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**TAXONOMIC DESCRIPTION**

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# *Sphingobacterium phlebotomi* sp. nov., a new member of family *Sphingobacteriaceae* isolated from sand fly rearing substrate

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**Abstract**

A Gram-stain-negative, rod-shaped, non-motile, non-spore-forming, aerobic bacterium, designated type strain SSI9$^T$, was isolated from sand fly (*Phlebotomus papatasi* Scopoli; *Diptera: Psychodidae*) rearing substrate and subjected to polyphasic taxonomic analysis. Strain SSI9$^T$ contained phosphatidylethanolamine as a major polar lipid, MK-7 as the predominant quinone, and $C_{16:1}\omega6c/C_{16:1}\omega7c$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and $C_{16:0}$ as the major cellular fatty acids. Phylogenetic analysis based on 16S rRNA gene sequences revealed that SSI9$^T$ represents a member of the genus *Sphingobacterium*, of the family Sphingobacteriaceae sharing 96.5–88.0% sequence similarity with other species of the genus *Sphingobacterium*. The results of multilocus sequence analysis using the concatenated sequences of the housekeeping genes *rec*A, *rpl*C and *gro*L indicated that SSI9$^T$ formed a separate branch in the genus *Sphingobacterium*. The genome of SSI9$^T$ is 5197142 bp with a DNA G+C content of 41.8 mol% and encodes 4395 predicted coding sequences, 49 tRNAs, and three complete rRNAs and two partial rRNAs. SSI9$^T$ could be distinguished from other species of the genus *Sphingobacterium* with validly published names by several phenotypic, chemotaxonomic and genomic characteristics. On the basis of the results of this polyphasic taxonomic analysis, the bacterial isolate represents a novel species within the genus *Sphingobacterium*, for which the name *Sphingobacterium phlebotomi* sp. nov. is proposed. The type strain is SSI9$^T$ (=ATCC TSD-210$^T$=LMG 31664$^T$=NRRL B-65603$^T$).

The genus *Sphingobacterium* was first described by Yabuuchi *et al.* [1] and classified as part of the family *Sphigobacteriacaea*, of the phylum *Bacteroidetes*. In general, members of the genus *Sphingobacterium* are Gram-stain-negative rods that are positive for catalase and oxidase, negative for heparinase and gelatinase activities, and variable for indole production [2]. The DNA G+C contents of different species of the genus *Sphingobacterium* range from 35 to 44 mol% [3]. They have phosphatidylethanolamine as a major polar lipid and MK-7 as the major isoprenoid quinone. As of April 2021, the genus *Sphingobacterium* includes 58 species with validly published names (www.bacterio.net/sphingobacterium.html). Species of the genus *Sphingobacterium* have been isolated from a variety of habitats, including clinical specimens [1], activated sludge [4], compost [5–7], lake water and aquifers [8], soil [9, 10], lichen [3], oil-contaminated soil [11, 12], plants, including leaves, bark and stems [13–15], and raw milk [16]. A bacterium, designated strain SSI9$^T$, was isolated from the rearing substrate of second/third instar *Phlebotomus papatasi* sand flies. In this study, we characterize the isolate using a polyphasic approach and propose that it represents a novel member of the genus *Sphingobacterium*.

Leishmaniasis is an important vector-borne parasitic disease affecting millions of people each year in tropical and subtropical parts of the world. Phlebotomine sand flies are the primary vectors of these parasites. During a study to develop an ecologically based approach for sand fly control [17], the novel strain SSI9$^T$ was isolated from a rearing substrate of second/third instar larvae of *Phlebotomus papatasi*, an old-world sand fly species. The substrate primarily constituted a mixture of rabbit chow and rabbit faeces with frass material (shed cuticles, dead bodies, and faeces) from the sand fly

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**Keywords:** 16S rRNA gene; Polyphasic analysis; *Sphingobacterium phlebotomi* sp. nov.

**Abbreviations:** AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, Digital DNA–DNA hybridization; RAST, Rapid Annotation using Subsystem Technology.

The GenBank accession number for the 16S rRNA gene sequence of *Sphingobacterium phlebotomi* sp. nov. SSI9$^T$ is MN032123. The GenBank accession number for the whole genome sequence is VTAV00000000.

