gram-staining was performed by the standard Gram reaction and confirmed with the KOH lysis test [18]. Anaerobic growth was assessed on ISP 2 agar slants under anaerobic conditions. The pH range for growth was determined between pH 4.0 and 13.0 (with intervals of 1.0 pH unit) in ISP 2 broth prepared using the buffer system as described by Xu *et al.* [19]. Salt tolerance was observed by supplementing up to 13 % (w/v) NaCl (at intervals of 1 %, w/v) in ISP 2 medium. The ability for growth at different temperatures was investigated by incubating related strains on ISP 2 medium at 5–55 °C (at intervals of 5 °C, except with one more at 37 °C). Oxidase activity was detected by using the bioMérieux oxidase reagent according to the manufacturer's instructions. Catalase activity was tested by observing the formation of bubbles on addition of a drop of 3.0 % (v/v) H_2O_2. Tests for hydrolysis of casein, cellulose, starch and Tweens 20, 40, 60 and 80 were carried out as described by Smibert and Krieg [20]. The antifungal activity was evaluated by using the classic dual-culture method as described by Puopolo *et al.* [21] and *Fusarium oxysporum* was used for testing pathogenic fungi. Nematicidal ability was tested by incubating strains in IPS 2 broth medium at 30 °C for 3 days, and then 2.0 ml fermentation medium was added to 35-mm-diameter plates containing *Meloidogyne incognita*. These plates were incubated at 30 °C for 12 h and 24 h respectively. Nematicidal ability was evaluated based on the ratio of live to dead nematodes. The Biolog Gen III microPlate system was used for testing carbon source utilization according to the manufacturer's instructions. Physiological and biochemical properties were investigated by using API ZYM and API 20E test strips (bioMérieux) according to the manufacturer's instructions. For the above test kits, strain C8-1^T and related reference strains were incubated on ISP 2 medium at 30 °C for 3 days prior to the experiments.

Cells of strain C8-1^T were Gram-stain-negative, aerobic, non-motile and rod-shaped (0.3–0.4 µm in width and 1.4–1.8 µm in length) (Fig. 1). Colonies were yellow, smooth, circular and convex, measuring 2.0–5.0 mm in diameter. Growth of strain C8-1^T was observed at 10–40 °C and pH 6.0–9.0, optimally at 30 °C and pH 7.0. Strain C8-1^T was able to tolerate up to 1 % (w/v) NaCl when grown on ISP 2 medium. Catalase and oxidase reactions were both positive. Hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 was positive, while hydrolysis of cellulose was negative. Strain C8-1^T was not able to inhibit growth of *Fusarium oxysporum* or to kill *Meloidogyne incognita*. Differential physiological and biochemical characteristics of the new isolate compared with the type species and related type strains of the genus *Lysobacter* are shown in Table 1, while detailed characteristics of strain C8-1^T are listed in the species description below.

The respiratory quinones were extracted and purified by TLC as described by Collins *et al.* [22] and analysed by reversed-phase HPLC [23]. Polar lipids were extracted by two-dimensional TLC and identified following the method of Minnikin *et al.* [24]. For analysis of fatty acids, strain C8-1^T and the reference strains were cultured on TSA medium at 30 °C for 3 days. Cellular fatty acid analysis was performed by using the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6) as described by Sasser [25].

The predominant respiratory quinone was Q-8. The polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, five unidentified phospholipids and two unidentified aminophospholipids (Fig. S1, available in the online version of this article). The major cellular fatty acids (>10.0 %) were iso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{17:1}\omega 9c$. The fatty acid profiles of strain C8-1^T and of other related reference strains are given in Table S1.

Genomic DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out using the procedures described by Li *et al.* [26]. Multiple alignments with 16S rRNA gene sequences of members of the genus *Lysobacter* and calculations of sequence similarity were carried out using the EzBioCloud server [27]. Phylogenetic analysis was performed using three tree-making algorithms, the neighbour-joining [28], maximum-likelihood [29] and maximum-parsimony [30] methods, by using the software MEGA 7 [31] to determine the taxonomic position of strain C8-1^T. Evolutionary distances were calculated using Kimura's two-parameter method [32]. The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein [33] with 1000 replicates. DNA–DNA hybridization tests were carried out by the fluorometric micro-well method [34, 35]. The hybridization temperature for the experiment was set at 50 °C. Whole genome sequencing of strain C8-1^T was performed using a paired-end sequencing method with a Hiseq 2000 platform (Illumina). Reads of each data set were filtered, and high-quality reads were assembled using SOAPdenovo2 [36]. Contigs with length greater than 500 bp were kept for gene prediction by applying GLIMMER 3.0 [37]. The predicted coding sequences of this genome were translated and used to search the KEGG, COG and Pfam databases.

