Lysobacter chinensis sp. nov., a cellulose-degrading strain isolated from cow dung compost

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Received: 2 February 2022 / Accepted: 21 May 2022 / Published online: 14 June 2022
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Abstract A novel bacterial strain, TLK-CK17T, was isolated from cow dung compost sample. The strain was Gram-staining negative, non-gliding rods, aerobic, and displayed growth at 15–40 °C (optimally, 35 °C), with 0–5.0% (w/v) NaCl (optimally, 0.5) and at pH 6.5–8.5 (optimally, 7.0–7.5). The assembled genome of strain TLK-CK17T has a total length of 4.3 Mb with a G+C content of 68.2%. According to the genome analysis, strain TLK-CK17T encodes quite a few glycoside hydrolases that may play a role in the degradation of accumulated plant biomass in compost. On the basis 16S rRNA gene sequence analysis, strain TLK-CK17T showed the highest sequence similarity (98.9%) with L. penaei GDMCC 1.1817T, followed by L. maris KCTC 42381T (98.3%). Cells contained iso-C16:0, iso-C15:0 3-OH and summed feature 9 (comprising C17:1 ω9c and/or 10-methyl C16:0), as its major cellular fatty acids (> 10.0%) and ubiquinone-8 as the exclusively respiratory quinone. Diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol prevailed among phospholipids. Based on the phenotypic, genomic and phylogenetic data, strain TLK-CK17T represents a novel species of the genus Lysobacter, for which the name Lysobacter chinensis sp. nov. is proposed, and the type strain is TLK-CK17T (= CCTCC AB2021257T = KCTC 92122T).

Keywords Lysobacter chinensis · Draft genome sequencing · Cellulose-degrading · Compost

Introduction

The genus Lysobacter was proposed by Christensen and Cook (1978), and it belongs to the family Lysobacteraceae, phylum ‘Pseudomonadota’. As of December 2021, the genus includes 66 validly published species with correct name; the type species is Lysobacter enzymogenes (Christensen and Cook 1978). The main characteristics of the genus are Gram-staining negative, rod-shaped with pink to yellow colonies, and contain high DNA G+C content (61.7–70.1%) (Christensen 2005; Li et al. 2018). Strains of Lysobacter, thought to play vital roles in the environment for their high enzyme production capacity, are ubiquitous in various ecosystems. At present, they have been isolated from various habitats, including different types of soil such as cave soil
Here, we report a polyphasic taxonomical description of a novel, cellulose-degrading bacterium strain, designated TLK-CK17T. The phenotypic, chemotaxonomic and genotypic properties indicate that strain TLK-CK17T represents a novel species within the genus Lysobacter, for which the name Lysobacter chinensis sp. nov. is proposed.

Materials and methods

Isolation and cultivation

In our investigation of the diversity of cultured bacteria in cow dung compost, Xinjiang Uygur Autonomous Region, China (43°81′N, 87°57′E), strain TLK-CK17T was isolated. The compost sample was suspended in sterile water and serially diluted to 10⁻¹, 10⁻² and 10⁻³, then 100 µL from each dilution was spread onto 1/3 nutrient agar (NA) plates. The plates were incubated at 30 °C and checked for growth after 2–3 days. After incubation, a single colony was purified by sub-culturing under the same conditions. For further investigation, the isolate was sub-cultured on NA plates at 35 °C and preserved at −80 °C as glycerol suspension (20.0%, w/v). Meanwhile, closely related strains L. penaei GDMCC 1.1817T, and L. maris KCTC 42381T were obtained from the Guangdong Microbial Culture Collection Center (GDMCC) and the Korean Collection for Type Culture (KCTC), respectively.

DNA extraction and genome sequencing

Genomic DNA of strain TLK-CK17T was extracted and purified using a bacterial genomic DNA kit (Takara), following the manufacturer’s recommendations. The taxonomic position of strain TLK-CK17T was first determined by 16S rRNA gene sequence using the forward primer 27F and the reverse primer 1492R as described previously (Liu et al. 2014). Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. A-tailed, ligated to paired-end adaptors and PCR amplified with a 350 bp insert was used for the library reconstruction. The raw reads were assembled using SOAPdenovo version 2.04 software (Li et al. 2008, 2010). The genes of strain TLK-CK17T were identified by NCBI Prokaryotic Genome Annotation Pipeline server online and the Pfam database (Angiuoli et al. 2008; http://pfam.xfam.org/), and the genes involved in metabolic pathways were analyzed in detail using the information present in RAST (Rapid Annotation using Subsystem Technology; https://rast.nmpdr.org). The G+C content of the chromosomal DNA was calculated using genome sequencing. The digital DNA–DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC 2.0) (Meier-Kolthoff et al. 2013). The average nucleotide identity (ANI) values were calculated using the algorithm of Goris et al. (2007) by using the EzGenome web service (https://www.ezbiocloud.net/).

