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# *Sphingomonas caeni* sp. nov., a phenolic acid-degrading bacterium isolated from activated sludge

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**Abstract** A Gram-stain-negative, rod-shaped, polar flagellated or stalked and non-spore-forming bacterium, designated LB-2<sup>T</sup>, was isolated from activated sludge. Growth was observed at 20–30 °C (optimum 28 °C), pH 6.0–8.0 (optimum pH 7.0) and salinity of 0–0.5% (w/v; optimum 0.5%). Phylogenetic analysis based on the 16S rRNA gene indicated that strain LB-2<sup>T</sup> belongs to the genus *Sphingomonas* and showed the highest sequence similarity (96.7%) and less than 96.7% similarities to other type strains. The genome size of strain LB-2<sup>T</sup> was 4.10 Mb, with 66.8 mol% G+C content. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between strains LB-2<sup>T</sup> and *S. canadensis* FWC47<sup>T</sup> were 77.8% and 21%,

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respectively. The predominant cellular fatty acids were summed feature 8 ( $C_{18:1}\omega7c$  and/or  $C_{18:1}\omega6c$ ) and C<sub>16.0</sub>. The major polar lipids were aminolipid, phosphatidylchosphingoglycolipid, glycolipid, line, phosphatidylglycerol, four unidentified lipids, glycophospholipid, phosphatidylethanolamine and diphosphatidylglycerol. The predominant respiratory quinone was Q-10 and the major polyamine was sym-homospermidine. On the basis of phenotypic, genotypic and phylogenetic evidences, strain LB-2<sup>T</sup> represents a novel species in the genus Sphingomonas, for which the name Sphingomonas caeni sp. nov. is proposed. The type strain is  $LB-2^{T}$  (GDMCC  $1.3630^{\mathrm{T}} = \mathrm{NBRC} \ 115,102^{\mathrm{T}}$ ).

**Keywords** Sphingomonas caeni sp. nov. · Sludge · Polyphasic taxonomy · 16S rRNA gene sequence

#### Introduction

The genus *Sphingomonas* (family *Sphingomona-daceae*, order *Sphingomonadales*, class *Alphaproteo-bacteria*, phylum *Proteobacteria*) was first proposed by Yabuuchi et al. in 1990 (Yabuuchi et al. 1990), and was divided into four genera: *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* by Takeuchi et al. in 2001 (Takeuchi et al. 2001). At the time of writing, the genus *Sphingomonas* comprises 201 species with validly published names according to the List of Prokaryotic names with Standing in

Nomenclature website (https://lpsn.dsmz.de/genus/ sphingomonas). *Sphingomonas* species are characterized by aerobic, Gram-negative, yellow- or orangepigmented, non-sporulating rods, motile or nonmotile, sphingoglycolipids as the characteristic polar lipid, Q-10 as the predominant isoprenoid quinone and *sym*-homospermidine or spermidine as the major polyamine (Yabuuchi et al. 1990, 2002; Takeuchi et al. 2001). Members of the genus have been isolated from various habitats such as soil (Huang et al. 2009; Kim et al. 2014), water (Lee et al. 2017), air (Xue et al. 2018), the plant phyllosphere and rhizosphere (Chung et al. 2011; Cha et al. 2019), contaminated environments (Niharika et al. 2012) and other locations.

In this study, a bacterial strain capable of degrading phenolic acids, designated LB-2<sup>T</sup>, was isolated from an activated sludge sample collected from an agricultural chemical plant. The taxonomic position of the strain was determined using a polyphasic taxonomic approach. The strain is affiliated to genus *Sphingomonas* but differs in some phenotypic and genetic features from the reported type strains in genus *Sphingomonas*. We propose to establish the species name *Sphingomonas caeni* sp. nov. for this strain.

