**Sanguibacter gelidistatuariae** sp. nov., a novel psychrotolerant anaerobe from an ice sculpture in Antarctica, and emendation of descriptions of the family **Sanguibacteraceae**, the genus **Sanguibacter** and species **S. antarcticus**, **S. inulinus**, **S. keddieii**, **S. marinus**, **S. soli** and **S. suarezii**

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**Abstract**

A novel psychrotolerant bacterium, strain ISLP-3⁷, was isolated from a sample of naturally formed ice sculpture on the shore of Lake Podprudnoye in Antarctica. Cells were motile, stained Gram-positive, non-spore-forming, straight or slightly curved rods with the shape of a baseball bat. The new isolate was facultatively anaerobic and catalase-positive. Growth occurred at 3–35 °C with an optimum at 22–24 °C, 0–2 % (w/v) NaCl with an optimum at 0.3 % and pH 6.2–9.5 with an optimum at pH 7.5. Strain ISLP-3⁷ grew on several carbon sources, with the best growth on cellobiose. The isolate possessed ureolytic activity but growth was inhibited by urea. The strain was sensitive to: ampicillin, gentamycin, kanamycin rifampicin, tetracycline and chloramphenicol. Major fatty acids were: anteiso-C₁₅ : ₀, iso-C₁₆ : ₀, C₁₆ : ₀, C₁₄ : ₀ and iso-C₁₅ : ₀. The predominant menaquinone was MK-9(H₄). The genomic G+C content was 69.5 mol%. The 16S rRNA gene showed 99 % sequence similarity to that of **Sanguibacter suarezii** ST-26⁸, but their recA genes shared ≤ 91 % sequence similarity, suggesting that this new isolate represents a novel species within the genus **Sanguibacter**. This conclusion was supported by average nucleotide identity, which was ≤ 91 % to the most closely related strain. The name **Sanguibacter gelidistatuariae** sp. nov. is proposed for the novel species with the type strain ISLP-3⁷=ATCC TSD-17⁹=DSM 100501⁰=JCM 30887¹. The complete genome draft sequence of ISLP-3⁷ was deposited under IMG OID 2657245272. Emendments to the descriptions of related taxa have been made based on experimental data from our comparative analysis.

The genus **Sanguibacter** was proposed and described by Fernandez-Garayzabal et al. [1] and currently includes six saccharolytic, facultatively anaerobic species: **Sanguibacter antarcticus**, **Sanguibacter inulinus**, **Sanguibacter keddieii**, **Sanguibacter marinus**, **Sanguibacter soli** and **Sanguibacter suarezii**. All species of the genus are mesophilic neutrophiles with optimal growth temperatures within the range 15–30 °C and pH 7.0–7.2. Two thalassic species, **S. marinus** and **S. antarcticus**, have been described so far. They require NaCl for growth, and both were isolated from marine ecosystems [2, 3]. The remaining species were described as athenalassic, with optimal growth at low (0.25–0.5 g l⁻¹) NaCl concentrations and the inability to grow in seawater. **S. soli** was isolated from soil [4]. Although **S. inulinus**, **S. keddieii** and **S. suarezii** were isolated from a blood sample from a healthy cow, their maximum growth temperature is 30 °C, well below the bovine body temperature [1, 5].

Here, we describe a novel isolate, ISLP-3⁷, that phylogenetically clusters within the genus **Sanguibacter**. This strain was isolated from a polar lacustral ecosystem, an ice sculpture naturally formed on the shore of Lake Podprudnoye in Antarctica. The 16S rRNA gene of the isolate showed 99 % sequence similarity to that from **S. suarezii** ST-26⁸. Five supplementary figures and two supplementary tables are available with the online Supplementary Material.

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**Keywords:** Actinomycetales; Antarctica; psychrotolerant; facultative anaerobe; ureolytic; chitinase.