Processing of sequence data and phylogenetic analysis

The complete 16S rRNA sequence of strain TLK-CK17T was uploaded to the EzGenome web service and the NCBI GenBank to indentify the strain based on the sequences available. Multiple alignments with corresponding sequences of the closely related strains were aligned using CLUSTAL_X (Thompson et al. 1997). Phylogenetic analysis was conducted by neighbour-joining (NJ) (Saitou et al. 1987), maximum-parsimony (MP) (Fitch et al. 1971) and maximum-likelihood (ML) (Felsenstein et al. 1981) methods in MEGA 7.0 program (Kumar et al. 2016) using the Kimura two-parameter model (Kimura et al. 1980), and the gaps were treated using a partial deletion method. Bootstrap analysis with 1000 replications was conducted, aimed at estimating the topology of the phylogenetic tree (Felsenstein et al. 1985). Moreover, the phylogenetic relationship based on nucleotide sequences was analysed via UBCG (Na et al. 2018), and the phylogenetic trees were constructed by using FastTree (Price et al. 2010) with GTR+CAT parameters and IQTree (Trifinopoulos et al. 2016) with GTR+F+I+G4 model and 1000 bootstrap replicates on the basis of 25 genomes.

Phenotypic and biochemical characteristics

Gram-staining and morphological features were tested with cells grown on NA plates at 35 °C for
24 h. Gram-staining was performed using a Gram stain kit (bioMérieux) according to the manufacturer’s instructions. Cell morphology and size were examined with light microscopy (E600; Nikon) and transmission electron microscopy (JEM-1200; JEOL). Motility was determined using the hanging-drop method and gliding motility was determined as described by Bowman 2000. Reduction of nitrate was performed as described by Cowan and Steel 1974. Catalase, oxidase and lipase (Tweens 20 and 80) activities and hydrolysis of alginate, starch and CM-cellulose were tested as described previously (Dong and Cai et al. 2001). Anaerobic growth was tested after incubation for 2 weeks at 35 °C on NA with or without 0.1% (w/v) KNO₃, in an anaerobic chamber filled with a gas mixture (10% H₂, 10% CO₂ and 80% N₂). Growth at different temperatures (0, 4, 10, 15, 20, 28, 30, 33, 40, 45 and 50 °C) and at different concentrations of NaCl (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0%, w/v) were investigated on nutrient broth (NB) for up to 10 days. Growth at pH 5.5–9.5 (at intervals of 0.5 pH unit) was determined by measuring the optical density (wavelength 600 nm) of cultures in NB with the pH adjusted prior to sterilization by adding the appropriate buffers, including MES (for pH 5.5 and 6.0), PIPES (for pH 6.5 and 7.0), HEPES (for pH 7.5 and 8.0), Tricine (for pH 8.5) and CAPSO (for pH 9.0 and 9.5). Antibiotic sensitivity was assessed as described by the Clinical and Laboratory Standards Institute (CLSI et al. 2012), and inoculated plates were incubated at 35 °C for up to 48 h. Other physiological and biochemical characteristics of strain TLK-CK17T and closely related strains were determined using the API 20E, API ZYM and API 50CHB identification systems (bioMérieux) and the Biolog GEN III identification system, according to the manufacturers’ instructions.

Chemotaxonomic analysis

Cell biomass of strains TLK-CK17T, L. penaei GDMCC 1.1817T, and L. maris KCTC 42381T were obtained from cultures grown in NB and on a rotary shaker (150 r.p.m) at 35 °C. After 24-h growth cells were centrifuged, washed three times in sterile distilled water and freeze-dried. The cellular fatty acid methyl esters were prepared and identified according to the Microbial Identification System (Sherlock version 4.5; database: TSBA40; MIDI; Sasser et al. 1990). The isoprenoid quinone strain TLK-CK17T and two closely related strains were extracted from freeze-dried cell material using the two-stage method described by Tindall et al. (2007) and subsequently analysed by High performance liquid chromatographic (HPLC; Kroppenstedt et al. 1982). Polar lipids were extracted using a chloroform/methanol system and analysed by using two-dimensional thin-layer chromatography, as described previously (Minnikin et al. 1984). For two dimensional Thin Layer Chromatography (TLC) of polar lipid analysis, TLC on silica gel 60 F254 plates (Merck) were developed with chloroform/methanol/water (65:25:4, by vol.) for the first dimension and chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) for the second dimension. The total lipid profiles were stained with 10% molybdatophosphoric acid and the functional groups were determined using spray reagents specific for each one. Complete details are provided by Fang et al. (2017).

