**Sphingobacterium cellulitidis** sp. nov., isolated from clinical and environmental sources

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**Abstract**

The taxonomic position of two isolates belonging to the genus *Sphingobacterium* was determined. The first isolate, R-53603T, was obtained from purulent discharge from the toe of a cellulitis patient in Kuwait. Comparative 16S rRNA gene sequence analysis revealed 99.87% similarity of R-53603 with environmental isolate P031 (=R-53745) originating from activated sludge in Singapore. The two isolates were phylogenetically positioned on the same sub-branch. Highest 16S rRNA gene sequence similarity was found with the type strains of *Sphingobacterium mizutaii* (98.23%), *Sphingobacterium lactis* (97.78%) and *Sphingobacterium daejeonense* (97.14%). DNA–DNA hybridizations revealed <70% relatedness between the two isolates and the type strains of the close phylogenetic neighbours *S. mizutaii* (18.0–24.5%), *S. lactis* (20.3–25.9%) and *S. daejeonense* (13.2–20.0%). The high relative contribution of iso-C15:0 3-OH and summed feature 3 (iso-C15:0 2-OH and/or C16:1 ω7c) in the cellular fatty acid profiles of R-53603 and R-53745, the presence of sphingophospholipids, MK-7 as the dominant menaquinone and phosphatidylethanolamine as the major polar lipid in strain R-53603T are typical chemotaxonomic characteristics for members of the genus *Sphingobacterium*. Phenotypic features most useful for differentiation of the two novel strains from the most closely related species *S. mizutaii* include growth on MacConkey agar, and utilization of stachyose, guanidine HCl and lithium chloride in Biolog GEN III tests. Strains R-53603T and R-53745 thus represent a novel species, for which the name *Sphingobacterium cellulitidis* sp. nov. is proposed. The type strain is R-53603T (=LMG 28764T =DSM 102028).

Cellulitis is one of the most frequent forms of skin and soft tissue infections worldwide [1]. For many decades, cases of cellulitis have been mainly attributed to β-haemolytic streptococci [2]. However, recent reviews of cellulitis reports revealed that the share of Gram-negative organisms in positive skin cultures is more significant than previously thought [3, 4].

Two short case descriptions of cellulitis have reported the isolation of *Sphingobacterium*, a Gram-negative organism ubiquitous in soil and aquatic ecosystems that has been isolated from clinical specimens only in very rare cases [5]. The first case concerned an elderly man from the USA with sepsis and cellulitis from whose blood cultures yielded *Sphingobacterium spiritivorum* [6]. The infection was probably acquired by walking barefoot in his backyard. In a second report, a strain representing a possible novel *Sphingobacterium* species most closely resembling *Sphingobacterium mizutaii* was isolated from foot cellulitis in an elderly man from France [7]. Also in this case, the source of infection was probably environmental, suggesting that the bacterium may behave as an opportunistic pathogen and produce infection in compromised patients.

In the present study, we report another rare case of cellulitis caused by an unclassified *Sphingobacterium* strain in Kuwait. Comparative analysis of its 16S rRNA gene sequence indicated a close relationship with an environmental isolate from an activated sludge sample from Singapore which was also included in this investigation. Detailed taxonomic characterization indicated that the two isolates are members of a novel species most closely related to *S. mizutaii*.