One supplementary table and five supplementary figures are available with the online version of this article.

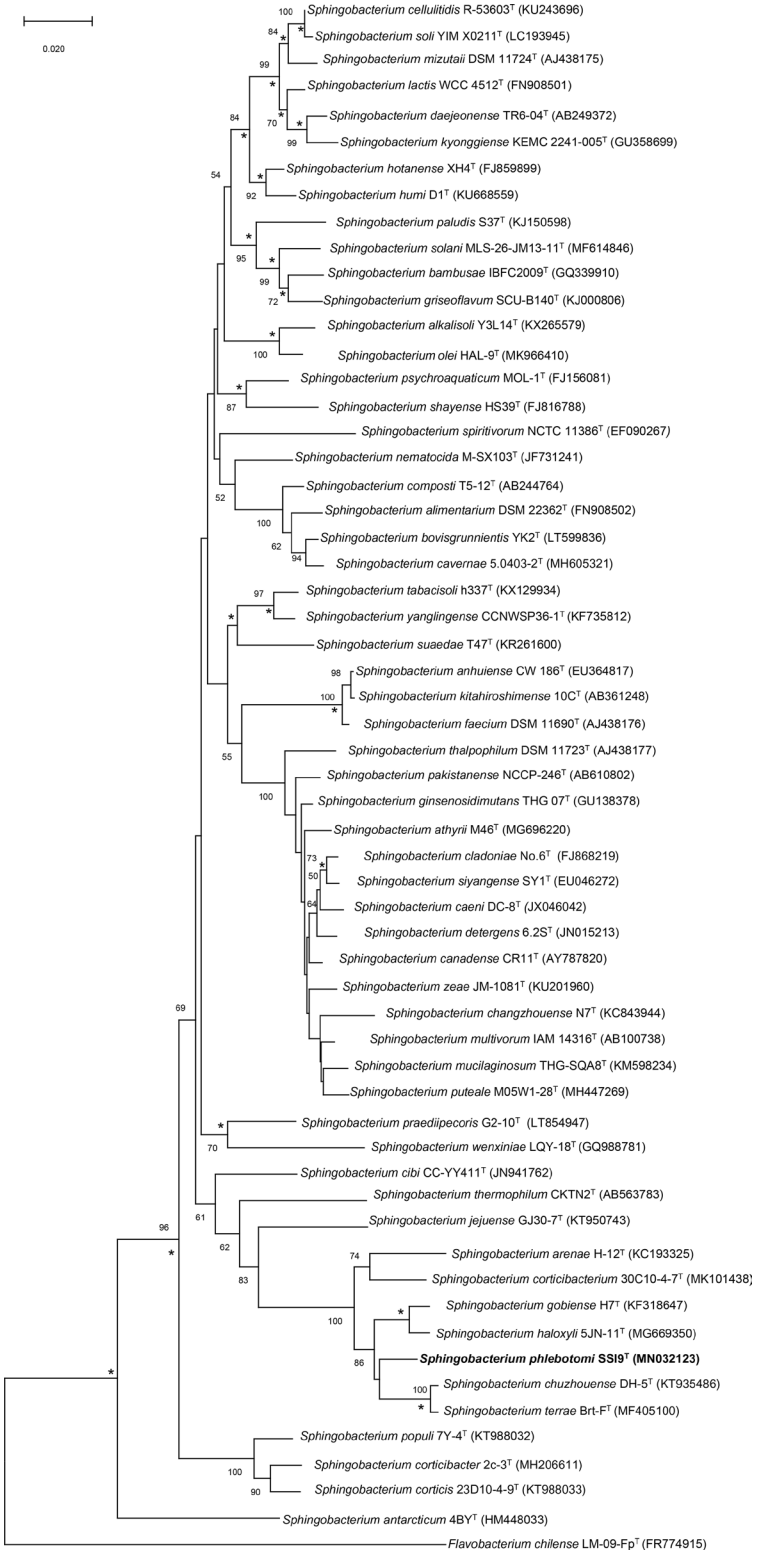


**Fig. 1.** Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences (1385 bp) showing the relationship of strain SSI9$^T$ with related type strains of known species of the genus *Sphingobacterium*. Bootstrap values ≥50% (based on 1000 replications) are shown at the branch nodes. The type strains *Parapedobacter luteus* DSM 22899$^T$, *Parapedobacter indicus* DSM 28470$^T$ and *Flavobacterium chilense* LM-09-Fp$^T$ were used as outgroups. Asterisks indicate branches that were also recovered using maximum likelihood methods. Accession numbers of sequences are given in parenthesis. Bar, 0.02 substitutions per nucleotide position.