Results and discussion

Phylogenetic analysis

According to the comparisons with the complete 16S rRNA gene sequence (1545 bp) in the EzTaxon database, the highest level of sequence similarity occurred with L. penaei GDMCC 1.1817T (98.9%) and L. maris KCTC 42381T (98.3%). The phylogenetic position of the novel isolate, determined using various tree-making algorithms, confirmed that strain TLK-CK17T was a member of the genus Lysobacter, forming a coherent cluster with the two abovementioned members of this genus in the NJ, ML and MP trees with low bootstrap values, respectively (Fig. 1). Additional phylogenetic analyses based on a more comprehensive data set of validly published name strains genomes was presented in Fig. 2. On the basis of 16S rRNA gene sequence phylogenetic and phylogenomic analysis, two strains L. penaei GDMCC 1.1817T and L. maris KCTC 42381T were chosen as reference strains in this study.

Phenotypic and biochemical characterisation

NA and NB medium was used for general laboratory cultivation, but the novel strain also grows well on
TSA and R2A media. After 24 h growth on NA at 35 °C, colonies were observed to be 1.0–1.5 mm in diameter, circular, smooth and apricot. Strain TLK-CK17T was found to be Gram-staining negative and catalase negative bacterium. Meanwhile, it showed a positive reaction for oxidase (weakly) and nitrate reduction, nitrate can be reduced to nitrite. Cells are aerobic, grow in 0–5.0% (w/v) NaCl, at a pH range from 6.5 to 8.5 and at temperatures between 15 and 40 °C. Optimal growth was observed at 35 °C, 0.5% (w/v) NaCl and pH 7.0–7.5. Cells of strain TLK-CK17T are non-sliding rods, the mean cell size is 0.3–0.5 μm in width and 1.5–2.0 μm in length (Supplementary Fig. 2). The strain was positive for the hydrolysis of Tweens 20, 80, casein and CM-cellulose, but negative for alginate and starch. Antibiotic susceptibility test indicated that the strain was sensitive to chloramphenicol (30 μg), ceftriaxone (30 μg), ofloxacin (5 μg) and ciprofloxacin (5 μg). However, it was resistant to penicillin (10 μg), tetracycline (30 μg), vancomycin (30 μg), ampicillin (10 μg), streptomycin (10 μg), clindamycin (2 μg), amoxicillin (25 μg) and cephalaxin (30 μg).

**Fig. 1** Neighbour-joining phylogenetic tree based on full-length 16S rRNA gene sequence (1545 bp), showing the phylogenetic position of strain TLK-CK17T among members of the genus *Lysobacter*. Numbers on nodes represent bootstrap values (NJ) based on 1000 replications. Only bootstrap values higher than 70.0% are marked on the branches. Filled circles indicate nodes also obtained in both maximum-likelihood and maximum-parsimony trees. Bar, 0.05 substitutions per nucleotide position.
Chemotaxonomic characteristics

Strain TLK-CK17\textsuperscript{T} contained iso-C\textsubscript{16:0} (24.3%), iso-C\textsubscript{15:0} (23.8%), and summed feature 9 (comprising C\textsubscript{17:1} \omega\textsubscript{9c} and/or 10-methyl C\textsubscript{16:0}, 15.4%) as predominant fatty acids (> 10.0%) in common with closely related strains. However, the ratios of the different components are different. The complete fatty acid composition was shown in Table 1. Ubiquinone-8 (Q-8) was the exclusively respiratory quinone. The polar lipid profile of strain TLK-CK17\textsuperscript{T} consists in phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) as dominant elements, and one unknown polar lipid (L), which was similar to two closely related strains (Supplementary Fig. 2). However, strain TLK-CK17\textsuperscript{T} and closely related strains were distinguishable from each other based on differences in other polar lipids. Strain TLK-CK17\textsuperscript{T} differed from \textit{L. penaei} GDMCC 1.1817\textsuperscript{T} by the absence of one unidentified phospholipid (PL). Strain TLK-CK17\textsuperscript{T} differed from \textit{L. maris} KCTC 42381\textsuperscript{T} by the existence or absence of two unknown polar lipids (L1 and L2).