A 23-year-old Kuwaiti female with a past history of recurrent skin infections presented in May 2013 to the...
Assad Al-Hamad Center for Dermatology, Al-Sabah Hospital, Kuwait. The patient showed scaly and erythematous lesions on the dorsa of both feet, presumptively diagnosed as psoriatic lesions. The patient was prescribed with calcipotriol/betamethasone dipropionate ointment for local application and cetrizine 10 mg orally. The patient returned after 3 weeks with extensive erythema and purulent discharge from the base of the right big toe. The patient was treated with 1 g oral amoxicillin/clavulanate for 7 days, after which the condition of the feet ameliorated and resulted in entire clearing of the lesions. Prior to antibiotic treatment, swabs from purulent discharge from the base of the right big toe were collected and processed for direct microscopy, and bacteriological and mycological culture. The swabs were inoculated on one or two plates of 5 % sheep blood agar (BA), chocolate agar (CA), MacConkey agar (MA), Sabouraud dextrose agar (SDA) (all from Oxoid) and BA with gentamicin (GBA). Plates of BA, MA and SDA were incubated aerobically at 37 °C. Second plates of BA and GBA were incubated anaerobically for 5 days at 37 °C. A second SDA plate was incubated at room temperature for 10 days. The CA plate was incubated in 3–10 % carbon dioxide at 37 °C. Pure growth of pale, greyish colonies (2–5 mm) on BA and CA resulted in the recovery of clinical isolate R-53603T (=LMG 28764T). After initial 16S rRNA gene sequence comparison of LMG 28764T via the BLAST program, a second isolate (GenBank accession no. KC252768) showing very close phylogenetic affiliation with the cellulis isolate was later also included in the study. This second environmental isolate P031 (=R-53745=LMG 28765) was recovered in 2012 from a bioreactor system undergoing simultaneous nitrification, denitrification and phosphorus removal processes that was seeded with a floccular sludge community from a water reclamation plant in Ulu Pandan, Singapore [8]. Strain R-53745 was originally isolated on nutrient agar (NA; Oxoid) at 25 °C.

The type strains S. mizutaii LMG 8340T, Sphingobacterium daejeonense LMG 23402T and Sphingobacterium canadense LMG 23726T used for DNA–DNA hybridizations, fatty acid analyses, (GTG)5-PCR fingerprinting and/or phenotypic characterization were obtained from the BCCM/LMG Bacteria Collection, Ghent University, Gent, Belgium.

Whole genome sequencing of isolates R-53603T and R-53745 was performed on an Illumina NextSeq 500 instrument using Nextera XT library preparation and 2×150 bp paired-end sequencing chemistry. Sequence reads were de novo assembled using SPAdes v3.7.1 [9]. The 16S rRNA gene sequence of the two isolates was determined from the assembled contig sequences using RNAmmer v1.2 [10]. Sequence similarity was determined using the identification service of the EzTaxon server [11]. Alignment with CLUSTAL W and phylogenetic analysis were performed using MEGA v7.0 [12] according to the Tamura–Nei model with elimination of all positions with less than 95 % site coverage and clustering with the maximum-likelihood [13], neighbour-joining [14] and minimum-evolution [15] methods, supported with bootstrap values based on 500 replications.

A BLAST analysis of the full-length 16S rRNA gene sequence (accession no. KU243696, 1533 nt) of clinical isolate R-53603T indicated highest similarity with a sequence previously determined for the environmental isolate R-53745 (accession no. KC252768, 1533 nt). The two isolates shared 99.87 % sequence identity, and showed highest sequence similarity to the type strain of S. mizutaii (98.23 % with R-53603T, accession no. AJ438175) followed by Sphingobacterium lactis DSM 22361T (97.78 % with R-53603T, accession no. FN908501) and S. daejeonense LMG 23402T (97.14 % with R-53603T, accession no. AB249372), and less than 97 % similarity to the type strains of other Sphingobacterium species. A phylogenetic tree based on the maximum-likelihood method was reconstructed based on 16S rRNA gene sequences of the majority of Sphingobacterium species with validly published names (Fig. 1), and showed that both strains are phylogenetically positioned on the same sub-branch within that genus. This topology was confirmed by the neighbour-joining and minimum-evolution trees (Figs S1 and S2, available in the online Supplementary Material).

Repetitive DNA element PCR fingerprinting targeting the (GTG)5 element [(GTG)5-PCR] was performed on isolates R-53603T and R-53745 and the type strains of the two most closely related Sphingobacterium species using genomic DNA extracted as previously described [16]. (GTG)5-PCR fingerprints were generated essentially as previously described [17, 18]. Visual inspection of the fingerprints indicated that isolates R-53603T and R-53745 are genotypically different and thus represent two different strains (Fig. S3).

