

# *Sphingobacterium tenebrionis* sp. nov., isolated from intestine of mealworm

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## Abstract

A bacterial strain designated PU5-4<sup>T</sup> was isolated from the mealworm (the larvae of *Tenebrio molitor*) intestines. It was identified to be Gram-stain-negative, strictly aerobic, rod-shaped, non-motile, and non-spore-forming. Strain PU5-4<sup>T</sup> was observed to grow at 10–40°C, at pH 7.0–10.0, and in the presence of 0–3.0% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain PU5-4<sup>T</sup> should be assigned to the genus *Sphingobacterium*. The 16S rRNA gene sequence similarity analysis showed that strain PU5-4<sup>T</sup> was closely related to the type strains of *Sphingobacterium lactis* DSM 22361<sup>T</sup> (98.49%), *Sphingobacterium endophyticum* NYYP31<sup>T</sup> (98.11%), *Sphingobacterium soli* NCCP 698<sup>T</sup> (97.69%) and *Sphingobacterium olei* HAL-9<sup>T</sup> (95.73%). The predominant isoprenoid quinone is MK-7. The major fatty acids were identified as iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH and summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c) and summed feature 9 (iso-C<sub>17:0</sub> ω9c). The polar lipids are phosphatidylethanolamine, one unidentified phospholipid, and six unidentified lipids. The genomic DNA G+C content of strain PU5-4<sup>T</sup> is 40.24 mol%. The average nucleotide identity of strain PU5-4<sup>T</sup> exhibited respective values of 73.88, 73.37, 73.36 and 70.84% comparing to the type strains of *S. lactis* DSM 22361<sup>T</sup>, *S. soli* NCCP 698<sup>T</sup>, *S. endophyticum* NYYP31<sup>T</sup> and *S. olei* HAL-9<sup>T</sup>, which are below the cut-off level (95–96%) for species delineation. Based on the above results, strain PU5-4<sup>T</sup> represents a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium temoinsis* sp. nov. is proposed. The type strain is PU5-4<sup>T</sup> (=CGMCC 1.61908<sup>T</sup>=JCM 36663<sup>T</sup>).

## INTRODUCTION

Yabuuchi *et al.* [1] reclassified *Flavobacterium spiritivorum* and *Flavobacterium multivorum* as *Sphingobacterium spiritivorum* and *Sphingobacterium multivorum*, respectively, leading to the establishment of the genus *Sphingobacterium*. Most members of the genus *Sphingobacterium* are oxidase-positive rods without flagella. They contain a high content of sphingophospholipids and iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 2-OH, C<sub>16:1</sub> ω7c and C<sub>17:0</sub> 3-OH as the major fatty acids and menaquinone-7 (MK-7) as the main respiratory quinone [2, 3]. The description of genus *Sphingobacterium* was emended by Wauters *et al.* from indole-production-positive to variable for indole production [4]. At the time of writing, this genus encompasses 69 validly published species (<https://lpsn.dsmz.de/genus/sphingobacterium>, accessed 6 May, 2024). Members of the genus *Sphingobacterium* have been identified in diverse environments, including leaf tissues, soil, lakes, compost, insects, raw milk and nodule surface of soybean [5–13]. Herein we describe the isolation and characterization of a new member within the genus *Sphingobacterium*, which was isolated during our study investigating plastic degradation in the intestines of yellow mealworms.

The yellow mealworm, the larva stage of *Tenebrio molitor*, exhibits a yellow body with a length ranging from 2 to 3 cm. This common edible insect is artificially cultivated to serve as a dietary source for birds, reptiles, and other small pets. These omnivorous larvae demonstrate a feeding preference for wheat bran, grains, and other materials. Notably, they have garnered attention in recent

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**Keywords:** 16S rRNA gene; intestines; *Sphingobacterium tenebrionis* sp. nov.; taxonomy; *Tenebrio molitor*.

**Abbreviations:** ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; LCFBM, liquid carbon-free basal medium; TYGS, Type Strain Genome Server.

The GenBank/EMBL/DDBJ/PIR accession numbers for the 16S rRNA gene sequence and the draft genome sequence of strain PU5-4<sup>T</sup> are PP092110 and JAYLLN000000000, respectively.