#### Materials and methods

Isolation and culture conditions

Strain LB-2<sup>T</sup> was isolated from activated sludge collected from an agricultural chemical plant located in Binzhou, Shandong Province, PR China (118° 1' E, 37° 29' N). The activated sludge sample was collected from the bioreactor treating pesticide-manufacturing wastewater. The sample was serially diluted and 100 µl of each dilution was plated on Reasoner's 2 A (R2A) agar (BD Difco) plates. After incubation at 30 °C for 5 days, a bacterial strain that formed convex with light yellow-coloured colonies was obtained and further purified. The strain, designated LB-2<sup>T</sup>, was preserved at - 80 °C in R2A broth supplemented with 20% (v/v) glycerol. For taxonomic purpose, S. canadensis FWC47<sup>T</sup>, obtained from Culture Collection University of Gothenburg (CCUG 62,982<sup>T</sup>), was selected as a reference strain.

### 16S rRNA gene sequencing and phylogenetic analysis

For 16S rRNA gene sequencing, the genomic DNA of the strain LB-2<sup>T</sup> was extracted using the method described by Sambrook et al. (Sambrook et al. 1989). PCR amplification of the 16 S rRNA gene was performed using the universal primers 27F (5'-GAG TTTGATCCTGGCTCAG-3') and 1492R (5'-ACG GCTACCTTGTTACGACTT-3'). The PCR product was purified by the PCR gel extraction kit (Omega Bio-Tek) and inserted into pMD19-T vector (TaKaRa Biotechnology). The inserted fragment was sequenced using an automated sequencer (model 3730XL; Applied Biosystems). The sequence similarity was analyzed using a global alignment algorithm implemented at the EzBioCloud (www.ezbiocloud. net). The phylogenetic trees of the 16S rRNA gene sequence of strain LB-2<sup>T</sup> and related type strains were constructed using the neighbour-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981) and minimum-evolution (ME) algorithms (Rzhetsky and Nei 1992) in MEGA version 7.0 software (Kumar et al. 2016). Kimura's two-parameter model (Kimura 1980) was used to calculate the distances and bootstrap values were set as 1000 replications (Felsenstein 1985).

#### Genome sequencing and genomic analysis

The draft genomes of strain LB-2<sup>T</sup> and the closely related type strain S. canadensis FWC47<sup>T</sup> were sequenced at Shanghai Biozeron Biothchnology Co., Ltd. (Shanghai, China.) using the Illumina HiSeq PE150 platform. Paired-end libraries with average insert length of 400 bp were constructed; then,  $100 \times$ libraries were obtained from clean paired-end read data. Raw sequencing data assembly was performed using the ABySS with multiple-Kmer parameters (Jackman et al. 2017). Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup> were calculated by using OrthoANIu algorithm (www.ezbiocloud.net/tools/ani) (Lee et al. 2016) and the Genome-to-Genome Distance Calculator (GGDC) web server (http://ggdc.dsmz.de/distc alc2.php) (Chun et al. 2018). The phylogenomic tree was also constructed based on amino acid sequences of core genes identified using BPGA 1.3 pipeline (Chaudhari et al. 2016). The missing data and poorly aligned regions from concatenated protein sequence alignment of the core genome were removed using Gblock (Castresana 2000). A ML tree was constructed from the resulting alignment of 19 967 amino acid sequences using IQ-Tree (Nguyen et al. 2015) with the best-fit substitution model (LG+F+R4) and 1000 ultrafast bootstrap replicates. The assembled genomes were annotated with the Rapid Annotation with Subsystem Technology (RAST) server (Aziz et al. 2008). Comparative genomic analysis was carried out by the compare-function-based tool of the SEED Viewer (Overbeek et al. 2014).