**Abbreviations:** ANI, average nucleotide identity; TEM, transmission electron microscopy.

¹Present address: Genome Sequencing Center, HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ISLP-3⁷ is KM406784. The draft genome sequence of ISLP-3⁷ was deposited under IMG number OID 2657245272.
However, their recA genes shared less than 91% sequence similarity, suggesting that the new isolate represents a novel species, for which the name *Sanguibacter gelidistatuariae* sp. nov. is proposed.

Sample collection and preliminary results of the study of Antarctica’s microbial biodiversity during the Antarctica 2008 Expedition were described previously [6–9]. The sample of a glacier containing small embedded stones was asexually collected from one of the 0.5-m-tall ‘ice sculptures’ naturally formed by the summer melt and wind erosion (Fig. S1, available in the online Supplementary Material). A large field of these ‘ice sculptures’ was observed in the vicinity of Lake Podprudnoye, a small lake so named by a Soviet Antarctic Expedition in 1962, and it is also known as Proglacial Lake 21 (70° 44′ 31″ S 11° 47′ 39″ E). The lake is located in the Schirmacher Hills of Drongon Queen Maud Land in East Antarctica, south-east of the larger Prelednikoye Lake. Lake Podprudnoye is spread along the edge of the continental ice sheet, and wind erosion produces spectacular ice sculptures on its shore. These formations contain multiple small black stones and dust particles captured within the ice. After collection, the samples were kept frozen prior to delivery to the astrobiology laboratory at the National Space Science and Technology Center in Huntsville, Alabama.

Procedures of enrichment and isolation were described previously [7]. The anaerobic medium (AM) composition was (g 1−1): NaCl 5.0, KCl 0.3, KH2PO4 0.3, NH4Cl 1.0, MgSO4·7H2O 0.1, CaSO4·7H2O 0.0125, NaHCO3 0.2, Na2S·9H2O 0.5, resazurin 0.005% (w/v), 2 ml vitamin solution [10], 1 ml trace element solution [11] and yeast extract 0.1. The final pH 7.2 was adjusted by using 2 M H2SO4 or 6 M NaOH. The gas phase in Hungate tubes was filled with high-purity nitrogen gas. The medium was autoclaved at 1 atm, 121°C for 1 h. d-Glucose (5 g 1−1) was added separately as a growth substrate. All experiments, except for isolation and purification, were performed in triplicate at room temperature (24°C).

Culture growth was estimated by cell counting with a spectrophotometer (Genesis 5; Spectronic Instruments).

Strain ISLP-3T was isolated from one of the ice sculptures described above. A glacial fragment (~0.4 g) containing embedded dark grey dust particles was slowly melted at 3°C in a sterile sealed flask under an atmosphere of pure nitrogen. The melted liquid (0.5 ml) then was injected into a Hungate tube with anaerobic medium and incubated at 3°C for 2–3 weeks. A pure culture was obtained by serial dilutions on AM medium and isolation from colonies was performed with the roll-tube technique in AM medium, in which sodium salts were replaced by equimolar amounts of potassium salts. Additional NaCl was then added to achieve a final concentration of: 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 5.0%. The strain was incubated at different temperatures (intervals of 3–5°C) to determine the optimum. The isolate was tested for the ability to grow on different substrates, including sugars, proteolysis and protein products, organic acids, and alcohols. Each substrate was sterilized separately and added in a final concentration of 3 g l−1. Staining with Nile blue A [12], Loeffler methylene blue and toluidine blue was performed to verify the composition of inclusion bodies [13]. Urea decomposition was measured colorimetrically at 340 nm on a spectrophotometer (GENESIS 10vis; Thermo Spectronic) with BUN Urea Nitrogen Reagent following the kit’s protocol (Infinity Urea Liquid Stable Reagent; Thermo Scientific).

The cellular ultrastructure of strain ISLP-3T was examined with transmission electron microscopy (TEM) by negative staining of thin sections as described previously [14].