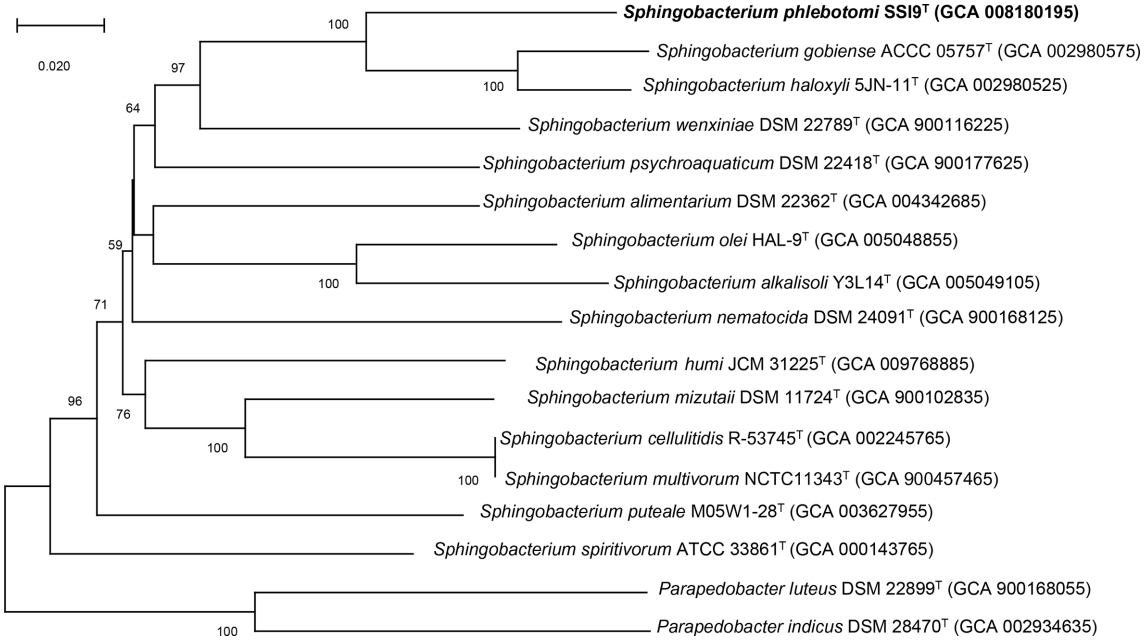


**Fig. 2.** Phylogenetic tree reflecting the phylogenetic position between SSI9$^T$ and type strains of the known species of the genus *Sphingobacterium*. The tree based on the concatenated sequences of core genes *rec*A, *rpl*C and *gro*L was reconstructed by the neighbor-joining method. The type strains *Parapedobacter luteus* DSM 22899$^T$ and *Parapedobacter indicus* DSM 28470$^T$ are included as an outgroup. Bootstrap values ≥50% (based on 1000 replications) are indicated at branch nodes. Bar, 0.02 substitutions per nucleotide position.

rearing colony [17]. Using the serial dilution plating technique, the rearing substrate suspension diluents were spread onto nutrient agar (NA) and tryptic soy Agar (TSA) (BD) plates and incubated at 28 °C for a week. Single colonies were isolated, and the bacteria were repeatedly streaked on TSA medium for purification. The pure cultures obtained on the TSA plates were stored at 4 °C for short-term maintenance and re-streaked every 2–3 weeks. Before use, the strain was routinely cultivated by streaking on TSA and incubating at 28 °C for 48 h. For long-term maintenance, the pure cultures of SSI9$^T$ were preserved at −80 °C using Microbank vials containing porous beads and a specially formulated cryopreservative (Pro-Lab Diagnostics).