Five supplementary figures and three supplementary tables are available with the online version of this article.

years for their remarkable ability to degrade plastic wastes [14–16], positioning them as environmentally beneficial insects. In the course of our investigation into polyurethane degradation and ingestion by yellow mealworms, we isolated a strain, designated PU5-4<sup>T</sup>, from the intestine of mealworm.

## ISOLATION AND ECOLOGY

The strain originates from the State Key Laboratory of Microbial Technology, Shandong University (120° 41' 1" E, 36° 21' 55" N), Qingdao Campus, Shandong Province, PR China. Thirty mealworms, exclusively fed a polyurethane foam diet for a duration of 3 months, were collected to prepare a gut suspension. This suspension was transferred to a 250 ml Erlenmeyer flask that contained 2 g polyurethane powders and 100 ml liquid carbon-free basal medium (LCFBM), which was prepared with deionized water and contained 0.7 g l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.7 g l<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.7 g l<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.0 g l<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>, 0.005 g l<sup>-1</sup> NaCl, 0.002 g l<sup>-1</sup> FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.002 g l<sup>-1</sup> ZnSO<sub>4</sub>·7H<sub>2</sub>O, and 0.001 g l<sup>-1</sup> MnSO<sub>4</sub>·H<sub>2</sub>O, adhering to the American Society for Testing and Materials standard for evaluating plastic resistance to bacteria (ASTM G22-76). This flask was incubated on a rotary shaker (120 r.p.m.) at 30°C. After 60 days, the enriched suspension was spread on nutrient agar (10.0 g peptone, 3.0 g beef extract, 5.0 g NaCl, 20 g agar, per litre), and incubated at 30°C for 48 h. Colonies were subsequently picked and transferred to fresh nutrient agar, and observed until pure isolates were confirmed based on colony morphology and microscopic examinations of cell morphology. Strain PU5-4<sup>T</sup> identified through preliminary 16S rRNA gene sequencing, was selected for this study. The pure culture of strain PU5-4<sup>T</sup> was preserved at -80°C as a glycerol suspension (20%, v/v). Seventeen other bacterial strains isolated from the same sample are listed in Table S1, available in the online Supplementary Material.