The almost-complete 16S rRNA gene sequence (1506 bp) was determined for strain C8-1^T. Analysis indicated that strain C8-1^T shared 16S rRNA gene sequence similarities of 98.6, 97.6, 97.3, 97.3, 97.3 and 97.1 % with *L. cavernae* YIM C01544^T, *L. soli* DCY21^T, *L. panacisoli* CJ29^T, *Lysobacter*

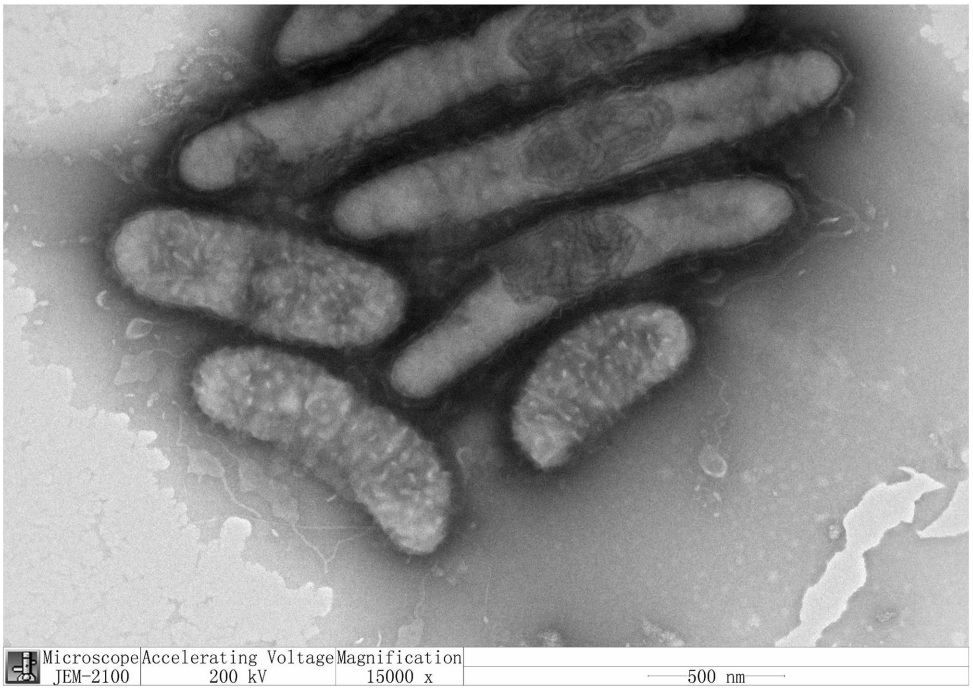

Fig. 1. Transmission electron micrograph of cells of strain C8-1^T grown on ISP 2 medium at 30 °C for 3 days. Bar, 500 nm.

firmicutimachus PB-6250^T, *L. niastensis* GH41-7^T and *Lysobacter gummosus* KCTC 12132^T, respectively. Levels of similarity were less than 97.0 % with other members of the genus *Lysobacter*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain C8-1^T belonged to the genus *Lysobacter*. The neighbor-joining phylogenetic tree showed that strain C8-1^T clustered with *L. cavernae* YIM C01544^T and was close to *L. soli* DCY21^T, *L. niastensis* GH41-7^T and *L. panacisoli* CJ29^T with high bootstrap values (Fig. 2). Similar relationships were also recovered in phylogenetic trees reconstructed with the maximum-parsimony and maximum-likelihood algorithms (Figs S2 and S3). Strain C8-1^T displayed DNA–DNA relatedness values of 62.2±1.2 % with *L. cavernae* YIM C01544^T, 53.5±1.1 % with *L. soli* KCTC 22011^T, 60.4±1.3 % with *L. niastensis* DSM 18481^T and 56.2±0.6 % with *L. panacisoli* JCM 19212^T. All values were lower than 70 %, which is the threshold value for prokaryotic species delineation [38]. The genome size of strain C8-1^T was approximately 3 120 411 bp, depth of coverage was 100×, number of scaffolds was 9, mean length of scaffolds was 3 46 712 bp and N_{50} length was 1 736 244 bp. The number of protein-coding genes was 2900, the number of genes assigned to COGs was 2319 and the number of genes assigned to KEGG was 1936. The genomic DNA G+C content of strain C8-1^T was 70.7 mol%.