Genomic DNA of SSI9$^T$ was extracted using a DNeasy Blood and Tissue extraction kit (Qiagen) according to the manufacturer's instructions. The 16S rRNA gene was amplified using universal 27F and 1492R primers [18] and PCR conditions were as described by Ponnusamy *et al.* [19]. Both strands of the amplicons were Sanger sequenced using five primers, 27F, 520F, 518R, 968F and 1492R at Eton Bioscience (Research Triangle Park, NC, USA) and we obtained a 1396 bp long fragment of the 16S rRNA gene of the novel strain. The 16S rRNA gene sequence of the related type strains were obtained from the EzBioCloud server and GenBank database [20, 21]. Multiple sequence alignments were performed using CLUSTAL_W in MEGA X software [22]. Evolutionary distances were calculated using Kimura's two-parameter model [23], and phylogenetic trees were inferred using maximum-likelihood [24] and neighbor-joining [25] analyses with MEGA X software [22]. Kimura's two-parameter model with complete deletion of gaps/missing data and uniform rates was used in neighbor-joining analysis, and nearest-neighbour-interchange was used in the maximum-likelihood analysis. A bootstrap analysis was performed by 1000 re-samplings to estimate the confidence values of tree topologies [26]. To refine the taxonomic position of SSI9$^T$, sequences of conserved marker genes (*gro*L, *rec*A, and *rpl*C) were retrieved from NCBI whole genome assemblies. and phylogenetic analyses were performed using concatenated sequences with MEGA X [22]. Evolutionary distances were calculated using Kimura's two-parameter model [23], and phylogenetic trees were inferred using maximum-likelihood [24] and neighbor-joining [25] analyses with MEGA X software.

Comparison of the 16S rRNA gene sequence of SSI9$^T$ with the available 16S rRNA gene sequences from GenBank revealed that SSI9$^T$ represents a member of the genus *Sphingobacterium*. It exhibited the highest sequence similarity with *Sphingobacterium haloxyli* 5JN-11$^T$ MG669350 (96.5% homology) [27] and *Sphingobacterium chuzhouense* DH-5$^T$ KT935486 (96.1%) [28], which were lower than the 98.7% threshold for differentiating two bacterial species, as recommended by Stackebrandt and Ebers [29] and recently reinforced by Kim *et al.* [30]. In the neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, SSI9$^T$ formed a separate branch within the genus *Sphingobacterium* (Fig. 1). A similar cluster was observed in a 16S rRNA gene sequence phylogenetic tree derived from the maximum-likelihood tree (Fig. S1, available in the online version of this article). The housekeeping genes

**Table 1.** Comparison table showing the physiological and biochemical properties of SSI9$^T$ and closely related species of the genus *Sphingobacterium*.

Strains: 1, SSI9$^T$; 2, *S. haloxyli* 5JN-11$^T$; 3, *S. chuzhouense* DH-5$^T$; 4, *S. gobiense* H7$^T$; 5, *S. terrae* Brt-F$^T$; 6, *S. arenae* H-12$^T$. Data for strains 1 and 6 are from this study, except for data indicated in parentheses which were taken from literature. Data for 5JN-11$^T$ [27], DH-5$^T$ [28], H7$^T$ [44], Brt-F$^T$ [12], and H-12$^T$ [9], were taken from the respective cited articles. +, Positive; –, negative; ND, no data available; W, weakly positive.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Growth at: | | | | | | |
| 4 °C | – | – | – | – | – | – |
| 42 °C | – | + | – | + | + | – |
| pH 9 | + | + | + | + | + | + |
| Enzyme activities | | | | | | |
| Valine arylamidase | W | – | + | ND | W | – |
| Cystine arylamidase | | – | + | ND | W | – |
| Trypsin | + | + | + | ND | + | – |
| α-Galactosidase | + | – | _ | ND | W | + |
| β-Galactosidase | + | – | _ | ND | _ | + |
| α-Glucosidase | + | – | _ | ND | + | + |
| β-Glucosidase | + | – | _ | ND | + | + |
| β-Glucuronidase | – | W | W | ND | – | – |
| *N*-acetyl-D-glucosaminidase | + | – | + | + | – | – |
| α-Mannosidase | + | – | _ | ND | + | + |
| α-Fucosidase | W | – | | ND | + | – |
| Oxidase | – | + | + | + | + | + |
| Catalase | + | + | + | + | + | + |
| Assimilation of: | | | | | | |
| D-Glucose | + | + | + | + | + | + |
| *N*-acetyl-D-glucosamine | + | + | + | + | + | + |
| Tween 40 | W | – | + | – | ND | – |
| Tween 80 | W | – | W | – | ND | – |
| Dextrin | + | + | + | + | ND | + |
| L-Arabinose | + | W | + | + | + | + |
| D-Fructose | + | + | + | ND | ND | – |
| L-Fucose | W | – | + | – | – | + |
| Melibose | + | W | + | + | + | + |
| Sucrose | + | + | + | + | + | + |
| D-Mannitol | – | – | + | – | – | – |
| DNA G+C content (mol%) | 41.8 | 42.8 | 42.8 | 44.3 | 43.4 | 44.2 |