#### Phenotypic and chemotaxonomic characterization

To analyze the biochemical and physiological characteristics, strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup> were cultivated on R2A agar or in R2A broth at 30 °C. The Gram-stain type was determined using the method described by Beveridge et al. (Beveridge et al. 2007). Cell size and morphology was observed by transmission electron microscopy (H-7650; Hitachi). Growth at various temperatures was determined at 4-42 °C (4, 10, 15, 20, 25, 28, 30, 37 and 42 °C) in R2A broth. Tolerance of NaCl was tested by growing the isolates in R2A broth supplemented with 0-2.0% (w/v) NaCl at ntervls of 0.1%. The pH range for grwth was assessed in R2A broth adjusted to pH 4.0-11.0 (at intervals of 1.0 pH unit) by using citrate/Na<sub>2</sub>HPO<sub>4</sub> buffer (pH range 4.0-5.0), Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> buffer (pH range 6.0-8.0), and glycine/NaOH buffer (pH range 9.0-11.0). Growth was monitored by measuring OD<sub>600</sub> nm. Susceptibility to antibiotics was tested using the diffusion method (Fraser and Jorgensen 1997) on R2A agar with filter-paper discs containing the following antibiotics: cefradine (30 µg), rifampicin (5 µg), chloromycetin (30 µg), neomycin (30 µg), penicillin (10 U), doxycycline (30 µg), lincomycin (2 µg), gentamicin (10 µg), streptomycin (10 µg), ampicillin (10 µg), norfloxacin (10 µg), erythromycin (15 µg), clindamycin (2 µg), novobiocin (30 µg), spectinomycin (100 µg), kanamycin (30 µg), vancomycin (30 µg), tetracycline (30 µg), polymyxin B (300 IU), amoxicillin (25 µg). The susceptibility of the strain was determined by measuring the dimension of the inhibition zone (>10 mm). Motility was examined by the hanging-drop method (Bernardet et al. 2002). Oxidase activity was evaluated using oxidase reagent (bioMérieux); catalase activity was measured by assessing bubble formation in 3% (v/v)  $H_2O_2$  (Dong and Cai 2001). Anaerobic growth was assessed in serum bottles containingR2A broth and sodium thioglycolate (1 g l<sup>-1</sup>) with the upper air layer replaced with nitrogen gas (Zhang et al. 2012). Other biochemical properties and enzyme activities were assayed using API 20NE, API ZYM and API 50CH kits and the Biolog GEN III MicroPlate (bioMérieux) in accordance with the manufacturer's instructions.

For analysis of fatty acids, cells of strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup> were cultured in R2A broth at 30 °C and harvested at exponential phase. The fatty acid methyl esters were prepared and separated following the instructions of the Microbial Identification System (MIDI) as described previously (Sasser 1990) and identified using the MIDI Sherlock MIS (Library: TSBA6; version, 6.0B). The polar lipids were extracted from freeze-dried cells using the method as described previously (Minnikin et al. 1984), and analyzed by two-dimensional thinlayer chromatography after spraying with the following reagents: molybdatophosphoric acid (10% w/v) in absolute ethanol, ninhydin (0.2% w/v) in acetone, molybdenum blueand  $\alpha$ -naphthol for detection of total lipids, amino lipids, phospholipids and glycolipids, respectively, as described by Kates at al. (Kates 1986). The respiratory quinones were extracted and purified in accordance with the method of Collins et al. (Collins et al. 1977) and identified via highperformance liquid chromatography (HPLC) as described by Tamaoka et al. (Tamaoka et al. 1983). The polyamine of strain LB-2<sup>T</sup>was extracted and analysed as described previously (Busse and Auling 1988; Busse et al. 1997).

#### **Results and discussion**

#### 16S rRNA gene phylogeny

The 16S rRNA gene sequence of strain  $LB-2^{T}$  was continuous stretches of 1451 nt, and was submitted to GenBank as OP610062. Comparisons of the 16S rRNA gene sequence in the EzBioCloud database showed that strain  $LB-2^{T}$  shared the highest similarity with *S. canadensis* (96.7%), and shared 95.5–96.7%

similarities with other type strains of the genus *Sphingomonas*, these similarity values were below the 98.7% threshold suggested for proposing a novel species (Bernardet et al. 2002). Phylogenetic analysis based on the NJ tree showed that strain LB-2<sup>T</sup> belonged to the genus *Sphingomonas* and formed a subclade with *S. canadensis* FWC47<sup>T</sup> (Fig. S1). In addition, the ME and ML trees also showed similar topologies (Figs. S2 and S3).