Fatty acid methyl esters were extracted from lyophilized cells at exponential growth on AM medium for 48 h at 25°C using the Sherlock Microbial Identification System (MINI) version 6.1 as described previously [15–17]. Analysis was carried out with an Agilent Technologies 6890 N gas chromatograph equipped with a phenyl methyl silicone-fused silica capillary column (HP 2.25 m×0.2 mm×0.33 μm film) and flame ionization detector. Hydrogen was the carrier gas. The temperature programme was initiated at 170°C and increased at 5°C min−1 to a final temperature of 270°C. The relative amount of each fatty acid was expressed in terms of the percentage of total fatty acids using the QTSi1 peak naming library.

For extraction of quinones, cells were grown in AM medium and harvested at the late logarithmic growth phase. Extraction and analysis of quinones was carried out according to the protocol of Tindall et al. [18, 19] and Altenburger et al. [20]. The HPLC equipment applied for analysis of quinones was described by Stolz et al. [21].

Extraction of DNA was performed according to the E.Z.N. A. Bacterial DNA Kit (Omega). The purity of DNA was checked by gel electrophoresis; additional RNAase treatment was performed for the strain.

PCR was performed as described previously [14] with the following primers: B16S-R: 5′-TACGGYTAATCCTGTTACGAC-3 (Escherichia coli positions 8–27) and B16S-F: 5′-AGAGTTTGATCMTGCTCAG-3 (E. coli positions 1513–1494). PCR products were purified with Zymo DNA Clean and Concentrator-5 column following the manufacturer’s instructions and prepared for 16S rRNA gene sequencing. Sequencing was done at the Georgia Genomics facility at the University of Georgia in Athens. The sequence was deposited in GenBank, and then was processed by the program MEGA 6 [22] the final format was released in CHROMAS LIGHT.
Reference strains were ordered from the JCM and NBRC culture collections: *S. suarezii* ST26^T^ NBRC 16159^T^, *S. keddieii* ST74^T^ NBRC 16159^T^, *S. inulins* ST50^T^ JCM 19122^T^ (formerly JCM 11442^T^), *S. antarcticus* KOPRI 21702^T^ JCM 14623^T^, *S. marinus* 1-19^T^ JCM 12547^T^ and *S. soli* DCY22^T^ JCM 14841^T^.

Cells were straight or slightly curved rods (Fig. 1a), arranged singly, in pairs or in short chains (Fig. S2). Cells were either motile or non-motile, 0.4–0.5 × 1.2–3.5 μm in size and stained Gram-positive. Some cells had a swollen polar region similar in shape to a baseball bat (Fig. 1b, c) and the original culture was dominated by this morphology. The ends of cells were rounded and slightly pointed (Fig. 1e). Sometimes, dichotomic branching could be observed (Fig. 1d), and light-refracting inclusions were observed at the swollen poles of the cell. However, Nile Blue A staining for poly-β-hydroxybutyrate and Löfleir methylene blue/toluidine blue staining for polyphosphates or volutin granules were negative. Cell division was by septation with formation of two daughter cells (Fig. S3). Nucleoids had pseudomembranous electron-dense layers (Fig. 1e).

On agar surfaces, colonies of strain ISLP-3^T^ were circular, 1–3 mm in diameter, flat, not raised, cream to brownish, opaque, smooth and not glossy. Grown within agar, the colonies had the shape of a convex lens with smooth and even edges.

Strain ISLP-3^T^ was catalase-positive and facultatively anaerobic. Under aerobic conditions, growth was 2–3 times faster. The strain was psychrotolerant and grew well within a temperature range of 3–35 °C with an optimum at 22–24 °C (Fig. S4a). No growth occurred at −3 or 0 °C. The strain had optimum of growth at 0.3 % (w/v) NaCl and growth range of 0–2 % (w/v) NaCl (Fig. S4b). The strain did not grow on medium with 3 % NaCl, which unambiguously confirms an athalassic origin. The pH range for growth was between 6.0 and 9.5 with an optimum at pH 7.5 (Fig. S4c).