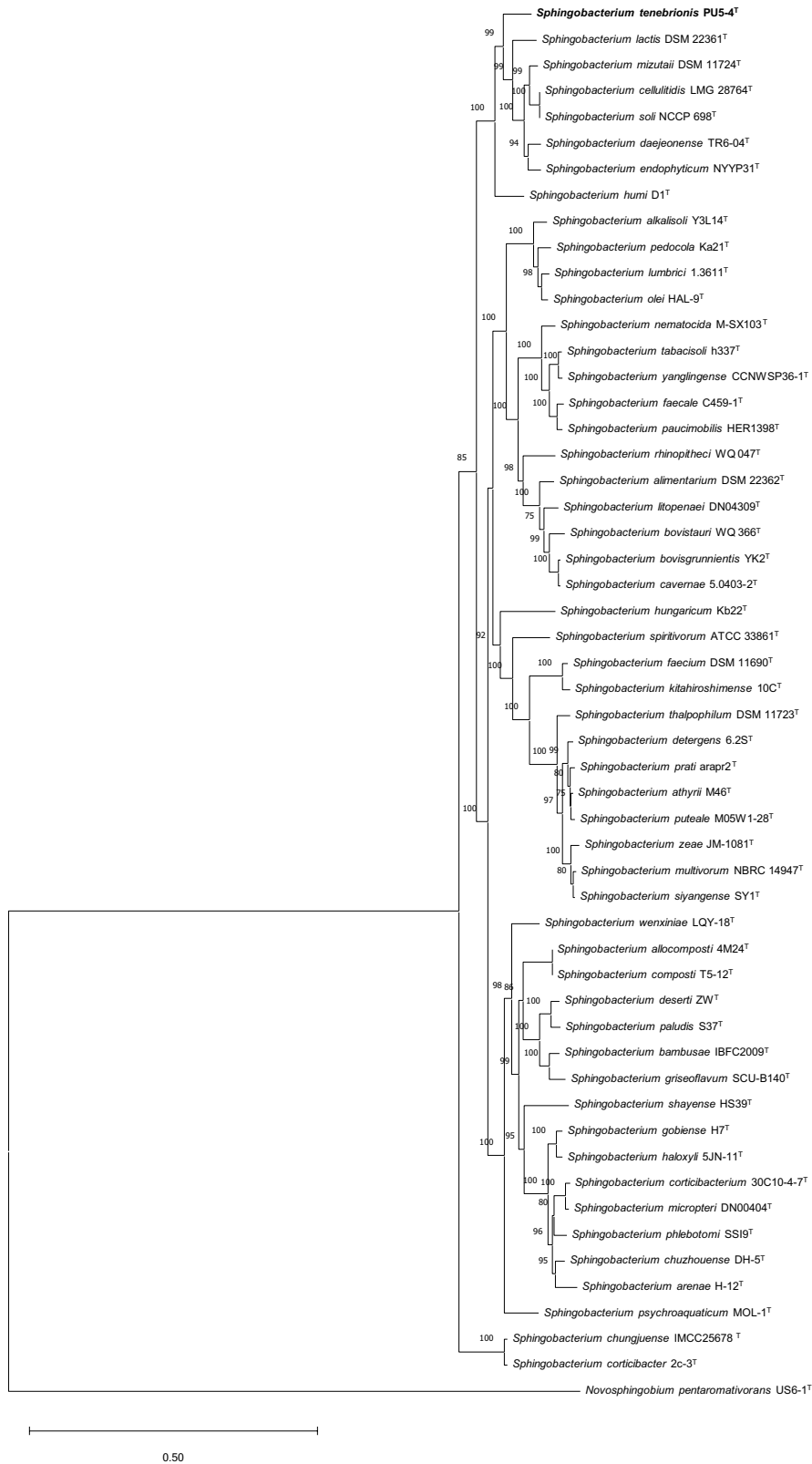
## 16S rRNA GENE PHYLOGENY

To accurately determine the taxonomic status of strain PU5-4<sup>T</sup>, we performed genomic DNA extraction and 16S rRNA gene amplification as described by Rao *et al.* [17]. The obtained 1401 bp sequence was then compared with available data on EzBioCloud ([www.ezbiocloud.net/identify](http://www.ezbiocloud.net/identify)) [18], and analysed using the Genome-to-Genome Distance Calculator ([ggdc.dsmz.de/ggdc.php#](http://ggdc.dsmz.de/ggdc.php#)). We used FastTree to build the maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences, and used MEGA11 [19–21] to build the maximum-parsimony phylogenetic tree (Fig. S1) and neighbour-joining tree [22] (Fig. S2), demonstrating the position of strain PU5-4<sup>T</sup> within the genus. The results revealed that the 16S rRNA gene sequence of strain PU5-4<sup>T</sup> exhibited the highest similarity to the type strains *Sphingobacterium lactis* DSM 22361<sup>T</sup> (98.49%) and *Sphingobacterium endophyticum* NYYP31<sup>T</sup> (98.11%) and below 98% to other related type strains, which were all below the threshold value (98.70%) for species delineation [23]. In the phylogenetic tree based on the maximum-parsimony algorithm (Fig. S1), strain PU5-4<sup>T</sup> formed a branch with the *Sphingobacterium* species, including *S. lactis* DSM 22361<sup>T</sup>. The phylogenetic trees based on the maximum-parsimony (Fig. S1) and maximum-likelihood (Fig. 1) algorithms yielded consistent results.