In addition to its close phylogenetic relationship with *L. cavernae* YIM C0 1544^T, strain C8-1^T had some typical characteristics of the genus *Lysobacter*: Gram-stain-negative, non-motile and rod-shaped cells, Q-8 as the respiratory quinone, major fatty acids of iso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{17:1}\omega 9c$, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as polar lipids, and G+C content of 70.7 mol%. Furthermore, phylogenetic analysis based on 16S rRNA gene sequences indicated that strain C8-1^T was affiliated to the clade encompassing the genus *Lysobacter* (Figs S2 and S3). However, strain C8-1^T had some distinctive characteristics from its phylogenetically related neighbours and the type species of the genus *Lysobacter*, *L. enzymogenes* LMG 8672^T. Strain C8-1^T was able to hydrolyse starch, while other strains except *L. firmicutimachus* PB-6250^T were not. The isolate showed growth at 40 °C, whereas *L. cavernae* YIM C0 1544^T did not. *L. soli* KCTC 22011^T could tolerate up to 3 % (w/v) NaCl, while strain C8-1^T could not. Strain C8-1^T was positive for hydrolysis of Tweens 20, 40, 60 and 80, whereas *L. soli* KCTC 22011^T, *L. panacisoli* JCM 19212^T and *L. niastensis* DSM 18481^T were negative. In API ZYM tests, the isolate showed positive reactions for β-galactosidase and Voges–Proskauer reaction, while *L. gummosus* LMG 8763^T and *L. enzymogenes* LMG 8762^T showed negative reactions. These different characteristics of strain C8-1^T from closely related strains and the type species are shown in Table 1. In addition, DNA–DNA hybridization values between strain C8-1^T and *L. cavernae* YIM C01544^T, *L. soli* KCTC 22011^T, *L. niastensis* DSM 18481^T and *L. panacisoli* JCM 19212^T were lower than the threshold value (70 %) for prokaryotic species delineation, indicating that strain C8-1^T represents a novel genomic species. In

Table 1. Differential phenotypic and genotypic chatacteristics between strain C8-1^T and closely related species of the genus *Lysobacter*

Strains: 1, C8-1^T; 2, *L. cavernae* YIM C01544^T; 3, *L. soli* KCTC 22011^T; 4, *L. panacisoli* JCM 19212^T; 5, *L. niastensis* DSM 18481^T; 6, *L. firmicutimachus* PB-6250^T [8]; 7, *L. gummosus* LMG 8763^T [1]; 8, *L. enzymogenes* LMG 8762^T [1, 8]. All data for strains 1–5 are from this study except the genomic DNA G+C contents. +, Positive; –, negative; W, weakly positive; ND, no data. Strains 1–5 are positive for tryptophan deaminase, gelatin hydrolysis, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, hydrolysis of casein and oxidase, but negative for L-arginine dihydrolase, ornithine decarboxylase, citric acid utilization, H_2S production, urease, indole reaction, α-galactosidase, β-glucuronidase, α-mannosidase, β-fucosidase and hydrolysis of cellulose. All physiological and biochemical data are from API 20E and API ZYM tests. Strains 1–5 can utilize acetic acid, acetoacetic acid, L-aspartic acid, dextrin, D-fructose, D-galactose, gelatin, *N*-acetyl-D-glucosamine, α-D-glucose, D-glucose 6-phosphate, glucuronamide, L-glutamic acid, maltose, D-mannose and glycyl L-proline, but not D-arabitol, L-arginine, γ-aminobutyric acid, α-hydroxybutyric acid, formic acid, *myo*-inositol, *N*-acetyl-D-galactosamine, gentiobiose, D-gluconic acid, glycerol, L-lactic acid, lactose, D-malic acid, L-malic acid, D-mannitol, *N*-acetyl neuraminic acid, *p*-hydroxyphenylacetic acid, quinic acid, D-saccharic acid, D-sorbitol or stachyose, according to results for the Biolog Gen III microplate.