*rec*A, *rpl*C and *gro*L of SSI9$^T$ showed sequence similarities with the *S. haloxyli* strain 5JN-11$^T$ of 89.6, 93.5, and 87.8%, respectively. Furthermore, the results of concatenated and aligned phylogenetic analysis based on the neighbor-joining tree (Fig. 2) and maximum-likelihood algorithms (Fig. S2), strongly indicated that the novel strain represented a novel species within the genus *Sphingobacterium*.

Genome sequencing was performed on the MiniSeq platform (Illumina) with 150 bp paired-end reads according

**Table 2.** Cellular fatty acid profiles of SSI9$^T$ and phylogenetically closely related species of the genus *Sphingobacterium*

Strains: 1, SSI9$^T$; 2, *S. haloxyli* 5JN-11$^T$; 3, *S. chuzhouense* DH-5$^T$; 4, *S. gobiense* H7$^T$; 5, *S. terrae* Brt-F$^T$; 6, *S. arenae* H-12 $^T$. Values are percentages of total fatty acids. Data for SSI9$^T$ are from this study and data for strains 5JN-11$^T$ [27], DH-5T [28], H7$^T$ [44], Brt-F$^T$ [12], and H-12 $^T$ [9] were taken from the respective cited articles. TR, Trace amount <0.25%; –, no data available or not determined.

| Fatty acid (%) | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $C_{14:0}$ | 0.6 | – | 0.9 | 0.6 | 0.3 | 1.4 |
| iso-$C_{15:1}$F | TR | – | 0.6 | – | TR | – |
| anteiso-$C_{15:0}$ | TR | – | – | 0.6 | 0.6 | 0.4 |
| iso-$C_{15:0}$ | 26.8 | 25.3 | 43.4 | 32.6 | 27.8 | 27.2 |
| $C_{15:1}\omega 6c$ | – | – | – | TR | 0.41 | 0.4 |
| $C_{14:0}$ 2-OH | 0.3 | – | 0.5 | TR | TR | 0.5 |
| $C_{16:1}\omega 5c$ | – | 1.4 | 0.8 | 1.1 | 0.8 | 0.5 |
| $C_{16:0}$ | 7.7 | – | 7.5 | 3.3 | 4.8 | 7.3 |
| iso-$C_{16:0}$ | – | 4.1 | – | – | – | – |
| iso-$C_{17:0}$ | 0.3 | – | 1.1 | TR | 0.4 | – |
| iso-$C_{15:0}$ 3-OH | 3.1 | 1.9 | 1.4 | 2.4 | 3.1 | 3.1 |
| iso-$C_{16:0}$ 3-OH | 0.3 | – | – | 0.3 | TR | 0.3 |
| $C_{16:0}$ 3-OH | 2.1 | 1.5 | 1.9 | 2.2 | 1.0 | 4.8 |
| iso-$C_{17:0}$ 3-OH | 16.5 | 9.4 | 14.4 | 18.1 | 19.1 | 15.4 |
| Summed feature 1* | TR | – | 1.2 | TR | – | TR |
| Summed feature 2* | TR | – | – | – | – | 0.3 |
| Summed feature 3* | 38.3 | 48.1 | 20.2 | 36.2 | 36.3 | 37.8 |
| Summed feature 4* | 0.3 | 0.8 | 0.9 | 0.4 | 0.4 | – |
| Summed feature 8* | 0.9 | – | – | – | 1.3 | – |
| Summed feature 9* | 1.3 | 2.2 | 2.8 | 1.2 | 2.1 | 0.6 |