Strain ISLP-3^T^ grew on: triethylamine, chitin, N-acetylglucosamine, D-glucose, D-fructose, D-arabinose, trehalose, maltose, sucrose, D-ribose, D-mannose, lactose, starch and D-cellobiose. The best growth was on D-cellobiose, and noticeably weak growth was observed on D-fructose with a prolonged 3-day lag phase. Growth was not observed on: H₂.
Antibiotic susceptibility was tested at concentrations of 250 µg ml⁻¹ (ampicillin, gentamycin, rifampicin, tetracycline and kanamycin) and 100 µg ml⁻¹ (chloramphenicol). Strain ISLP-3T was sensitive to all tested antibiotics, indicative of its wild species level than the 16S rRNA gene [23–25]. recA genes encoding recombinase A were PCR amplified from Sanguibacter strains by Phusion High-Fidelity DNA Polymerase (NEB M0530S) using the primers Sg-recA-F (5’-TTGGAGGCTCGATCATG-3’) and Sg-recA-R (5’-TCGCGTCTGAGTGAAACCA-3’) designed from the complete genome sequence of S. keddii DSM 784T (GenBank CP001819.1). The S. keddii recA sequence was analysed before primer design to ensure that the 3’-end of both primers could be anchored to codons without wobble sites to minimize their degeneracy. For wobble positions internal to the primer, the choice of the nucleotides was made according to codons of related nucleotide sequences. Various annealing temperatures (55, 58, 60 and 65 °C) for PCR were applied to ensure successful amplifications for all Sanguibacter strains. For samples having low PCR yield, the recA PCR band was pooled using a micro-pipette tip and re-suspended in double distilled H₂O, and served as the DNA template for a second round of PCR amplification. All recA PCR bands were gel-purified using a Zymoclean Gel DNA Recovery Kit (#D4001). The novel isolate showed more than 91 % recA gene sequence similarity to all six described species of the genus Sanguibacter (Table S2). Their phylogeny closely resembled that of the 16S rRNA gene, and S. suarezii was the closest relative of strain ISLP-3T (Figs 3 and S5).

Strain ISLP-3T is the second strain in the genus Sanguibacter found in Antarctica. The previously described S. antarcticus KOPRI 21702T is athalassic, while ISLP-3T is athalassic, which agreed with its isolation from a freshwater lacustrine ecosystem. In Table 1, phenotypic features of the novel isolate ISLP-3T are compared with all currently known Sanguibacter species. In this study, S. soli DCY22T demonstrated very good anaerobic growth, suggesting that its previous diagnosis as strictly aerobic needs correction. All athalassic Sanguibacter species were inhibited by marine levels of salinity as well as the novel isolate ISLP-3T (Fig. S4b). In our experiments, the optimum growth temperature /CO₂ (80:20), formate, acetate, pyruvate, lactate, propionate, butyrate, citrate, oxalate, methanol, ethanol, glycerol, D-mannitol, acetone, betaine, trimethylamine, peptone, yeast extract, Casamino acids and pectin. Cells possessed ureolytic activity, and 2 g l⁻¹ was degraded by culture during 3 days of incubation. Urea (3 g l⁻¹) inhibited growth in medium with D-glucose as a carbon source.

Antibiotic susceptibility was tested at concentrations of 250 µg ml⁻¹ (ampicillin, gentamycin, rifampicin, tetracycline and kanamycin) and 100 µg ml⁻¹ (chloramphenicol). Strain ISLP-3T was sensitive to all tested antibiotics, indicative of its wild origin from an ecologically balanced environment without a predominance of antimicrobial-producing species.