Characteristic	1	2	3	4	5	6 [8]	7 [1]	8 [1,8]
Temperature range for growth (°C)	10–40	4–30	10–40	4–37	10–37	15–37	10–40	5–40
NaCl tolerance range (%, w/v)	0–1	0–1	0–3	0–3	0–1	0–0.5	0–2	0–3
Hydrolysis of:								
Tween 20	+	+	–	–	–	ND	+	+
Tween 40	+	–	–	–	–	ND	+	+
Tween 60	+	–	–	–	–	ND	+	+
Tween 80	+	–	–	–	–	ND	+	+
Starch	+	–	–	–	–	ND	–	–
Enzyme activities								
Catalase	+	–	+	+	–	+	ND	ND
β-Galactosidase	+	+	–	+	+	–	–	–
Lysine decarboxylase	–	+	–	+	–	ND	ND	ND
Voges–Proskauer reaction	+	+	+	–	–	–	–	–
Nitrate reduction	–	+	+	+	W	–	–	–
Alkaline phosphatase	–	+	+	+	+	+	ND	ND
Valine arylamidase	–	+	+	+	–	–	ND	ND
Cystine arylamidase	–	W	+	–	–	–	ND	ND
Chymotrypsin	–	+	W	–	+	–	–	+
β-Galactosidase	–	+	–	+	–	–	–	–
α-Glucosidase	+	–	+	–	+	–	–	+
Carbon source utilization								
Sucrose	+	–	–	–	–	ND	+	+
Turanose	+	–	–	–	–	ND	ND	ND
L-Aspartic acid	–	+	+	+	+	ND	ND	ND
L-Glutamic	–	+	+	+	+	ND	ND	ND
Bromosuccinic acid	+	–	–	–	–	ND	ND	ND
DNA G+C content (mol%)	70.7	64.9 [6]	65.4 [3]	65.6 [7]	66.6 [4]	68.7	65.7	69.0

conclusion, strain C8-1^T had distinctive characteristics from other closely related species and therefore represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter tabacisoli* sp. nov. is proposed.

DESCRIPTION OF *LYSOBACTER TABACISOLI* SP. NOV.

Lysobacter tabacisoli (ta.ba.ci.so'li. N.L. neut. n. *tabacum* tobacco; L. neut. n. *solum* soil; N.L. gen. n. *tabacisoli* of tobacco field soil).

Cells are Gram-stain-negative, aerobic, non-motile and rod-shaped. Cells are 0.3–0.4 µm in width and 1.4–1.8 µm in length. Colonies are yellow, smooth, convex and circular with diameters of 2–5 mm after 3 days on ISP 2 medium at 30 °C. Growth occurs at 10–40 °C and pH 6.0–9.0, optimally at 30 °C and pH 7.0. Cells are able to tolerate up to 1 % (w/v) NaCl on ISP 2 medium. Catalase-positive and oxidase-positive. Positive for hydrolysis of casein, starch and Tweens 20, 40, 60 and 80, but negative for hydrolysis of cellulose. In API 20E test strips, β-galactosidase, tryptophan deaminase, Voges–Proskauer reaction and gelatin hydrolysis are positive, while L-arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, indole reaction and urease are negative. In API ZYM test strips, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-