Whole genome sequence analysis

The genome size and G+C content of strain TLK-CK17\textsuperscript{T} are 4,300,099 \text{bp} and 68.2\%, respectively. Despite the close relationship, large difference in the genome size was found, the genome of strain TLK-CK17\textsuperscript{T} was larger than the genomes of \textit{L. penaei} GDMCC 1.1817\textsuperscript{T} (3.2 \text{Mb}), and \textit{L. maris} KCTC 42381\textsuperscript{T} (4.1 \text{Mb}), respectively. By contrast, strain TLK-CK17\textsuperscript{T} and them have a similar DNA G+C
content of 68.0–69.0%. Furthermore, 3705 CDSs (with protein) were contained in the genome, which were further annotated in NR, Swiss-Prot, COGs, KEGGs, GO and Pfam, and their numbers were 3630, 1485, 2762, 3421, 2419 and 2419, respectively (Table S1). The ANI values of strains TLK-CK17\textsuperscript{T} with \textit{L. penaei} GDMCC 1.1817 \textsuperscript{T} and \textit{L. maris} KCTC 42381 \textsuperscript{T} were 79.9\% and 85.6\%, respectively, while the GGDC values were 29.6\% and 23.8\%, respectively. For species delineation, ANI values of 95–96\% and dDDH values of 70\%, respectively, are normally accepted (Wayne et al. 1988; Thompson et al. 2013). These results indicated that strain TLK-CK17\textsuperscript{T} represents a novel species of the genus \textit{Lysobacter}. The related genome data strain TLK-CK17\textsuperscript{T} and two closely related type strains are listed in Tables S1 and S2.

The genome contributes to an important understanding of the genetic evolution of bacteria, disease prevention and treatment, and the development of antibiotics; thus, the genome of strain TLK-CK17\textsuperscript{T} was analysed to decipher the genetic code involved in the environmental suitability. The RAST analysis revealed the presence of 302 subsystems, the subsystem coverage was 25\% (total 977, non-hypothetical 936, hypothetical 41), and 67 glycoside hydrolase (GHs), 42 glycosyltransferases (GTs), 44 carbohydrate-binding modules (CBMs), 6 carbohydrate esterases (CEs) and 4 auxiliary activities (AAs) were identified. Because cow dung is rich in lignocellulose, the coexistence of these genes suggests that they play important roles in the breakdown and modification of carbohydrates in cow dung composting. Compared with \textit{L. penaei} GDMCC 1.1817 \textsuperscript{T} and \textit{L. maris} KCTC 42381 \textsuperscript{T}, result showed that GH, GT and CBM family numbers were more in strain TLK-CK17\textsuperscript{T}, while the \textit{L. penaei} GDMCC 1.1817 \textsuperscript{T} contained more CE and AA family members. We considered that it might be closely related to their isolated environment, strains \textit{L. penaei} GDMCC 1.1817 \textsuperscript{T} and \textit{L. maris} KCTC 42381 \textsuperscript{T} were isolated from the pacific white shrimp and seawater, respectively. \textit{Lysobacter} spp. has been identified as heterotrophic with a wide range of extracellular enzymes and other metabolites against other microorganisms, including fungi and nematodes, so it played an important role in the suppression of pathogenic bacteria (de Bruijn et al. 2015; Xie et al. 2012; Pidot et al. 2014). Our results showed that the strain TLK-CK17\textsuperscript{T} possessed chitinase, protease and glucanase activity, confirming and extending previous research (Zhang et al. 2001; Palumbo et al. 2005). Chitinase, glucanase and protease activities may contribute to antimicrobial activity, since chitin, \(\alpha\) and \(\beta\)-glucans and glycoproteins are the major components of the cell walls of fungi (Figueiredo et al. 2014). Moreover, we analysed that \textit{Lysobacter} strains showed a high genetic diversity, which could confer an advantage under adverse environmental conditions (Foster et al. 2005). To better understand the potential effect