*Summed feature 1 comprises $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$h; Summed feature 2 comprises $C_{14:0}$ 3-OH and/or $C_{16:1}$iso I; summed feature 3 comprises $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$; Summed feature 4 contained iso-$C_{17:1}$ I/anteiso-$C_{17:1}$, Summed feature 8 comprises $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$ and summed feature 9 contained $C_{16:0}$ 10- methyl and/or iso-$C_{17:1}$ $\omega$9c.

to the manufacturer's instructions at the Sequencing Centre, Fort Collins Colorado, USA. Sequence reads were quality-filtered with FastQC (version 0.11.5) and assembled using SPAdes-3.11.1 [31]. The draft genome of SSI9$^T$ was annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [32] and the Rapid Annotations using Subsystems Technology (RAST) server [33]. To determine the Digital DNA–DNA hybridization (dDDH) values, the genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available at https://tygs.dsmz.de, for a whole genome-based taxonomic analysis [34]; DNA G+C content was calculated using GGDC 2.1 [35]. In order to calculate the average amino acid identity (AAI) between SSI9$^T$ and *S. haloxyli* 5JN-11$^T$ [27], the online calculator from the Konstantinidis group (http://enve-omics.ce.gatech.edu/aai/) was used [36].

The draft genome of SSI9$^T$ consists of 69 scaffolds with a genome size of 5197142 bp. A total of 4395 genes, including 4290 protein-coding and 57 RNA genes were identified (Table S1). The RAST annotation identified 236 subsystems with amino acids and derivatives as the dominant (19.3%) category. The distribution of the genes into clusters of orthologous groups (COGs) functional categories is presented in Fig. S3. The DNA G+C content of strain SSI9$^T$ was calculated as 41.8 mol% based on the draft genome assembly, whereas *S. haloxyli* 5JN-11$^T$ has a G+C content of 42.8 mol%. The dDDH value between SSI9$^T$ and *S. haloxyli* 5JN-11$^T$ was 25.1% (Formula 2) and the AAI value calculated was 84.4%. All of these values are well below the commonly accepted AAI/dDDH cut-off values for species delineation [37, 38] supporting the hypothesis that SSI9$^T$ represents a novel species within the genus *Sphingobacterium*.

Cell morphology was observed using light microscopy and scanning electron microscopy. For scanning electron microscopy imaging, 2-day-old cultures from TSA plates were collected, pelleted and cells were fixed with 6% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 6.8) at 4 °C for 24 h. Cells were then triple washed with 0.1 M sodium cacodylate buffer (pH 6.8) at 4 °C for 30 min. Following rinsing, the bacterial cells were dehydrated with successive 30, 50, 70, 95 and 100% ethanol (v/v) washes. The sample was critical point dried using liquid carbon dioxide. Filters were quartered, secured to stubs and placed in a desiccator. Finally, samples were sputter coated with 50 Å of Au/Pd, stored in a desiccator and examined under a 5900LV SEM (JEOL) at 20 kV. SSI9$^T$ is a rod-shaped bacteria, 0.8–1.1 µm long and 0.25–0.45 µm wide (Fig. S4).

To investigate the physiological and biochemical characteristics, SSI9$^T$ was routinely cultivated on TSA media or in broth at 28 °C for 2 days under aerobic conditions. Aerobic growth of SSI9$^T$ was monitored on six different media, TSA, R2A, NA, brain heart infusion (BHI) agar, LB agar and MacConkey agar, incubated at 28 °C for 3 days. To determine the aerotolerance of SSI9$^T$, it was incubated at 28 °C for 72 h in fluid thioglycollate medium (FTM; Difco). Growth of SSI9$^T$ was monitored at different temperatures between 4 and 42 °C on TSA medium. Salinity tolerance of SSI9$^T$ was tested in TSA supplemented with 0–7% NaCl (w/v) at 28 °C and growth at the pH range of 3–10 was examined on TSA medium. For hydrolysis experiments, cultures were grown on basal media supplemented with starch or gelatin, incubated at 28 °C for 48 h and observed for presence of a clearing zone. Iodine was added to the plates to detect starch hydrolysis. Gram staining was carried out using a BD BBL Gram staining kit (Fisher Scientific) as per the manufacturer's protocol. Catalase activity was tested by adding 3% $H_2O_2$ (v/v) to a freshly cultured cell smear and observing for gas production. Oxidase activity was evaluated by smearing freshly cultured cells onto filter