#### Genomic characterisation

The genomic characteristics of the two draft genomes were shown in Table S1. For draft genome of  $LB-2^{T}$ , the number of contigs was 24, and the N50 length was 0.58 Mb, the genome coverage was 367×; for draft genome of S. canadensis FWC47<sup>T</sup>, the number of contigs was 29, the N50 length was 0.29 Mb, the genome coverage was  $426 \times$ . These data showed that the qualities of the two genomic sequences met the standard and could be applied for taxonomic purposes (Chun et al. 2018). Besides, the size of the genome and protein coding genes of strain LB-2<sup>T</sup> were 4.10 Mb and 4032, respectively, which were a little smaller than those of S. canadensis FWC47<sup>T</sup> (4.20 Mb and 4067, respectively); The DNA G+Ccontent and number of RNA of strain LB-2<sup>T</sup> (66.8% and 53, respectively) were very closed to that of S. canadensis FWC47<sup>T</sup> (68.3% and 54, respectively). Phylogenomic analysis based on the concatenated amino acid sequences of the core genes (Fig. 1) also revealed that strain LB-2<sup>T</sup> was located in the genus Sphingomonas and was most closely related to S. canadensis FWC47<sup>T</sup> with a robust bootstrap value (98%), which was in ine with that of 16S rRNA gene phylogeny. The calculated ANI and dDDH values between strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup> were 77.8% and 21%, respetively. byiously, the ANI and dDDH values were far below the cut-off values (<95–96% for ANI, and <7% for dDDH) for rcommended for general species delineation, supporting that strain LB-2<sup>T</sup> represent a novel species within the genus Sphingomonas.

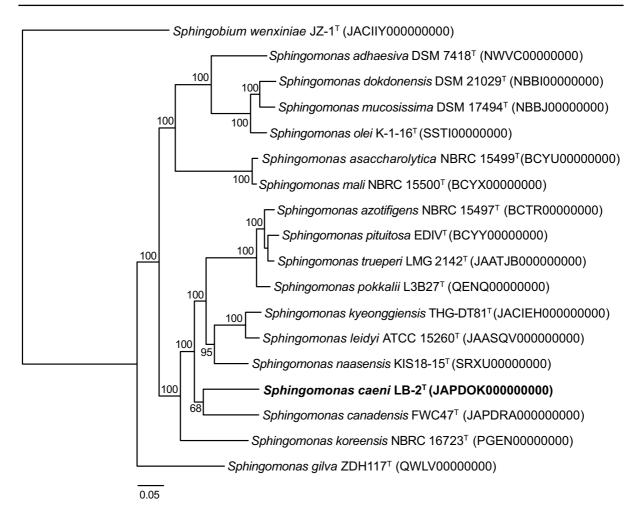
The gene compositions between strain  $LB-2^{T}$  and *S. canadensis* FWC47<sup>T</sup> are shown in Table 1. In nitrogen metabolism annotation, strain  $LB-2^{T}$  possessed genes encoding nitrate reductases NarGHIJ and nitrite reductases NiR1a1b responsible for nitrate and nitrite ammonification, whereas *S. canadensis* 

**Table 1** Comparison of the presence and absence of selected genes between strains LB- $2^{T}$  and *S. canadensis* FWC47<sup>T</sup>.

Genes putatively encoding	LB-2 <sup>T</sup>	S. canadensis FWC47 <sup>T</sup>	
Metabolism of carbohydrates			
Malyl-CoA lyase	+	_	
Trehalase	_	+	
D-lactate dehydrogenase	_	+	
Polygalacturonase	+	_	
Gluconokinase	+	-	
Glucose dehydrogenase	+	_	
Metabolism of nitrogen			
Nitrite reductases	+	_	
Nitrate reductases	+	_	
Oxidative stress			
Dye-decolorizing peroxidase	+	_	
Cytochrome c peroxidase	+	_	
Membrane Transport			
Copper resistance protein CopC	_	+	
Copper resistance protein CopD	_	+	
Mg <sup>2+</sup> transport ATPase, P-type	_	+	
HupE-UreJ family metal transporte	+	-	

FWC47<sup>T</sup> lacked these genes. This result was confirmed by the observation that the reductase activity is positive for strain LB-2<sup>T</sup> but negative for *S. canadensis* FWC47<sup>T</sup>. Dye-decolorizing peroxidase and cytochrome c peroxidase, which could protect bacteria from oxidative stress, were only found in strain LB-2<sup>T</sup>. Furthermore, strain LB-2<sup>T</sup> could also be distinguished from *S. canadensis* FWC47<sup>T</sup> in putative genes involved in membrane transport and metabolism of carbohydrates.