### Table 1: Cellular fatty acid compositions of strain TLK-CK17\textsuperscript{T} and phylogenetically related species of the genus \textit{Lysobacter}

| Fatty acid          | Strain TLK-CK17\textsuperscript{T} | \textit{L. penaei} GDMCC 1.1817 \textsuperscript{T} | \textit{L. maris} KCTC 42381 \textsuperscript{T} |
|---------------------|------------------------------------|-------------------------------------------------|-------------------------------------------------|
| C\textsubscript{16}:0 | 2.3                                | 4.2                                             | 2.1                                             |
| C\textsubscript{16}:0 cyclo | 1.6                                | TR                                             | TR                                              |
| C\textsubscript{17}:0 cyclo | TR                                 | TR                                             | 1.5                                             |
| iso-C\textsubscript{11}:0 | 6.5                                | 5.8                                             | 6.4                                             |
| iso-C\textsubscript{12}:0 | 0.6                                | TR                                             | TR                                              |
| iso-C\textsubscript{14}:0 | 1.1                                | 1.8                                             | 1.1                                             |
| iso-C\textsubscript{15}:0 | 23.8                               | 20.6                                           | 20.3                                            |
| iso-C\textsubscript{16}:0 | 24.3                               | 19.3                                           | 28.4                                            |
| iso-C\textsubscript{17}:0 | 3.1                                | 7.8                                             | 5.1                                             |
| anteiso-C\textsubscript{15}:0 | TR                                 | TR                                             | 0.8                                             |
| anteiso-C\textsubscript{17}:0 | TR                                 | TR                                             | 0.7                                             |
| iso-C\textsubscript{11}:0 3-OH | 7.8                                | 6.6                                             | 7.1                                             |
| iso-C\textsubscript{15}:0 3-OH | 0.5                                | TR                                             | TR                                              |
| iso-C\textsubscript{16}:1 H | 1.8                                | 0.5                                             | 0.7                                             |

*Summed feature

| Feature | 1 | 2 | 3 |
|---------|---|---|---|
| 1       | 0.5 | TR | TR |
| 3       | 5.0 | 8.0 | 4.3 |
| 8       | 0.7 | 1.5 | 1.0 |
| 9       | 15.4 | 20.2 | 6.0 |

* Strains: 1, TLK-CK17\textsuperscript{T}; 2, \textit{L. penaei} GDMCC 1.1817 \textsuperscript{T}; 3, \textit{L. maris} KCTC 42381 \textsuperscript{T}. Data were obtained in the present study unless indicated. Values are percentages of the total fatty acids, and only fatty acids comprising > 0.5\% are shown.

The fatty acids in bold are the major cellular fatty acid (> 10.0\%). Results are scored as follows: TR, Trace (<0.5\%); –, not detected.

* Summed features are groups of two or three fatty acids that could not be separated by gas/liquid chromatography with the Microbial Identification System (MIDI). Summed feature 1 contains iso-C\textsubscript{15}:1 H and/or C\textsubscript{13}:0 3-OH; summed feature 3 contains C\textsubscript{16}:1 \(\omega\)9c and/or C\textsubscript{16}:1 \(\omega\)7c; summed feature 8 contains C\textsubscript{18}:1 \(\omega\)7c and/or C\textsubscript{18}:1 \(\omega\)6c; summed feature 9 contains iso-C\textsubscript{17}:1 \(\omega\)9c and/or 10-methyl C\textsubscript{16}:0.
of strain TLK-CK17T to the overall activities of the microbial communities in cow dung compost, our future work will include testing it with other bacterial genera abundant in compost. Interactions of strain TLK-CK17T with other bacteria whether or not stimulate the production of antimicrobial compounds, so as to quickly remove pathogenic microorganisms in livestock manure.

**Conclusion**

The results of the phylogenetic analysis, phenotypic analysis and chemotaxonomic studies presented above support the view that strain TLK-CK17T should be assigned to the genus *Lysobacter*. Based on the phenotypic analysis a comparison was made between the characteristics of strain TLK-CK17T and closely related strains and a number of differences were observed, as shown in Table 2. Overall, considering its 16S rRNA gene sequence similarity (<98.9%) to members of closely related taxa, its unique branching position in phylogenetic trees and the differences that the isolate exhibited described above, the isolate cannot be assigned to any recognized species. Therefore, strain TLK-CK17T represents a novel species of the genus *Lysobacter*, for which the names *Lysobacter chinensis* sp. nov., is proposed. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and whole-genome shotgun project of strain TLK-CK17T is OK143236.