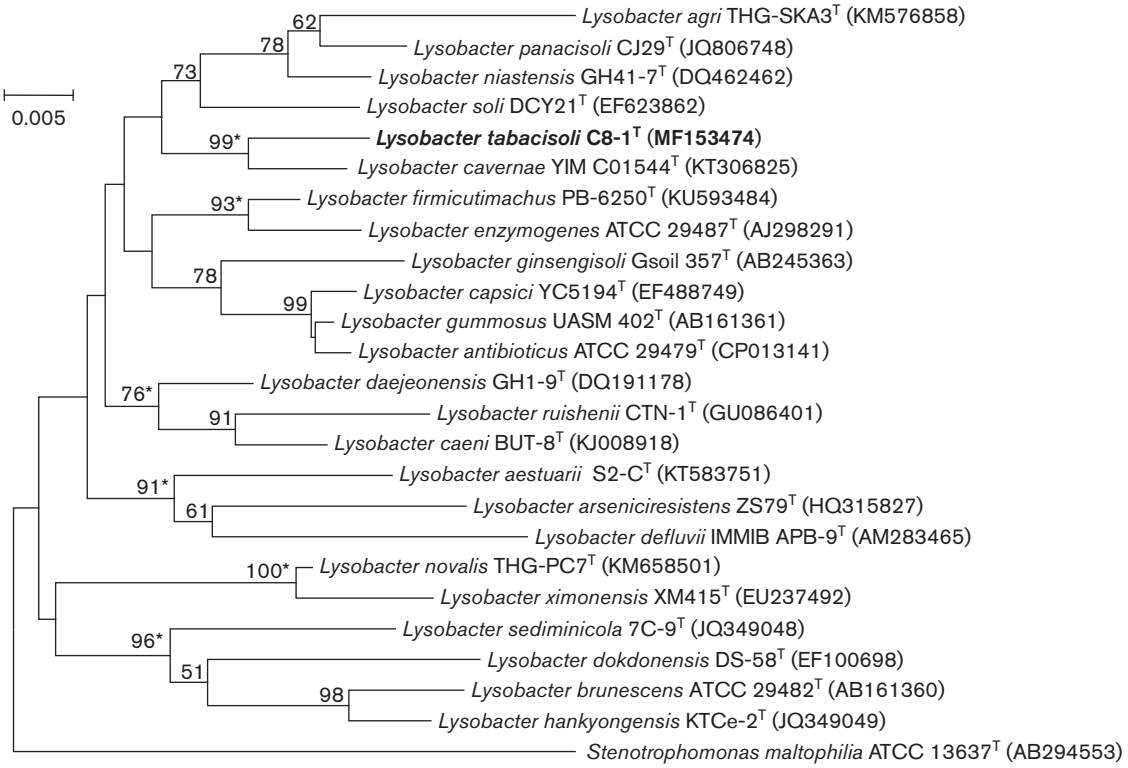

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain C8-1^T. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at branch nodes. Asterisks indicate the same clades were retrieved in phylogenetic trees reconstructed with the maximum-likelihood and maximum-parsimony algorithms. *Stenotrophomonas maltophilia* ATCC 13637^T was used as an outgroup. Bar, 0.005 sequence divergence indicating 0.005 nt changes per position.

phosphohydrolase, α-glucosidase and β-glucosidase are positive, whereas alkaline phosphatase, valine arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase are negative. In the Biolog Gen III microPlate system, the following carbon sources are utilized: acetic acid, acetoacetic acid, L-aspartic acid, dextrin, D-fructose, D-galactose, gelatin, *N*-acetyl-D-glucosamine, α-D-glucose, D-glucose 6-phosphate, glucuronamide, L-glutamic acid, maltose, D-mannose, glycyl L-proline, bromosuccinic acid, sucrose and turanose; D-arabitol, L-arginine, γ-aminobutryric acid, α-hydroxybutyric acid, formic acid, *myo*-inositol, *N*-acetyl-D-galactosamine, gentiobiose, D-gluconic acid, L-glutamic acid, glycerol, L-lactic acid, lactose, D-malic acid, L-malic acid, D-mannitol, *N*-acetyl neuraminic acid, *p*-hydroxyphenylacetic acid, quinic acid, D-saccharic acid, D-sorbitol and stachyose are not utilized. The predominant respiratory quinone is Q-8. The cellular polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, five unidentified phospholipids and two unidentified aminophospholipids. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{17:1}\omega 9c$.

The type strain, C8-1^T (=KCTC 62034^T=CGMCC 1.16271^T), was isolated from a rhizosphere soil sample of *Nicotiana tabacum* L. in Kunming, south-west China. The genomic DNA G+C content of the type strain is 70.7 mol %. The 16S rRNA gene and genome sequences of strain C8-1^T were submitted to GenBank with accession numbers MF153474 and QXJI00000000, respectively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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