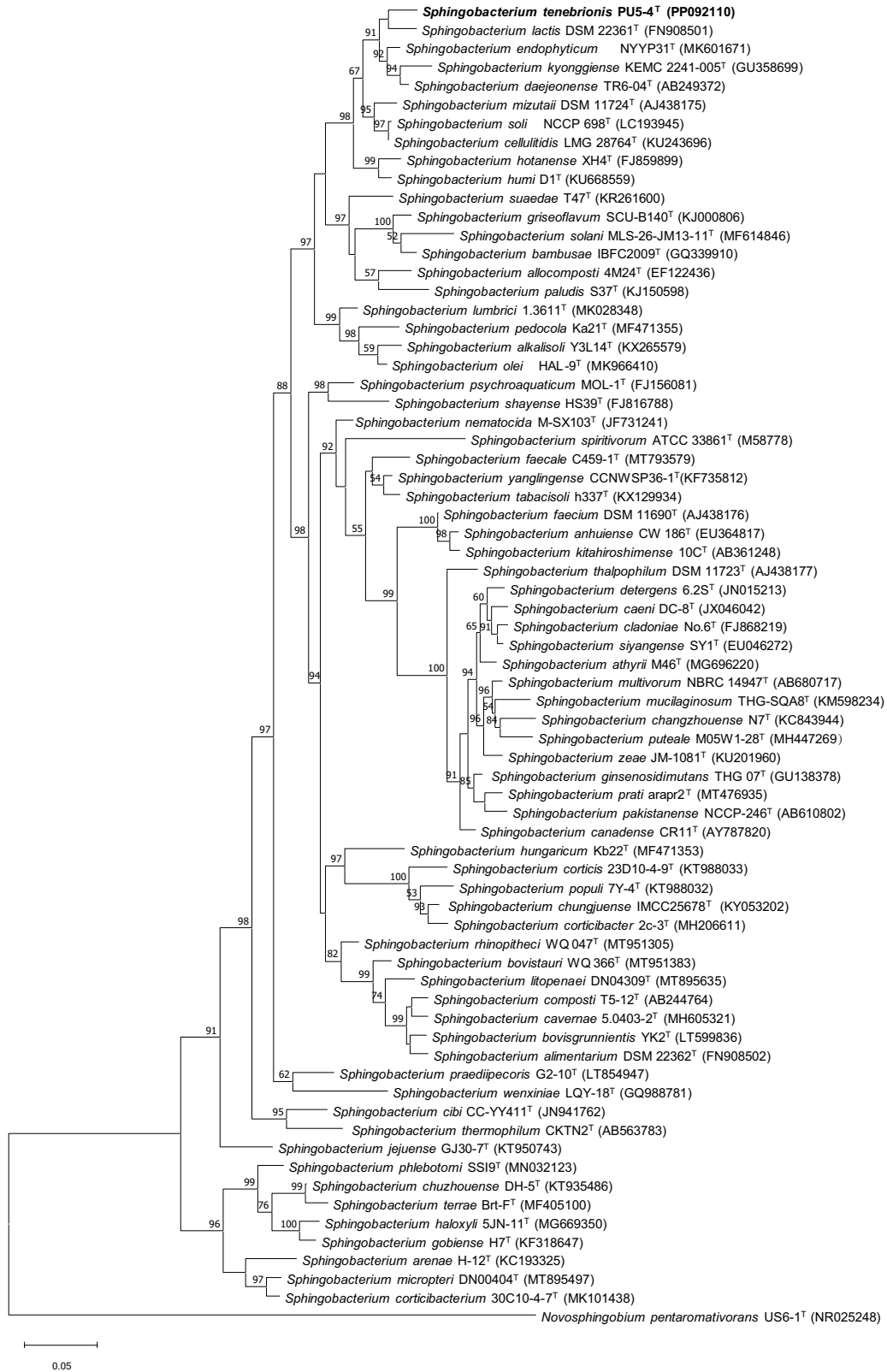
## GENOME FEATURES

The genome of strain PU5-4<sup>T</sup> was sequenced and analysed by Sangon Biotech (Shanghai, PR China) using the MGISEQ-2000 platform (MGI). The original data of sequencing were counted and evaluated by Fastp TP, with the quality cut at the same time, and relatively accurate and effective data were obtained. Sequencing data splicing was performed using SPAdes. Gap Filler was employed to complement gaps in the concatenated contig. Pilon was utilized for sequence correction, rectifying editing errors and missing insertions of small segments introduced during the splicing process. NCBI BLAST+ was used to compare gene/protein sequences with various databases including CDD, KOG, COG, NR, NT, PFAM, Swissprot, and TrEMBL to obtain functional annotation information. GO functional annotation information was derived based on gene annotation results from Swissprot and TrEMBL. Additionally, KEGG gene annotation information was obtained using KAAS. The genome sequence has been deposited in the NCBI GenBank database. We assessed the genome using QUAST, and using CheckM to evaluate the genome completeness and determined the genome contamination. A total of 120 single-copy ortholog sequences were searched by the GTDB-Tk in all strains containing the outgroup strain *Novosphingobium pentaromativorans* US6-1<sup>T</sup>. The maximum-likelihood phylogenetic tree based on concatenated protein sequence of the 120 core orthologue genes was reconstructed by using FastTree. As shown in Fig. 2, strain PU5-4<sup>T</sup> is most closely related to *S. lactis* DSM 22361<sup>T</sup>, corroborating the results in Fig. 1. A phylogenomic tree, featuring genomes of strain PU5-4<sup>T</sup> and closely related species, was reconstructed utilizing the Type Strain Genome Server (<https://tygs.dsmz.de/>) [24, 25]. The genome-based phylogenomic tree (Fig. S3) revealed that strain PU5-4<sup>T</sup> forms a separated branch with five closely related *Sphingobacterium* species, three of which are consistent with results in Fig. 2, including *Sphingobacterium mizutaii*, *Sphingobacterium cellulitidis* and *Sphingobacterium soli*.