Strain LB-2<sup>T</sup> was isolated from activated sludge treating pesticide-manufacturing wastewater in a chemical plant. Aromatic compounds are widely found in wastewater, and phenolic acid are byproducts of the degradation of aromatic compounds (Zhang et al. 2019). Furthermore, the genome analysis revealed that the genome of strain LB-2<sup>T</sup> contained genes putatively encoding dioxygenase, hydroxylase, O-demethylase and decarboxylase responsible for the metabolism of aromatic compounds, especially phenolic acid, such as benzoate, p-hydroxybenzoate, protocatechuate and vanillate (Table S2). Therefore, degradation of some

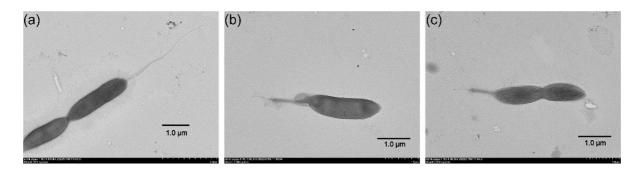


**Fig. 1** Phylogenomic tree based on concatenated amino acid sequences of the core genes are reconstructed by the maximum-likelihood method. Genome accession numbers are indicated in parentheses. *Sphingobium wenxiniae* JZ-1<sup>T</sup> was used

as an outgroup. Bootstrap values (based on 1000 replications) above 50% are indicated at branch nodes. Bar, 0.05 substitutions per nucleotide position

phenolic acids (benzoate, gentisate, p-hydroxybenzoate, protocatechuate and vanillate) by strain LB-2<sup>T</sup> were investigated as described previously (Gai et al. 2007; Chen et al. 2015). The results showed that strain LB-2<sup>T</sup> was able to degrade benzoate, p-hydroxybenzoate, protocatechuate and vanillate (Fig. S4). However, strain LB-2<sup>T</sup> could not degrade gentisate. The result is consistent with the results of the genomic analysis. Consequently, the ability to degrade phenolic acids enabled the strain LB-2<sup>T</sup> to utilize various aromatic hydrocarbon pollutants in wastewater for growth and thus survive in the wastewater environment. Phenotypic and chemotaxonomic characterization

Colonies of strain LB-2<sup>T</sup> grown on R2A agar were round, smooth, mucoid and light yellow-pigmented after 5 days of incubation at 30 °C. Strain LB-2<sup>T</sup> was Gram-stain-negative, non-spore-forming and vibrioid-shaped ( $0.4-0.6 \times 1.0-2.3 \mu m$ ). The cells were dimorphic: one was motile by means of a single polar flagellum, and the other one was non-motile with a stalk (Fig. 2). Growth was observed at 20–30 °C (optimum 28 °C), pH 6.0–8.0 (optimum pH 7.0) and salinity of 0–0.5% (w/v; optimum 0.5%). Oxidase and catalase were positive. Strain LB-2<sup>T</sup> was resistant to polymyxin B, penicillin, gentamicin and ampicillin.



**Fig. 2** Transmission electron micrograph of a cell of strain LB-2<sup>T</sup>, grown on R2A agar for 5 days at 30 °C. The cell bears a polar stalk and a single polar flagellum. **a** Motile cell with a single polar flagellum. **b** Stalked cell. **c** Stalked daughter cell. Bar, 1.0  $\mu$ m

Some detailed phenotypic and biochemical characteristics are summarized in the species description. Differential characteristics between strains LB- $2^{T}$  and *S. canadensis* FWC47<sup>T</sup> are also listed in Table 2.