### Table 2 Physiological and chemotaxonomic properties of strain TLK-CK17T compared with the closely related species of the genus *Lysobacter*

| Characteristics                        | 1               | 2               | 3               |
|----------------------------------------|------------------|------------------|------------------|
| isolated                              | Cow dung compost | Pacific white shrimp | Rhizosphere of pepper |
| Pigmentation                          | Apricot          | Light yellow     | Apricot          |
| NaCl range for growth (%)             | 0–5.0            | 0–6.0            | 0–7.0            |
| Tween 20                              | +                | +                | –                |
| Tween 80                              | +                | –                | +                |
| Oxidase                               | w                | +                | –                |
| Catalase                              | –                | +                | +                |
| API ZYM assay:                        |                  |                  |                  |
| Lipase (C14)                          | –                | –                | w                |
| Valine arylamidase                    | +                | w                | –                |
| Trypsin                               | –                | –                | w                |
| α-Galactosidase                       | +                | –                | –                |
| α-Glucosidase                         | +                | –                | +                |
| N-Acetyl-β-glucosaminidase            | –                | –                | +                |
| API 20NE assay:                       |                  |                  |                  |
| Reduction of nitrate to nitrite nitrite| +                | +                | –                |
| β-Galactosidase                       | +                | w                | w                |
| α-Glucose                            | +                | w                | w                |
| L-Arabinose                           | +                | –                | –                |
| D-Mannose                             | w                | –                | –                |
| N-Acetyl-glucosamine                  | –                | +                | +                |
| Maltose                               | w                | +                | +                |
| DNA G+C content (%)                   | 68.2             | 68.8             | 69.0             |
| Genome size (Mb)                      | 4.3              | 3.2              | 4.1              |
| Genomic genes                         | 3630             | 2872             | 3578             |

Strains: 1, TLK-CK17T; 2, *L. penaei* GDMCC 1.1817T; 3, *L. maris* KCTC 42381T. Data were obtained in the present study unless indicated. +, Positive; w, weakly positive; –, negative
Description of Lysobacter chinensis sp. nov.

_Lysobacter chinensis_ (chin.en’sisis. N.L. masc. adj. chinensis pertaining to China, where the type strain was isolated).

Cells are Gram-staining negative, aerobic, non-gliding rods, 0.3–0.5 μm in width and 1.5–2.0 μm in length. Good growth is observed on R2A agar, TSA and NA, but not on MA. Colonies on NA are beige to apricot, smooth, opaque, and circular (approximately 1.0–1.5 mm in diameter) with entire edges and convex. Growth occurs on NA at temperatures of 15–40 °C (optimum 35 °C). The pH range for growth is from pH 6.5 to 8.5 (optimum pH 7.0–7.5). Growth occurs at 0–5.0% NaCl concentrations (optimum 0.5). Oxidase-positive (weakly) and catalase-negative. Nitrate can be reduced to nitrite. CM-cellulose, casein, Tweens 20 and 80 are hydrolysed, but alginate and starch are not. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, acid phosphatase, and β-glucosidase, but negative for esterase lipase (C8), lipase (C14), cystine arylamidase, α-chymotrypsin, acid phosphatase, β-glucuronidase, N-acetyl-glucosaminidase and α-mannosidase. Positive for ONPG test, indole production and gelatinase, but negative for H₂S production, Voges-Proskauer reaction, Simmons’ citrate utilization, Acid is produced from L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, amygdalin, esculin, D-cellobiose, D-maltose (weakly), D-lactose, D-melibiose, D-sucrose, D-raffinose, glycogen, D-gentiobiose. Positive for oxidation of D-trehalose, D-cellobiose (weakly), gentiobiose, N-acetyl-β-D-mannosamine (weakly), d-mannose, D-galactose. The major cellular fatty acids are iso-C₁₆:₀, iso-C₁₅:₀, and feature 9 (comprising C₁₇:₁ ω₉c and / or 10-methyl C₁₆:₀). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The exclusively respiratory quinone is Q-8.

The type strain TLK-CK17ᵀ (= CCTCC AB2021257ᵀ = KCTC 92122ᵀ) was isolated from a cow dung compost sample collected from the Xinjiang Uygur Autonomous Region, China (43°81’N, 87°57’E). The genomic DNA G+C content of the type strain was 68.2%.

Author contributions All authors contributed to the study conception and design. YYL wrote the manuscript and analysed the cultivation data. LYZ performed the genomic and phylogenetic analysis. YXP and PBL isolated the strain and performed the initial cultivation and strain deposition. YXX, JPD and ZQS contributed to text preparation and revised the manuscript. LF performed the electron microscopic analysis and prepared the SEM pictures. XWW and ZFW took the samples. All authors read and approved the final manuscript.