Given the origins of strain PU5-4<sup>T</sup> from polyurethane-fed mealworm survivors and the recognized role of mealworm gut micro-organisms in plastic digestion [14], we conducted a genome analysis to identify potential enzymes responsible for polyurethane degradation. Esterase, amidase, protease, and urease were reported to be the primary enzymes involved in catalysing the hydrolysis of polyurethane chemical bonds [26]. Our analysis of strain PU5-4<sup>T</sup> revealed the presence of 24 esterases, four amidases, and 13 proteases. Notably, among these enzymes, 16 esterases and four amidases were found to



**Fig. 1.** Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain PU5-4<sup>T</sup>. Numbers at nodes are based on 1000 resamplings; only values above 50 % are presented. *Novosphingobium pentaromatorans* US6-1<sup>T</sup> was used as an outgroup.



**Fig. 2.** Phylogenomic tree based on concatenated protein sequences of the 120 core ortholog genes. The maximum-likelihood phylogenetic tree shows the position of strain PU5-4<sup>T</sup> within the genus. *Novosphingobium pentaromativorans* US6-1<sup>T</sup> was used as an outgroup. Bar, 0.50 substitutions per amino acid.

possess signal peptides for protein secretion (Table S3), which is a critical characteristic facilitating extracellular degradation of high molecular weight polymers [27].

The total draft genome size of strain PU5-4<sup>T</sup> was 3.94 Mb, with 3522 coding sequences, three rRNA genes, 45 tRNA genes, one ncRNA and 83 contigs. Notably, 18 contigs were used to form L50, and the length of N50 was 68640 bp. The DNA G+C content was calculated as 40.24 mol%. The CheckM results showed that the genome integrity and contamination of strain PU5-4<sup>T</sup> were 98.41 and 0.48%, respectively. This indicates that its genome quality is relatively good. The digital DNA–DNA hybridization (dDDH) values demonstrated that strain PU5-4<sup>T</sup> shared 20.1% similarity with *S. lactis* DSM 22361<sup>T</sup>, 19.1% with *S. soli* NCCP 698<sup>T</sup>, 18.9% with *S. endophyticum* NYYP31<sup>T</sup>, and 19.9% with *S. olei* HAL-9<sup>T</sup>. Additionally, the average nucleotide identity (ANI) values revealed 73.88, 73.37, 73.36 and 70.84% similarity of strain PU5-4<sup>T</sup> to *S. lactis* DSM 22361<sup>T</sup>, *S. soli* NCCP 698<sup>T</sup>, *S. endophyticum* NYYP31<sup>T</sup> and *S. olei* HAL-9<sup>T</sup>, respectively. All ANI and dDDH values of strain PU5-4<sup>T</sup> comparing to other *Spingobacterium* species are summarized in Table S2. These values are below the threshold established to classify strain PU5-4<sup>T</sup> as representing a new species [23].