The cellular fatty acid profiles of strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup> are presented in Table S3. Strain LB-2<sup>T</sup> contained summed feature 8 ( $C_{18:1}\omega7c$ and/or  $C_{18:1}\omega 6c$ ) and  $C_{16:0}$  as the major fatty acids. The fatty acid profile of strain LB-2<sup>T</sup> were similar to that of S. canadensis FWC47<sup>T</sup>. However, some qualitative and quantitative differences in fatty acid content could be observed between strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup>. For example, the concentrations of C<sub>16:0</sub> 2-OH, C<sub>17:1</sub>*w6c*, C<sub>18:1</sub>*w5c* and C<sub>18:1</sub>*w7c* 11-methyl in strain  $LB-2^{T}$  were much lower than those in S. canadensis FWC47<sup>T</sup>, while the concentration of  $C_{16:0}$  and summed feature 3 ( $C_{16:1}\omega7c$  and/ or  $C_{16:1}\omega 6c$ ) in strain LB-2<sup>T</sup> was much higher than that in S. canadensis FWC47<sup>T</sup>.  $C_{13:0}$ ,  $C_{17:1}\omega7c$  and summed feature 4 (iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B) were present in strain LB-2<sup>T</sup> but absent in S. canadensis FWC47<sup>T</sup>, while iso- $C_{17:0}$  3-OH were present in S. canadensis FWC47<sup>T</sup> but absent in strain LB-2<sup>T</sup>. The polar lipids detected were aminolipid (AL), glycolipid (GL), sphingoglycolipid (SGL), phosphatidylcholine (PC), phosphatidylglycerol (PG), four unidentified lipids (L1-4), glycophospholipid (GPL), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) (Fig. S5). In comparison of the polar lipid profiles, AL, GL, SGL, PC, L, GPL and PE were present in strain LB-2<sup>T</sup> but not in *S. canadensis* FWC47<sup>T</sup>. The predominant respiratory quinone was Q-10 and the major polyamine was sym-homospermidine, in line with previously reported chemotaxonomic data for the genus Sphingomonas.

In summary, based on phylogenetic, genomic, phenotypic and chemotaxonomic analysis, strain LB-2<sup>T</sup> represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas caeni* sp. nov is proposed.

#### Description of sphingomonas caenisp. nov.

## Sphingomonas caeni ((cae'ni. L. gen. n. caeni of sludge)

Cells are Gram-stain-negative, aerobic, non-sporeforming, rod-shaped and motile with a single polar flagellum or non-motile with a stalk. Cells are about 0.4-0.6×1.0-2.3 µm. Colonies are circular, 2-3 mm in diameter, smooth, mucoid, convex, opaque and light yellow-coloured after 5 days of incubation on R2A agar at 30 °C. Growth occurs at 20-30 °C (optimum 28 °C), pH 6.0-8.0 (optimum pH 7.0) and salinity of 0-0.5% (w/v) NaCl (optimum, 0.5%). Positive for oxidase, catalase and nitrate reduction, but negative for glucose fermentation and indole production. Hydrolyses aesculin and esculin but not arginine, gelatin and urea. In the API 20 NE test, positive for assimilation of L-arabinose, N-acetyl-glucosamine, D-maltose, adipic acid and trisodium citrate, while negative for D-glucose, D-mannitiol, potassium gluconate, capric acid, malic acid and phenylacetic acid. In the Biolog GEN III MicroPlate system, the following substrates can be used as sole carbon sources for growth:  $\alpha$ -D-lactose, D-melibiose,  $\alpha$ -D-glucose, D-mannose, D-sorbitol, myo-inositol, L-aspartic acid, glucuronamide, D-saccharic acid and  $\alpha$ -ketoglutaric acid. Insensitive to the following chemicals: fusidic acid, tetrazolium violet, tetrazolium blue and **Table 2** Differential characteristics between strains LB-2<sup>T</sup> and *S. canadensis* FWC47<sup>T</sup>. Both strains are Gram-stain-negative, aerobic, non-spore-forming, non-motile and rod-shaped; positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and  $\beta$ -galactosidase; aesculin hydrolysis and assimilation of D-maltose, L-aspartic acid and  $\alpha$ -ketoglutaric acid. Both strains negative for: activities of lipase (C14),  $\alpha$ -galactosidase,  $\alpha$ -glucosidase,  $\alpha$ -mannosidase; gelatin hydrolysis; and assimilation of dextrin, trehalose, cellobiose, gentiobiose, turanose,

 $\alpha$ -D-lactose, *N*-acetyl-D-glucosamine, D-fructose, D-fructose, L-fucose, L-rhamnose, inosine, D-mannitol, D-arabitol, glycerol, D-glucose-6-phosphate, D-serine, L-Serine, L-glutamic acid, L-histidine, L-pyroglutamic acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, quinic acid, *p*-hydroxyphenylacetic acid, methyl pyruvate, L-lactic acid, citric acid, bromosuccinic acid,  $\gamma$ -aminobutyric acid,  $\beta$ -hydroxybutyric acid,  $\alpha$ -ketobutyric acid, propionic acid, acetic acid and formic acid. All data are from this study. +, positive; –, negative; w, weakly positive