paper discs wetted with Gordon-McLeod reagent (Sigma). Motility testing was conducted by stabbing the bacteria into the medium (Motility medium, catalogue number: L97921; Fisher Scientific), incubating for 24–48 h and examining for extension of the pink colour and colony growth. Assimilation of various carbon sources by SSI9$^T$ was assessed by incubating fresh cultures of SSI9$^T$ with the GN2 Microplate system (Biolog) as per the manufacturer's protocol. The results were recorded at multiple time points (4, 6, and 24 h) with the naked eye and the analysis was replicated three times. Additional enzyme activities and biochemical properties were examined using API ZYM, following the manufacturer's instructions (Biomerieux). Antibiotic susceptibility of SSI9$^T$ was tested using disc diffusion assays on TSA medium at 28 °C. The antibiotics (Fisher Scientific) tested were ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), oxytetracycline (30 µg), penicillin (10 units), streptomycin (10 µg) and tetracycline (30 µg). Discs with no antibiotics were used as controls and the assays were replicated three times.

Cells of SSI9$^T$ are Gram-stain-negative, rod shaped (Fig. S4), and non-motile. The bacterium grows optimally on TSA medium at between 28 °C and 30 °C under aerobic conditions. SSI9$^T$ is an obligate aerobe, as determined by incubation in thioglycollate medium. The colony colour of SSI9$^T$ is beige when grown on TSA medium for 48 h and it forms smooth colonies with substantial exopolysaccharide production. SSI9$^T$ also grows well on R2A, NA, BHI agar and LB agar, but not on MacConkey agar. Growth was observed at a temperature range of 10–40 °C (optimal between 20 and 37 °C), but no growth was detected at ≤4 and ≥42 °C. The pH range for growth is 7–10 and the optimum pH is 7.0. The novel bacterium is salt tolerant up to 6% (0–6%) NaCl (w/v) in the TSA medium. It is positive for catalase activity and negative for oxidase activity and no hydrolysis of starch or gelatin was observed. In antibiotic assays, SSI9$^T$ was sensitive to tetracycline and oxytetracycline, but resistant to kanamycin, chloramphenicol, erythromycin, penicillin, ampicillin and streptomycin. SSI9$^T$ showed several distinct physiological and biochemical characteristics that distinguished it from the most closely related species *S. haloxyli* 5JN-11$^T$ and other members of the genus *Sphingobacterium*. The detailed results from the phenotypic and biochemical analyses are summarized in Table 1 and in the species description.

To analyse the whole cellular fatty acid composition, SSI9$^T$ was grown aerobically on TSA medium at 28 °C until the mid-exponential phase and the fatty acids were extracted, saponified and methylated using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6). Fatty acid methyl esters were then analysed by GC (model 6890; Hewlett Packard) and identified using the RTSBA6 database of the Sherlock Microbial Identification System as described by Sasser [39]. Polar lipids and respiratory quinones of SSI9$^T$ were analysed by the Identification Service, Leibniz_ Institut DSMZ (Braunschweig, Germany). Polar lipids were extracted from 100 mg of lyophilized SSI9$^T$ cell material using a choroform:methanol:0.3% aqueous NaCl mixture (modified after the method of Bligh and Dyer [40]). Polar lipids extracted in the chloroform phase were then separated by two dimensional silica gel thin layer chromatography (TLC). In TLC, the first direction was developed in chloroform:methanol:water, and the second in chloroform:methanol:acetic acid:water. Total lipid material was detected using molybdatophosphoric acid and functional groups were detected using spray reagents specific for defined functional groups [41]. Respiratory quinones were extracted using methanol:hexane [42, 43], followed by phase separation into hexane.