For DNA–DNA hybridization, total genomic DNA was prepared from strains R-53603T and R-53745 and from the type strains S. mizutaii LMG 8340T and S. daejeonense LMG 23402T using a combination of the protocols of Marmur [19] and Pitcher et al. [16] as described previously [20]. Total DNA of S. lactis DSM 22361T was prepared from a crude lysate according to the method of Cashon and co-workers [21]. Except for the combination R-53603T×S. lactis DSM 22361T, all DNA–DNA hybridizations were performed with biotin-labelled probes in microplate wells according to Ezaki et al. [22] with modifications by Goris et al. [20] using an HTS7000 Bio Assay Reader (Perkin Elmer) for fluorescence measurements. The hybridization temperature was 36 °C, in the presence of 50 % formamide. Reciprocal experiments were performed for every pair of strains and standard deviations ranged from 1 to 12 %. Hybridizations were repeated three times and means of the resulting values were determined. Hybridization between R-53603T and S. lactis DSM 22361T was carried out in 2× SSC at 68 °C as described by De Ley et al. [23] under consideration of the modifications described by Huss et al. [24], using a model Cary 100 Bio UV/VIS-
spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with in situ temperature probe (Varian). A high DNA–DNA relatedness (89.7–95.8 %) was found between isolates R-53603 and R-53745. In contrast, a very low relatedness was obtained in hybridizations of both isolates with the close phylogenetic neighbours S. mizutaii LMG 8340T (18.0–24.5 %), S. lactis DSM 22361T (20.3–25.9 %), only with R-

![Fig. 1.](image-url)
53603T) and S. daejeonense LMG 23402T (13.2–20.0 %). These values are far below the threshold value of 70 % accepted for the delineation of a novel bacterial species [25].

The DNA G+C contents of isolates R-53603T and R-53745 were determined using the enzymatic degradation method described by Mesbah et al. [26]. The nucleoside mixture was separated by HPLC using a Waters SymmetryShield C8 column maintained at 37 °C. The solvent was 0.02 M (NH₄)₂HPO₄ (pH 4.0) with 1.5 % acetonitrile. Non-methylated λ-phage DNA (Sigma) was used as the calibration reference. The DNA G+C content of strains R-53603T and R-53745 was 37.3 and 36.9 mol%, respectively, which is within the DNA G+C content range of members of the genus Sphingobacterium (36.6–44.4 mol%) [27–29].

Whole-cell fatty acid composition was determined for isolates R-53603T and R-53745 using an Agilent Technologies 6890 N gas chromatograph. Cultivation of the isolates, and extraction and analysis of the fatty acid methyl esters were performed according to the recommendations of the Microbial Identification System, Sherlock version 3.10 (MIDI). Cells were harvested from cultures grown on NA for 24 h at 37 °C. The peaks of the profiles were identified using the TSBA50 identification library version 5.0. The predominant cellular fatty acids of strains R-53603T and R-53745 were the branched-chain saturated fatty acids iso-C₁₅:₀ (43.6–46.9 %), iso-C₁₇:₀ 3-OH (13.8–13.9 %) and iso-C₁₅:₀ 3-OH (1.2 %), the straight-chain monounsaturated fatty acids summed feature 3 (iso-C₁₅:₀ 2-OH and/or C₁₆:₁ω7c, 25.6–28.1 %), the straight-chain saturated fatty acids C₁₆:₀ (2.7–3.1 %) and C₁₆:₁ 3-OH (1.7–2.0 %), and the branched-chain monounsaturated fatty acid iso-C₁₇:₁ω9c (1.9–2.3 %). The high relative contribution of iso-C₁₅:₀, iso-C₁₇:₀ 3-OH and summed feature 3 in the cellular fatty acid profile is typical for the genus Sphingobacterium [5, 29, 30]. The cellular fatty acid profiles of strains R-53603T and R-53745 and the type strains of the most closely related Sphingobacterium species are presented in Table 1.

Analysis of the respiratory quinones of isolate R-53603T was carried out by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen – German Collection of Microorganisms and Cell Cultures). In short, respiratory quinones were extracted using methanol/hexane [31, 32], followed by phase separation into hexane. The dominant menaquinone of strain R-53603T was MK-7 (96 %), followed by MK-6 (4 %). This agrees with previously reported findings showing that MK-7 is the predominant respiratory quinone in Sphingobacterium species [5, 29, 30].