Characteristic	Strain LB-2 <sup>T</sup>	S. canadensis FWC47 <sup>T</sup>
Cell size (µm)	0.4-0.6×1.0-2.3	0.4–0.5×1.3–1.5
Salinity growth range (%)	0-0.5	0–2.0
Nitrate reduction	+	_
Utilization of:		
$\alpha$ -D-Lactose	+	_
D-Melibiose	+	_
$\alpha$ -D-Glucose	+	_
D-Mannose	+	_
D-Sorbitol	+	_
myo-Inositol	+	_
L-Alanine	_	+
D-Galactose	_	+
D-Sucrose	_	+
Glucuronamide	+	_
D-Saccharic acid	+	_
D-Raffinose	-	+
L-Arabinose	+	_
Tween 40	_	+
N-Acetyl-glucosamine	+	_
$\alpha$ -Hydroxybutyric acid,	-	+
Enzyme activity:		
$\alpha$ -Chymotrypsin	_	+
$\alpha$ -Galactosidase	-	+
Naphthol-AS-BI-phosphohydrolase	W	+
Valine arylamidase	+	_
$\beta$ -glucosidase	+	-
$N$ -acetyl- $\beta$ -glucosaminidase	+	-
$\alpha$ -Fucosidase	+	-
Cystine arylamidase	W	-
Trypsin	W	_

potassium tellurite. In the API ZYM test, activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, acid phosphatase,  $\beta$ -galactosidase,  $\beta$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase and  $\alpha$ -fucosidase are present, but lipase (C14),  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase and  $\alpha$ -mannosidase are absent. The major fatty acids are summed feature 8 (C<sub>18:1</sub> $\omega$ 7*c*  and/or  $C_{18:1}\omega 6c$ ) and  $C_{16:0}$ . The major polar lipids are aminolipid, glycolipid, sphingoglycolipid, phosphatidylcholine, phosphatidylglycerol, four unidentified lipids, glycophospholipid, phosphatidylethanolamine and diphosphatidylglycerol. The predominant respiratory quinone is Q-10 and the major polyamine is *sym*-homospermidine.

 $LB-2^{T}$ (=GDMCC)The type strain,  $1.3630^{\mathrm{T}} = \mathrm{NBRC} \ 115,102^{\mathrm{T}}$ ), was isolated from an activated sludge sample of an agricultural chemical plant, in Binzhou, Shandong Province, PR China. The DNA G+C content of the type strain is 66.8%. The GenBank/EMBL/DDBJ accession number for the 16 S rRNA gene sequence of strain  $LB-2^{T}$  is OP610062. The whole-genome shotgun sequence of strains LB-2<sup>T</sup> and S. canadensis FWC47<sup>T</sup> have been deposited in GenBank/EMBL/DDBJ under the accession numbers JAPDOK00000000 and JAP-DRA00000000, respectively.

Author contributions Conceptualization, funding acquisition, and supervision: HN; Laboratory work, data analysis and writing-original draft: BL; Writing-review and editing: YW, EC, MH, XC, JH. All authors read and approved the final manuscript.

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**Data availability** The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain  $LB-2^{T}$  is OP610062. The whole-genome shotgun sequence of strains LB-2<sup>T</sup> and *S. canadensis* FWC47<sup>T</sup> have been deposited in GenBank/EMBL/DDBJ under the accession numbers JAP-DOK000000000 and JAPDRA000000000, respectively.

#### Declarations

**Conflict of interest** The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All the authors declare that they have no conflict of interest.

**Ethical approval** The authors have declared that no ethical issues exist.

**Consent to participate** All authors agree to have participated in the research proposed to be published and agree to be published in the journal.

**Consent for publication** All authors agree to have participated in the research proposed to be published and agree to be published in the journal.

Human and animal participants This article does not contain any studies with human participants or animals performed.by any of the author.

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