## PHYSIOLOGY AND CHEMOTAXONOMY

Strain PU5-4<sup>T</sup> was cultured on nutrient agar at 30°C for 1–2 days for morphological, physiological and biochemical tests, unless otherwise indicated. The cellular morphology of strains was observed with a field emission SEM (FEI Quanta FEG 250) and STEM mode of electron microscopy (Zeiss Crossbeam550) after 2 days of culture in nutrient broth (10.0 g peptone, 3.0 g beef extract, 5.0 g NaCl, per litre). TEM and SEM images of strain PU5-4<sup>T</sup> are shown in Fig. S4. The yellow colonies of strain PU5-4<sup>T</sup> were covered with 20% aqueous KOH solution, and the production of flexirubin-type pigments was determined by observing whether the colour of the colonies reversibly changed to red or purple, or brown [28]. No significant colour change was observed after the treatment. Gram-staining was performed by following the classic Gram-staining procedure [29] and confirmed using the KOH lysis test [30]. Anaerobic growth was evaluated on nutrient broth agar plate, incubated at 30°C for 2 weeks in an anaerobic container equipped with oxygen-absorbing and carbon dioxide-generating reagents (Anaero Pack, Mitsubishi Gas Chemical). Cell motility was examined by semi-solid nutrient agar medium containing 0.6% agar, and no diffusion or spreading pattern was observed for PU5-4<sup>T</sup>. Growth range and optimal conditions of this strain were determined at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50 and 55°C) and pH (pH 4.0–11.0, at 1 pH unit intervals). The pH was adjusted using acetate buffer for pH 4.0–5.5, phosphate buffer for pH 6.0–8.0 and Tris buffer for pH 8.5–11.0. The NaCl tolerance test of strain PU5-4<sup>T</sup> was determined in nutrient broth medium supplemented with NaCl concentrations ranging from 0–5.0% (w/v, with 0.5% intervals).

According to the manufacturer's instructions, substrate utilization and enzymatic activity assays were performed using the API 20NE (bioMérieux), API ZYM (bioMérieux) and Gen III MicroPlate (Biolog) identification systems. Oxidase detection was performed by dropwise addition of OX reagent from API 20NE to observe whether a deep purple reaction occurred.

For whole-cell fatty acid analysis, strain PU5-4<sup>T</sup> and three closely related strains were inoculated on nutrient broth and harvested when cells reached the exponential phase, and washed with LCFBM medium. The fatty acids were saponified, methylated, and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.3). Cellular fatty acids were analysed using an Agilent 6890 N gas chromatograph and identified using the TSBA40 database of the microbial identification system [31]. Respiratory quinones were extracted from 300 mg freeze-dried cells [32] and purified with a silica gel TLC plate (Merck Kieselgel 60 F254), then analysed by HPLC [33]. Polar lipids were determined using 2D TLC [34].

Cells of strain PU5-4<sup>T</sup> were Gram-stain-negative, non-motile and rod-shaped (0.3–0.4×0.8–1.2 μm). After 3 days of culture at 30°C, colonies on nutrient agar were yellow, opaque, round, smooth and 2–4 mm in diameter. Strain PU5-4<sup>T</sup> grew well in nutrient broth, and was able to grow at the pH range of pH 7.0–10.0 and in the temperature range of 10–40°C. Optimal growth was at 30°C and pH 7.0. The range of NaCl concentrations for cell growth was determined as 0–3.0% (w/v), and growth was optimal without NaCl addition. Strain PU5-4<sup>T</sup> was detected as positive for oxidase. More detailed physiological and biochemical characteristics are listed in Table 1 and in the species description.

The major fatty acids were identified as iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub>3-OH and summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), which is similar to other related type strains with minor differences in the types and amounts of certain components, especially for saturated iso-C<sub>15:0</sub> and summed feature 3 and summed feature 9 (Table 2). The only respiratory quinone detected in strain PU5-4<sup>T</sup> was MK-7, and the polar lipids detected were phosphatidylethanolamine, and six other lipids (Fig. S5).

The quinone pattern, polar lipid profile and fatty acid composition of strain PU5-4<sup>T</sup> were similar to other *Spingobacterium* members. However, distinctions in the number of unknown aminolipids, unknown lipids, and fatty acid content set them apart [8, 12, 35–39]. Notably, the 16S rRNA gene sequence similarity between strain PU5-4<sup>T</sup> and closely related reference strains was consistently below 98.5%. Additionally, ANI and dDDH values fell below the threshold for new species delineation, and disparities in genome size and DNA G+C content further contributed to differentiation [18]. Differentiating phenotypic,