Funding This work was supported by the Xinjiang Academy of Agricultural Sciences Young Science and Technology Backbone Innovation Ability Training Project (xjnkq-2022019), Regional Collaborative Innovation Special Project of Xinjiang Uygur Autonomous Region (Nos. 2021E02022) and Forestry Development Subsidy Fund Project of Xinjiang Uygur Autonomous Region (Nos. XJLYKJ-2021–15).

Data availability The genome and 16S rRNA gene sequence are available from GenBank under the accession numbers provided in the manuscript.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethics approval This article does not contain any studies with animals performed by any of the authors.

References

Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D et al (2008) Toward an online repository of standard operating procedures (SOPs) for (meta) genomic annotation. OMICS 12:137–141. https://doi.org/10.1089/omi.2008.0017

Bowman JP (2000) Description of _Cellulophaga algicola_ sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of _Cytophaga uliginosa_ (ZoBell and Upham 1944) Reichenbach 1989 as _Cellulophaga uliginosa_ comb. nov. Int J Syst Evol Microbiol 50:1861–1868. https://doi.org/10.1099/00207713-50-5-1861

Chen W, Zhao YL, Cheng J, Zhou XK, Salam N et al (2016) _Lysobacter cavernae_ sp. nov., a novel bacterium isolated from a cave sample. Antonie Van Leeuwenhoek 109:1047–1053. https://doi.org/10.1007/s10482-016-0704-7

Christensen P, Cook FD (1978) _Lysobacter_, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int J Syst Bacteriol 28:367–393

Christensen P (2005) Genus IV _Lysobacter_ Christensen and Cook 1978 372AL. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey’s manual of systematic bacteriology, 2nd edn. Springer, New York, pp 95–101

CLSI (2012) Performance standards for antimicrobial susceptibility Testing, Twenty-second informational supplement.
CLSI document M100–S22. Clinical and Laboratory Standards Institute.

Cowan ST, Steel KJ (1974) Bacterial characters and characterization, 2nd edn. Cambridge University Press, Cambridge
de Bruijn I, Cheng X, de Jager V, Expósito RG (2015) Comparative genomics and metabolic profiling of the genus Lysobacter. BMC Genomics 16:991. https://doi.org/10.1186/s12864-015-2191-z

Dong XZ, Cai MY (2001) Determination of biochemical characteristics. In: Dong XZ, Cai MY (eds) Manual for the systematic identification of general bacteria. Science Press, Beijing, pp 370–398

Fang DB, Han JR, Liu Y, Du ZJ (2017) Seonanhaeicola marina sp. nov., isolated from marine algae. Int J Syst Evol Microbiol 67:4857–4861. https://doi.org/10.1099/ijs.0.002396

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17(6):368–376. https://doi.org/10.1007/BF01734359

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolut Int J Org Evolut 39(4):783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fitch WM (1971) Towards defining the course of evolution: minimal change for a specified tree topology. Syst Zool 20:406–416. https://doi.org/10.2307/2412116

Foster PL (2005) Stress responses and genetic variation in bacteria. Mutat Res 569(1–2):3–11. https://doi.org/10.1016/j.mrfmm.2004.07.017

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.001722

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Tamura K (2016) MEGA 7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:726–741. https://doi.org/10.1093/molbev/msw410

Lee D, Jang JH, Cha S, Seo T (2017) Lysobacter humi sp. nov., isolated from soil. Int J Syst Evol Microbiol 67:951–955. https://doi.org/10.1099/ijs.0.01722

Li R, Li Y, Kristiansen K, Wang J (2008) SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714. https://doi.org/10.1093/bioinformatics/btn025

Li R, Zhu H, Ruan J, Qian W, Fang X (2010) De novo assembly of human genomes with massively parallel short read sequencing. Genome Res 20:265–272. https://doi.org/10.1101/gr.097261.109

Li J, Han Y, Guo W, Wang Q, Wang G (2018) Lysobacter tongrenensis sp. nov., isolated from soil of a manganese factory. Arch Microbiol 200:439–444. https://doi.org/10.1007/s00203-017-1457-z

Liu M, Liu Y, Wang Y, Luo X, Daj J, Fang C (2011) Lysobacter xinjiangensis sp. nov., a moderately thermotolerant and alkalitolerant bacterium isolated from a gamma-irradiated sand soil sample. Int J Syst Evol Microbiol 61:433–437. https://doi.org/10.1099/ijs.0.016931-0