The polar lipid profile of isolate R-53603T was determined by the Identification Service of the DSMZ. Polar lipids were extracted from 100 mg of freeze dried cell material using a chloroform/methanol/0.3 % aqueous NaCl mixture (1 : 2 : 0.8, by vol.) (modified after Bligh and Dyer [33]). The extraction solvent was stirred overnight and the cell debris pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0.3 % aqueous NaCl mixture to a ratio of 1 : 1 : 0.9 (by vol.). Polar lipids were separated by two-dimensional silica gel TLC.

Table 1. Cellular fatty acid composition of isolates R-53603T and R-53745 and the type strains of phylogenetically closely related species of the genus Sphingobacterium

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>C₁₆:₀</td>
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<td>2.7</td>
<td>0.8</td>
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<tr>
<td>C₁₆:₀ 3-OH</td>
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<td>1.2</td>
<td>0.4</td>
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<tr>
<td>C₁₇:₀ 2-OH</td>
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<td></td>
<td></td>
<td>0.5</td>
<td>1.8</td>
<td>NR</td>
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<td>Branched saturated</td>
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<td>anteiso-C₁₅:₀</td>
<td>0.5</td>
<td>0.4</td>
<td>0.9</td>
<td>4.0</td>
<td>+</td>
<td>5.0</td>
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<tr>
<td>iso-C₁₅:₀</td>
<td>43.6</td>
<td>46.9</td>
<td>29.3</td>
<td>32.4</td>
<td>27.5</td>
<td>27.8</td>
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<tr>
<td>iso-C₁₇:₀ 3-OH</td>
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<td>1.2</td>
<td>2.5</td>
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<tr>
<td>iso-C₁₇:₀ 3-OH</td>
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<td>13.9</td>
<td>20.8</td>
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<td>16.6</td>
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<td>iso-C₁₅:₁</td>
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<td>1.2</td>
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<tr>
<td>iso-C₁₇:₁ω9c</td>
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<td>2.3</td>
<td>4.6</td>
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<td>4.0</td>
<td>2.4</td>
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<td>3</td>
<td>28.1</td>
<td>25.6</td>
<td>34.2</td>
<td>31.0</td>
<td>37.5</td>
<td>29.6</td>
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</table>

*Summed feature 3 comprises iso-C₁₅:₀ 2-OH and/or C₁₆:₁ω7c.
To investigate their morphological, biochemical and physiological characteristics, strains R-53603 and R-53745 were routinely cultivated on NA for 24 h at 37°C under aerobic conditions. Cell morphology and size were examined by phase-contrast microscopy. Gram staining, oxidase and catalase tests were performed as described by Cowan et al. [37]. Growth of both isolates at different temperatures (5, 15, 28, 37 and 42°C) was carried out in nutrient broth (NB; Oxoid). The pH range for growth of both isolates was examined in NB containing 100 mM Bis-Tris propane buffer (Sigma) in the range pH 3.0–12.0 (with intervals of 1 pH unit) at 37°C. pH values of the NB were adjusted by the addition of 1 M NaOH or HCl and verified after autoclaving. Salt tolerance was tested in NB at 37°C supplemented with 0–10% (w/v) NaCl. Anaerobic growth was verified on NA at 37°C. Aerobic growth on MacConkey agar (Difco) was verified at 37°C. Additional biochemical characterization was performed with API 20E and API 50CH (bio-Mérieux) and GEN III MicroPlate (Biolog) test panels at 37°C according to the respective manufacturer’s instructions. API 20E and API 50CH panels were read after 48 h and 4 days, respectively. GEN III MicroPlate panels were read after 48 h. The antibiotic susceptibility profile of R-53603 was determined with a VITEK 2 system (bio-Mérieux) according to the manufacturer’s instructions. Strain R-53603 showed susceptibility to amoxicillin/clavulanate, ciprofloxacin, piperacillin/tazobactam, ceftoxime, meropenem, imipenem, colistin, tetracycline and cotrimoxazole, and resistance to gentamicin, amikacin, ampicillin, cefuroxime and cephalexin.