**Table 1.** Different phenotypic and chemotaxonomic characteristics of strain PU5-4<sup>T</sup> and its closely related type strains of the genus *Sphingobacterium*. Strains: 1, PU5-4<sup>T</sup>; 2, *S. soli* NCCP 698<sup>T</sup>; 3, *S. endophyticum* NYYP31<sup>T</sup>; 4, *S. olei* HAL-9<sup>T</sup>; and 5, *S. lactis* DSM 22361<sup>T</sup>. All data were obtained from this study unless otherwise mentioned. +, Positive; –, negative; w, weakly positive; ND, not determined or no data available.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	10–40	15–37*	4–42†	10–35‡	10–41§
pH range for growth	7–10	5–9*	5–10†	6–10‡	6–9§
NaCl range for growth (% w/v)	0–3.0	0–5.0*	0–5.0†	0–5.0‡	0–5.0§
Genome size (Mb)	3.94	4.40*	4.70†	5.41‡	3.98§
DNA G+C content (mol%)	40.24	41.20*	36.40†	40.60‡	44.2§
Oxidase	+	+	–	+	+\$
API 20 NE:					
D-Glucose	–	–	–	+	+\$
D-Mannose	w	+	+	+	+\$
Maltose	+	+	+	w	+\$
Biolog GENIII MicroPlate:					
N-Acetylneuraminic acid	–	+	+	+	ND
D-Galactose	–	+	+	+	ND
L-Rhamnose	–	+	+	+	ND
L-Pyroglutamic acid	w	–	–	–	ND
API ZYM:					
Lipase (C14)	w	–	–	–	–\$
α-Glucosidase	w	–	w	–	ND
N-Acetyl-glucosaminidase	–	w	+	+	ND

\*Data taken from [35].

†Data taken from [36].

‡Data taken from [37].

§Data taken from [12].

genotypic and chemotaxonomic characteristics between strain PU5-4<sup>T</sup> and four closely related type strains are shown in Table 1. Considering morphological, physiological, and chemotaxonomic traits, along with comparisons of 16S rRNA gene and whole genome sequences, we propose that strain PU5-4<sup>T</sup> represents a novel species within the genus *Sphingobacterium*, for which the name *Sphingobacterium temoinsis* sp. nov. is proposed.

## DESCRIPTION OF *SPHINGOBACTERIUM TENEBRIONIS* SP. NOV.

*Sphingobacterium tenebrionis* (te.ne.bri.o'nis. N.L. gen. n. *tenebrionis*, of the mealworm *Tenebrio molitor*).

Cells are Gram-negative, strictly aerobic and rod-shaped (0.3–0.4×0.8–1.2 μm), with no motility. Growth occurs on nutrient agar. The colonies of this strain are round, smooth, yellow and non-transparent, with a diameter of 2–4 mm after incubation at 30°C for 3 days on nutrient agar. Growth occurs at 10–40°C (optimal, 30°C) and pH 7.0–10.0 (optimal, pH 7.0) and tolerates 0–3.0% (w/v) NaCl (optimal, 0%). In API 20NE tests, positive reactions were observed for assimilation of D-glucose, D-mannose, N-acetylglucosamine and maltose, and for hydrolysis of aesculin ferric citrate and 4-nitrophenyl-β-D-galactopyranoside. However, negative results were observed for nitrate reduction, indole production, gelatin hydrolysis, arginine dehydratase, urease activity, and assimilation of potassium gluconate, D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In API ZYM strips, strain PU5-4<sup>T</sup> displayed positive activities for alkaline phosphatase, esterase (C4), lipase (C8), leucine arylamidase and valine arylamidase. Weak positive reactions were observed for lipase (C14), cystine arylamidase, trypsin arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and β-glucosidase. Negative reactions were noted for α-chymotrypsin, acid phosphatase,

**Table 2.** Cellular fatty acid composition characteristics of strain PU5-4<sup>T</sup> and representatives of closely related *Sphingobacterium* species

Strains: 1, PU5-4<sup>T</sup>; 2, *S. soli* NCCP 698<sup>T</sup>; 3, *S. endophyticum* NYYP31<sup>T</sup>; 4, *S. olei* HAL-9<sup>T</sup>; and 5, *S. lactis* DSM 22361<sup>T</sup>. Data were obtained from this study unless otherwise mentioned and expressed as a percentage of the total fatty acids. Only fatty acids that account for more than 1.0% of the total fatty acids of at least one strain are shown. TR, Trace amounts (<1%); –, not detected; ND, not determined or no data available.

