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

Madhavi L. Kakumanu1, Bahjat Fadi Marayati2, Ayako Wada-Katsumata1, Gideon Wasserberg3, Coby Schal1,3, Charles S. Apperson1,3 and Loganathan Ponnusamy1,3,*

Abstract

A Gram-stain-negative, rod-shaped, non-motile, non-spore-forming, aerobic bacterium, designated type strain SSI97T, was isolated from sand fly (Phlebotomus papatasi Scopoli; Diptera: Psychodidae) rearing substrate and subjected to polyphasic taxonomic analysis. Strain SSI97 contained phosphatidylethanolamine as a major polar lipid, MK-7 as the predominant quinone, and C16:1ω6c/C16:ω7c, iso-C15:0 2-OH, and C16:0 3-OH as the major cellular fatty acids. Phylogenetic analysis based on 16S rRNA gene sequences revealed that SSI97 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 recA, rplC and groL indicated that SSI97T formed a separate branch in the genus Sphingobacterium. The genome of SSI97T 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. SSI97T could be distinguished from other species of the genus Sphingobacterium with validated published names by several phenotypic, chemotaxonomic and genomic characteristics. Based on 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 SSI97T (=ATCC TSD-210T=LMG 31664T=NRRL B-65603T).

The genus Sphingobacterium was first described by Yabuuchi et al. [1] and classified as part of the family Sphingobacteriaceae, 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 validated 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 SSI97, 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 SSI97 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...
Fig. 1. Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences (1385 bp) showing the relationship of strain SS19 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.
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 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 (groL, recA, and rplC) 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

![Phylogenetic tree reflecting the phylogenetic position between SSI9 T and type strains of the known species of the genus Sphingobacterium.](image-url)
recA, rplC and groL of SSI9T showed sequence similarities with the S. haloxyli strain 5JN-11T 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 SS19T and phylogenetically closely related species of the genus Sphingobacterium

| Fatty acid (%) | 1   | 2   | 3   | 4   | 5   | 6   |
|----------------|-----|-----|-----|-----|-----|-----|
| C14:0          | 0.6 | 0.9 | 0.6 | 0.3 | 1.4 |
| iso-C15:0      | TR  | 0.6 | TR  |     |     |     |
| anteiso-C15:0  | TR  |     | 0.6 | 0.6 | 0.4 |
| iso-C15:0      | 26.8| 25.3| 43.4| 32.6| 27.8| 27.2|
| C15:0 3-OH     | –   | –   | 1.4 |     | 0.8 | 0.5 |
| C14:0 2-OH     | 0.5 | TR  |     |     |     |     |
| C16:0 3-OH     | 1.4 | 0.8 | 1.1 | 0.8 | 0.5 |
| C16:0          | 7.7 | 7.5 | 3.3 | 4.8 | 7.3 |
| iso-C16:0      | –   | 4.1 | –   | –   | –   | –   |
| iso-C17:0      | 0.3 | 1.1 | TR  | 0.4 |     | –   |
| iso-C15:0,3-OH | 3.1 | 1.9 | 1.4 | 2.4 | 3.1 | 3.1 |
| iso-C16:0,3-OH | 0.3 | –   | –   | 0.3 | TR  | 0.3 |
| C16:0,3-OH     | 2.1 | 1.5 | 1.9 | 2.2 | 1.0 | 4.8 |
| iso-C17:0,3-OH | 16.5| 9.4 | 14.4| 18.1| 19.1| 15.4|
| Summed feature 1* | TR | – | 1.2 | TR  | – | – |
| Summed feature 2* | TR | – | 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_{15:0} 3-OH and/or iso-C_{15:0} h; Summed feature 2 comprises C_{14:0} 3-OH and/or C_{15:0} iso l; Summed feature 3 comprises C_{14:0} omega7c and/or C_{15:0} omega6c; Summed feature 4 contained iso-C_{17:0} l/anteiso-C_{17:0} t; Summed feature 8 comprises C_{16:0} omega7c and/or C_{16:0} omega6c and summed feature 9 contained C_{16:0} 10- methyl and/or iso-C_{17:0} omega9c.