Liu QQ, Li XL, Rooney AP, Du ZJ, Chen GJ (2014) Tanglefania diversioriginum gen. nov., sp. nov., a representative of the family Dracunobacteriaceae. Int J Syst Evol Microbiol 64:3473–3477. https://doi.org/10.1099/ijs.0.066902-0

Manges R, Zhang DC, Albuquerque L, Froufe HJ, Egas C (2018) Lysobacter silvestris sp. nov., isolated from alpine forest soil, and reclassification of Luteimonas tolerans as Lysobacter tolerans comb. nov. Int J Syst Evol Microbiol 68:1571–1577. https://doi.org/10.1099/ijsem.0.002710

Meier-Kolthoff JP, Auchi AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60. https://doi.org/10.1186/1471-2105-14-60

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M et al (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241. https://doi.org/10.1016/1670-7012(84)90018-6

Na SL, Kim YO, Yoon SH, Ha S, Baek I et al (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280–285. https://doi.org/10.1007/s12275-018-8014-6

Palumbo JD, Yuen GY, Jochem CC, Tatum K, Kobayashi DY (2005) Mutagenesis of beta-1, 3-glucanase genes in Lysobacter enzymogenes strain c3 results in reduced biological control activity toward bipolaris leaf spot of tall fescue and pythium damping-off of sugar beets. Phytopathology 95:701–707. https://doi.org/10.1094/PHYTO-95-0707

Pidot SJ, Coyne S, Kloss F, Hertweck C (2014) Antibiotics from neglected bacterial sources. Int J Med Microbiol 304:14–22. https://doi.org/10.1016/j.ijmm.2013.08.011

Price MN, Dehal PS, Arkin AP (2010) FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS ONE 2010(5):e9490. https://doi.org/10.1371/journal.pone.0009490

Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a0

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI technical note 101. Microbial ID Inc, Newark, DE

Siddiqi MZ, Im WT (2016) Lysobacter pocheonensis sp. nov., isolated from soil of a ginseng field. Arch Microbiol 198:551–557. https://doi.org/10.1007/s00203-016-1214-8

Spudich JL, Yang CS, Jung KH, Spudich EN (2000) Retinylidene proteins: structures and functions from archaea to humans. Annu Rev Cell Dev Biol 16:365–392. https://doi.org/10.1146/annurev.cellbio.16.1.365

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882. https://doi.org/10.1093/nar/25.24.4876

Thompson CC, Chimetto L, Edwards RA, Swings J, Stackebrandt E et al (2013) Microbial genomic taxonomy. BMC Genomics 14:913. https://doi.org/10.1186/1471-2164-14-913

Antoni van Leeuwenhoek (2022) 115:1031–1040

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Tindall BJ, Sikorski J, Smibert RM, Krieg NR (2007) Pheno-
typic characterization and the principles of comparative
systematics. In: Reddy CA, Beveridge TJ, Breznak JA,
Marzluf G, Schmidt TM et al (eds) Methods for general
and molecular microbiology, 3rd edn. ASM Press, Wash-
ington, DC, pp 330–393
Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016)
W-IQ-TREE: a fast online phylogenetic tool for maximum
likelihood analysis. Nucleic Acids Res 44:W232–W235.
https://doi.org/10.1093/nar/gkw256
Xie Y, Wright S, Shen Y, Du L (2012) Bioactive natural
products from Lysobacter. Nat Prod Rep 29:1277–1287.
https://doi.org/10.1039/c2np20064c
Zhang Z, Yuen GY, Sarath G, Penheiter AR (2001) Chitinases
from the plant disease biocontrol agent, Stenotropho-
monas maltophilia C3. Phytopathology 91(2):204–211.
https://doi.org/10.1094/PHYTO.2001.91.2.204
Kroppenstedt RM (1982) Separation of bacterial menaqui-
nones by HPLC using reverse phase (RP18) and a silver
loaded ion exchanger as stationary phases. J Liq Chro-
matography 5:2359–2367. https://doi.org/10.1080/01483
918208067640
Wayne LG (1988) International committee on systematic bac-
teriology: announcement of the report of the ad hoc com-
mittee on reconciliation of Approaches to bacterial sys-
tematics. Syst Appl Microbiol 10(2):99–100. https://doi.
org/10.1016/S0723-2020(88)80020-1

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