Fatty acid	1	2	3	4	5†
Saturated:					
C <sub>16:0</sub>	1.9	2.3	2.4	1.24	2.6
C <sub>18:0</sub>	1.0	TR	TR	1.2	ND
C <sub>16:0</sub> 3-OH	1.8	2.0	TR	TR	1.0
C <sub>17:0</sub> 2-OH	–	TR	2.6	–	ND
Unsaturated:					
C <sub>15:1</sub> ω6c	TR	1.5	1.1	TR	ND
Branched:					
Iso-C <sub>15:0</sub>	30.7	21.5	33.1	36.9	27.5
Iso-C <sub>15:0</sub> 3-OH	2.1	1.6	1.3	2.2	1.1
Iso-C <sub>17:0</sub> 3-OH	17.2	19.0	14.7	22.3	16.6
Iso-C <sub>15:1</sub> F	TR	–	–	1.1	TR
Iso-C <sub>15:1</sub> G	–	TR	1.5	–	–
Anteriso-C <sub>15:0</sub>	1.3	TR	7.6	TR	ND
Summed feature 3*	31.8	22.1	26.5	44.5	37.5
Summed feature 4*	TR	1.7	TR	TR	TR
Summed feature 9*	4.8	7.8	3.3	16.1	ND

\*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 comprises C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c, summed feature 4 comprises iso-C<sub>17:0</sub>I and/or iso-C<sub>17:1</sub>I, summed feature 5 comprises C<sub>18:2</sub>ω6,9c, and summed feature 9 comprises iso-C<sub>17:0</sub>ω9c.

†Data taken from [12].

α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and β-mannosidase. In the ID 32 GN system, the reactions of maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, lactose, melibiose, β-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, D-mannose, D-fructose, L-fucose, D-serine, troleandomycin, minocycline, gelatin, glycyl-L-proline, L-glutamic acid, L-histidine, L-serine, lincomycin, tetrezolium, nalidixic acid, lithium chloride, Tween 40, γ-aminobutyric acid, acetoacetic acid, acetic acid, aztreonam and sodium butyrate were positive; the reactions of dextrin, raffinose, N-acetylneuraminic acid, D-galactose, 3-methyl glucose, D-fucose, L-rhamnose, inosine, fusidic acid, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-PO<sub>4</sub>, D-fructose-6-PO<sub>4</sub>, D-aspartic acid, D-serine, rifamycin SV, L-alanine, L-arginine, L-aspartic acid, Niaproof 4, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, vancomycin, tetrazolium violet, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric, D-malic acid, L-malic acid, bromo-succinic acid, potassium tellurite, α-hydroxy-butyric acid, β-hydroxy-D,L-butyric acid, α-keto-butyric acid, propionic acid, formic acid and sodium bromate were negative; and the reactions of N-acetyl-β-D-mannosamine, L-pyroglutamic, D-saccharic acid and methyl pyruvate were weak. The predominant isoprenoid quinone was found to be MK-7. The major fatty acids were identified as iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub>3-OH and summed feature 3 (C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c) and summed feature 9 (iso-C<sub>17:0</sub>ω9c). The polar lipids were determined to be phosphatidylethanolamine, one unidentified phospholipid, and six unidentified lipids.

The type strain, PU5-4<sup>T</sup> (=CGMCC 1.61908<sup>T</sup>=JCM 36663<sup>T</sup>), was isolated from the intestines of the yellow mealworm at the State Key Laboratory of Microbial Technology, Shandong University Qingdao Campus (120° 41' 1" E, 36° 21' 55" N) in Shandong Province, PR China. The genome has a DNA G+C content of 40.24mol%. The GenBank/EMBL/DDBJ/PIR

accession numbers for the 16S rRNA gene sequence and the draft genome sequence of strain PU5-4<sup>T</sup> are PP092110 and JAYLLN000000000, respectively.

#### Funding information

This work was supported by the National Key Research and Development Program of China (2019YFA0706900); National Natural Science Foundation of China (32370124); and State Key Laboratory of Microbial Technology Open Projects Fund (Project NO. M2023-10).

#### Acknowledgements

We thank Sen Wang in Shandong University Core Facilities for Life and Environmental Sciences for the help in the SEM and TEM analysis.

#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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