Physiological, morphological and biochemical characteristics are summarized in Table 2 and in the species description. Several phenotypic differences allowed us to...
differentiate strains R-53603<sup>T</sup> and R-53745 as a group from the type strains of the phylogenetically most closely related species (Table 2). Characteristics most useful for differentiation of the two novel strains from the most closely related species <i>S. mizutaii</i> include growth on MacConkey agar, and utilization of stachyose, guanidine HCl and lithium chloride in Biolog GEN III panels.

In conclusion, genotypic analysis with (GTG)<sub>3</sub>-PCR fingerprinting indicated that R-53603<sup>T</sup> and R-53745 are different strains. Together, they formed a well-supported phylogenetic clade within the genus <i>Sphingobacterium</i> (Fig. 1) most closely related to <i>S. mizutaii</i>. However, DNA–DNA hybridizations indicated that the genomic relatedness of strains R-53603<sup>T</sup> and R-53745 with the type strain of <i>S. mizutaii</i> was far below the proposed species delineation threshold. The DNA G+C content, isoprenoid quinone composition, major polar lipid, presence of sphingophospholipids and major fatty acid profiles of R-53603<sup>T</sup> and R-53745 agree with those considered typical for the genus <i>Sphingobacterium</i>. A number of phenotypic characteristics allow the differentiation of both strains from the type strains of their closest phylogenetic neighbours. Therefore, R-53603<sup>T</sup> and R-53745 should be classified as members of a novel species within the genus <i>Sphingobacterium</i>, for which the name <i>Sphingobacterium cellulitidis</i> sp. nov. is proposed.

**DESCRIPTION OF SPHINGOBACTERIUM CELLULITIDIS SP. NOV.**

<i>Sphingobacterium cellulitidis</i> (cel.lu.li’ti.dis. N.L. gen. n. <i>cellulitidis</i> of cellulitis, pertaining to the isolation source of the type strain).

Cells are Gram-stain-negative, non-motile, non-spore-forming, aerobic short rods approximately 0.9 × 1.2–1.5 µm. On the surface of NA medium after 48 h of incubation at 37 °C, the bacterial colonies are 0.5 mm in diameter, circular with entire margins and convex. A pale yellow non-diffusible pigment is produced. Growth occurs at 15–37 °C with entire margins and convex. A pale yellow non-diffusible pigment is produced. Growth occurs at 15–37 °C with entire margins and convex. A pale yellow non-diffusible pigment is produced. Growth occurs at 15–37 °C.

The DNA G+C content, isoprenoid quinone composition, major polar lipid, presence of sphingophospholipids and major fatty acid profiles of R-53603<sup>T</sup> and R-53745 agree with those considered typical for the genus <i>Sphingobacterium</i>. A number of phenotypic characteristics allow the differentiation of both strains from the type strains of their closest phylogenetic neighbours. Therefore, R-53603<sup>T</sup> and R-53745 should be classified as members of a novel species within the genus <i>Sphingobacterium</i>, for which the name <i>Sphingobacterium cellulitidis</i> sp. nov. is proposed.

The type strain, R-53603<sup>T</sup> (=LMG 28764<sup>T</sup>=DSM 102028<sup>T</sup>), was isolated from a swab of purulent discharge from the base of the right big toe of a cellulitis patient at Assad Al-Hamad Center for Dermatology, Al-Sabah Hospital in Kuwait. The genomic DNA G+C content of this strain is 37.3 mol%.

With the description of <i>S. cellulitidis</i>, the first case of cellulitis associated with <i>Sphingobacterium</i> is reported in Kuwait. Mostly described in patients with compromising local or systemic immunity, this represents a rare cause of infection. The taxonomic allocation of a second strain to the novel species originating from sludge suggests an environmental source of infection.

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**Funding information**

This work received no specific grant from any funding agency.

**Acknowledgements**

We thank Aharon Oren and Bernhard Schink for nomenclatural assistance. We acknowledge the assistance of Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, for hybridization studies with <i>S. lactis</i> and tests for quinones and polar lipids.

**Conflicts of interest**

There is no conflict of interest.

**Ethical statement**

This work did not involve human or animal experimentation.

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