The cellular fatty acids profiles of SSI9$^T$ together with its closest phylogenetic neighbours are shown in Table 2. The data indicate that the major cellular fatty acids of SSI9$^T$ are $C_{16:1}\omega6c/C_{16:1}\omega7c$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and $C_{16:0}$. The polar lipids found in SSI9$^T$ were phosphatidylethanolamine, two unknown glycolipids, two unidentified phosphoglycoplipids, and three unidentified lipids (Fig. S5). Menaquinone-7 (MK-7) is the predominant isoprenoid quinone detected in SSI9$^T$. The overall fatty acids profile of SSI9$^T$ was similar to those of the reference taxa of the genus *Sphingobacterium*, but there were some differences in the respective proportions of some fatty acid components (Table 2). For example, iso-$C_{17:0}$ was detected from SSI9$^T$, but not in the reference strain *S. haloxyli* 5JN-11$^T$. In addition, $C_{16:0}$ was detected from SSI9$^T$, but it was absent in *S. haloxyli* 5JN-11$^T$ (Table 2). Phosphatidylethanolamine and MK-7, the predominant polar lipid and isoprenoid respiratory quinone, respectively, detected in SSI9$^T$ are consistent with the phenotypes of other species of the genus *Sphingobacterium*.

In summary, the novel strain SSI9$^T$ was found to be closely related to *S. haloxyli* 5JN-11$^T$ on the basis of the results of phylogenetic analysis of 16S rRNA gene sequences. The results of genomic analysis of the AAI genes also indicated that SSI9$^T$ was separated from *S. haloxyli* 5JN-11$^T$ and other members of the genus *Sphingobacterium*. Furthermore, the strain showed absence of oxidase enzyme activities; in contrast, *S. haloxyli* 5JN-11$^T$ is positive for oxidase. On the basis of the results of molecular analysis (16S rRNA gene sequence similarity, whole genome sequence analysis and DNA G+C content), phenotypic features, phylogenetic inference and genomic differences, it is proposed to assign strain SSI9$^T$ to a novel species within the genus *Sphingobacterium*, for which the name *Sphingobacterium phlebotomi* sp. nov. is proposed.

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

*Sphingobacterium phlebotomi* (phle.bo.to′mi. N.L. gen. n. *phlebotomi* of *Phlebotomus*, as the organism was isolated from *Phlebotomus* rearing substrate).

Cells are Gram-stain-negative, aerobic, non-motile, rod shaped (0.8–1.1 µm long and 0.25–0.45 µm wide) and oxidase-negative. Colonies on TSA are beige, smooth, circular, convex and 2–4 mm in diameter after 2 days of incubation. Optimal growth occurs at 20–37 °C, pH 7.0–10 (optimum pH 7.0) and 0–6.0% (w/v) NaCl (optimum 0–4.0%). Grows on NA,

R2A and TSA but not on MacConkey agar. On the API ZYM kit, positive for alkaline phosphatase, esterase lipase (C8), leucine acrylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, weakly positive for valine arylamidase and α-fucosidase activity, but negative for esterase (C4), lipase (C14), cystine arylamidase, chymotrypsin and β-glucuronidase activity. Assimilates α-cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, D-mannose, melibiose, β-methyl-D-glucoside, raffinose, sucrose, trehalose, turanose, pyruvic acid methyl ester, succinic acid monomethyl ester, DL-lactic acid and L-threonine. Cannot utilize D-arabitol, *meso*-erythritol, *myo*-inositol, D-mannitol, L-rhamnose, D-sorbitol, xylitol, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid, lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-, β- and γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D- and L-alanine, L- alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D- and L-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-amino ethanol, 2,3-butanediol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. However, the assimilation of glycogen, Tween 40, Tween 80, L-fucose, D-psicose, acetic acid and L-asparagine are weak and not clear. Has phosphatidylethanolamine as a major polar lipid, MK-7 as the predominant quinone, and $C_{16:1}\omega6c/C_{16:1}\omega7c$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and $C_{16:0}$ as the major cellular fatty acids.

The type strain is SSI9$^T$ (=ATCC TSD-210$^T$=LMG 31664$^T$=NRRL B-65603$^T$). The GenBank accession number for the 16S rRNA gene sequence of *Sphingobacterium phlebotomi* sp. nov. SSI9$^T$ is MN032123. The GenBank accession number for the whole genome sequence is VTAV00000000.

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

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