The draft genome of SS19T 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 SS19T was calculated as 41.8 mol% based on the draft genome assembly, whereas S. haloxyli 5JN-11T has a G+C content of 42.8 mol%. The dDDH value between SS19T and S. haloxyli 5JN-11T 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 supporting the hypothesis that SS19T 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. SS19T 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, SS19T was routinely cultivated on TSA media or in broth at 28 °C for 2 days under aerobic conditions. Aerobic growth of SS19T 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 aero-tolerance of SS19T, it was incubated at 28 °C for 72 h in fluid thioglycollate medium (FTM; Difco). Growth of SS19T was monitored at different temperatures between 4 and 42 °C on TSA medium. Salinity tolerance of SS19T 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% H2O2 (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 SSI9T was assessed by incubating fresh cultures of SSI9T 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 SSI9T 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 SSI9T 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. SSI9T is an obligate aerobe, as determined by incubation in thioglycollate medium. The colony colour of SSI9T is beige when grown on TSA medium for 48 h and it forms smooth colonies with substantial exopolysaccharide production. SSI9T grows on NA, NBHI 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, SSI9T was sensitive to tetracycline and oxytetracycline, but resistant to kanamycin, chloramphenicol, erythromycin, penicillin, ampicillin and streptomycin. SSI9T showed several distinct physiological and biochemical characteristics that distinguished it from the most closely related species S. haloxyli 5JN-11T 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, SSI9T 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 SSI9T were analysed by the Identification Service, Leibniz Institut DSMZ (Braunschweig, Germany). Polar lipids were extracted from 100 mg of lyophilized SSI9T cell material using a chloroform: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 SSI9T together with its closest phylogenetic neighbours are shown in Table 2. The data indicate that the major cellular fatty acids of SSI9T are C16:0, C16:1ω6c/C16:1ω7c, iso-C15:0 3-OH and C16:0. The polar lipids found in SSI9T were phosphatidyethanolamine, two unknown glycolipids, two unidentified phosphoglycolipids, and three unidentified lipids (Fig. S5). Menaquinone-7 (MK-7) is the predominant isoprenoid quinone detected in SSI9T. The overall fatty acids profile of SSI9T 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-C17:0 was detected from SSI9T, but not in the reference strain S. haloxyli 5JN-11T. In addition, C16:0 was detected from SSI9T, but it was absent in S. haloxyli 5JN-11T (Table 2). Phosphatidylethanolamine and MK-7, the predominant polar lipid and isoprenoid respiratory quinone, respectively, detected in SSI9T are consistent with the phenotypes of other species of the genus Sphingobacterium.

In summary, the novel strain SSI9T was found to be closely related to S. haloxyli 5JN-11T 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 SSI9T was separated from S. haloxyli 5JN-11T and other members of the genus Sphingobacterium. Furthermore, the strain showed absence of oxidase enzyme activities; in contrast, S. haloxyli 5JN-11T 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 SSI9T 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 NA 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,
The type strain is SS19\(^\text{T}\) (=ATCC TSD-210\(^\text{T}\)=LMG 31664\(^\text{T}\)=NRRL B-65603\(^\text{T}\)). The GenBank accession number for the 16S rRNA gene sequence of *Sphingobacterium phlei-botomi* sp. nov. SS19\(^\text{T}\) is MN032123. The GenBank accession number for the whole genome sequence is VTAV00000000.

Funding information

This work was supported by the National Institute of Allergy and Infectious Diseases, NIH (1R01AI123327-01) and the North Carolina Biotechnology Centre Multidisciplinary Research Grant (MRG) number 1104.

Acknowledgements

We acknowledge the Identification Service of the DSMZ in Germany for respiratory quinone analysis, the Microbial ID for fatty acid analysis and The Sequencing Centre, Colorado, for genome sequencing analysis.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Yabuuchi E, Kaneko T, Yano I, Moss CW, Miyoshi N. *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium mutilorum* comb. nov., *Sphingobacterium mizutaii* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting Gram-negative rods in CDC groups Ii-2 and Iib. *Int J Syst Bacteriol* 1983;33:580–598.
2. Wauters G, Janssens M, De Baere T, Vaneechoute M, Deschaght P. Isolates belonging to CDC group II-i belong predominantly to *Sphingobacterium mizutaii* Yabuuchi et al. 1983: emended descriptions of *S. mizutaii* and of the genus *Sphingobacterium*. *Int J Syst Evol Microbiol* 2012;62:2598–2601.
3. Lee D-H, Hur JS, Kahng H-Y. *Sphingobacterium cladoae* sp. nov., isolated from lichen, *Cladonia* sp., and emended description of *Sphingobacterium siyangense*. *Int J Syst Evol Microbiol* 2013;63:755–760.
4. Sun L-N, Zhang J, Chen Q, He J, Li S-P. *Sphingobacterium caeni* sp. nov., isolated from activated sludge. *Int J Syst Evol Microbiol* 2013;63:2260–2264.
5. Yoo S-H, Weon H-Y, Jang H-B, Kim BY, Kwon S-W et al. *Sphingobacterium* compositi sp. nov., isolated from cotton-cotton waste composts. *Int J Syst Evol Microbiol* 2007;57:1590–1593.
6. Yabe S, Aiba Y, Sakai Y, Hazaka M, Kawahara K et al. *Sphingobacterium* thermophilum sp. nov., of the phylum *Bacteroidetes*, isolated from compost. *Int J Syst Evol Microbiol* 2013;63:1584–1588.
7. Siddiqui MQ, Muhammad Shafi S, Choi KD, Im W-T, Aslam Z. *Sphingobacterium jejuense* sp. nov., with ginsenoside-converting activity, isolated from compost. *Int J Syst Evol Microbiol* 2016;66:4433–4439.
8. Albert RA, Waas NE, Pavlons SC, Pearson JL, Ketelboeter L et al. *Sphingobacterium* psychraquaticum sp. nov., a psychrophilic bacterium isolated from Lake Michigan water. *Int J Syst Evol Microbiol* 2013;63:952–958.
9. Jiang S, Chen M, Su S, Yang M, Li A et al. *Sphingobacterium* arenace sp. nov., isolated from sandy soil. *Int J Syst Evol Microbiol* 2014;64:248–253.
10. Xiao T, He X, Cheng G, Kuang H, Ma X et al. *Sphingobacterium* hotanense sp. nov., isolated from soil of a *Populus euphratica* forest, and emended descriptions of *Sphingobacterium daejeonense* and *Sphingobacterium shayense*. *Int J Syst Evol Microbiol* 2013;63:815–820.
11. Liu B, Yang X, Sheng M, Yang Z, Qiu J et al. *Sphingobacterium* olei sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* 2020;70:1931–1939.
12. Chaudhary DK, Kim J. *Sphingobacterium* terrae sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* 2018;68:609–615.
13. Liu J, Yang L-L, Xu C-K, Xi J-Q, Yang F-X et al. *Sphingobacterium* nematodaica sp. nov., a nematicidal endophytic bacterium isolated from tobacco. *Int J Syst Evol Microbiol* 2012;62:1809–1813.
14. Li Y, Xu G-T, Chang J-P, Guo L-M, Yang X-Q et al. *Sphingobacterium* corticus sp. nov., isolated from bark of *Populus × euramericana* from *euamericana*. *Int J Syst Evol Microbiol* 2017;67:3860–3864.
15. Li Y, Guo L-M, Chang J-P, Yang X-Q, Xie S-J et al. *Sphingobacterium* corticibacter sp. nov., isolated from bark of *Populus × euramericana*. *Int J Syst Evol Microbiol* 2019;69:1870–1874.
16. Schmidt VS, Wenning M, Scherer S, sp Siactis. *Sphingobacterium lactis* sp. nov. and *Sphingobacterium alimentarium* sp. nov., isolated from raw milk and a dairy environment. *Int J Syst Evol Microbiol* 2012;62:1506–1511.
17. Marayati BF, Schal C, Ponnusamy L, Apperson CS, Rowland TE et al. Attraction and oviposition preferences of *Phlebotomus papatasii* (Diptera: Psychodidae), vector of Old-World cutaneous leishmaniasis, to larval rearing media. *Parasit Vectors* 2015;8:663.
18. Lane D. 16S/23S rRNA sequencing. London, UK: John Wiley and Sons; 1991.
19. Ponnusamy L, Xu N, Nojima S, Wesson DM, Schal C et al. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proc Natl Acad Sci U S A* 2008;105:9262–9267.
20. Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with
phytotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.

21. Benson D, Karsch-Mizrachi I, Lipman D, Ostell J. Genbank. 2009. *Nucleic Acids Res* 2009:D26–D31.

22. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.

23. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.

24. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.

25. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.

26. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.

27. Liu L, Hui N, Liang L-X, Zhang X-X, Li L-B et al. *Sphingobacterium haloxyl* sp. nov., an endophytic bacterium isolated from *Haloxylon ammodendron* stems in Kumtag desert. *Int J Syst Evol Microbiol* 2018;68:3279–3284.

28. Wang X, Zhang C-F, Yu X, Hu G, Yang H-X. *Sphingobacterium chuzhouense* sp. nov., isolated from farmland soil. *Int J Syst Evol Microbiol* 2016;66:4968–4974.

29. Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006;33:152–155.

30. Kim M, Oh H-S, Park S-C, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.

31. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.

32. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufo S. Prokaryotic genome annotation pipeline. *The NCBI Handbook [Internet], 2nd edition*. US: National Center for Biotechnology Information; 2013.

33. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T et al. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75–15.

34. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.

35. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.

36. Rodriguez-R LM, Konstantinidis KT. Bypassing cultivation to identify bacterial species. *Microbe* 2014;9:111–118.

37. Oren A. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2006;56:1521–1525.

38. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR et al. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.

39. Grazziotin AM, Reis CA, Correa OE, Aguiar GM, Pereira FA et al. DNA–DNA reassociation and 16S rRNA gene sequence similarities of the type strains of *Nocardioides chlorophialae* sp. nov. and *Nocardioides chlorophialae* sp. nov. *Syst Appl Microbiol* 2010;33:399–405.

40. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.

41. Tindall BJ, Sikorski J, Smibert RA, Krieg NR. Phenotypic characterization and the principles of comparative systematics. *Methods General Mol Microbiol* 2007;330–393.

42. Tindall BJ. A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.

43. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.

44. Zhao P, Zhou Z, Chen M, Lin W, Zhang W et al. *Sphingobacterium gobiense* sp. nov., isolated from soil of the Gobi Desert. *Int J Syst Evol Microbiol* 2014;64:3931–3935.