The fatty acid profile of strain ISLP-3T is shown in Table S1. The major fatty acids were: anteiso-C₁₅:₀ (47.3 %), iso-C₁₆:₀ (8.5 %), C₁₆:₀ (8.5 %) and iso-C₁₅:₀ (3.9 %).

The quinone system of strain ISLP-3T consisted exclusively of menaquinones MK-(9)(H₄) (78.7 %), moderate amounts of MK-(8)(H₅) (9.5 %) and MK-(9)(H₆) (8.2 %), and minor amounts of MK-(9)(H₇) (2.5 %) and MK-(8)(H₇) (1.0 %). Quinone systems with MK-(9)(H₄) as the major menaquinone have been also reported for other Sanguibacter species [1–3, 5].

The sequence of the 16S rRNA gene (1492 bp) was deposited in GenBank under accession number KM406784. A BLAST analysis indicated a close relationship to species of the genus Sanguibacter: S. suarezii ST26T, 99.0 % sequence similarity; S. inulinus ST50T, 98.6 %; S. keddii DSM 10542T, 98.1 %; S. antarcticus KOPRI 21702T, 97.1 %; S. marinus 1-19T, 96.5 %; and S. soli DCY22T, 96.5 %. Sequence and phylogenetic analyses were performed in Geneious 8.0.5.

Phylogenetic analysis of the 16S rRNA gene showed the new isolate was most closely related to S. suarezii (Fig. 2). Because of the high sequence similarity of the 16S rRNA genes, the sequences of recA genes were also compared. The comparison of core gene sequences provides a higher resolution at the species level than the 16S rRNA gene [23–25]. recA genes encoding recombinase A were PCR amplified from Sanguibacter strains by Phusion High-Fidelity DNA Polymerase (NEB M0530S) using the primers Sg-recA-F (5’-TTGGAGGCTCGATCATG-3’) and Sg-recA-R (5’-TCGCGTCTGAGTGAAACCA-3’) designed from the complete genome sequence of S. keddii DSM 784T (GenBank CP001819.1). The S. keddii recA sequence was analysed before primer design to ensure that the 3’-end of both primers could be anchored to codons without wobble sites to minimize their degeneracy. For wobble positions internal to the primer, the choice of the nucleotides was made according to codons of related nucleotide sequences. Various annealing temperatures (55, 58, 60 and 65 °C) for PCR were applied to ensure successful amplifications for all Sanguibacter strains. For samples having low PCR yield, the recA PCR band was pooled using a micro-pipette tip and re-suspended in double distilled H₂O, and served as the DNA template for a second round of PCR amplification. All recA PCR bands were gel-purified using a Zymoclean Gel DNA Recovery Kit (#D4001). The novel isolate showed more than 91 % recA gene sequence similarity to all six described species of the genus Sanguibacter (Table S2). Their phylogeny closely resembled that of the 16S rRNA gene, and S. suarezii was the closest relative of strain ISLP-3T (Figs 3 and S5).

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for the athalassic species was 22–24 °C. Furthermore, *S. inulins* JCM 19122T, *S. keddieii* NBRC 16159T and isolate ISLP-3T did not grow at 37 °C although good growth was observed at room temperature and at 3–4 °C. *S. suarezii* NBRC 16159T grew at 37 °C only after a lag phase of 72 h. Cell yield was also very poor, and its morphology changed with loss of motility, cell swelling and formation of granules in the cytoplasm, all indicative of unfavourable cultivation conditions. These results exclude a pathogenicity for warm-blooded animals. In addition, the source of the three species *S. suarezii*, *S. inulins* and *S. keddieii* isolated from the blood of a healthy cow is questionable, and contamination of the sampling equipment or the procedure itself may have been a reason. Only two strains, *S. marinus* JCM 12547T and *S. soli JCM 14841T, showed good growth at 37 °C (the lag phase at 24 °C was twice that at 37 °C). *S. marinus* cannot be categorized as pathogenic due to thalassic physiology, but *S. soli’s* growth parameters would not exclude it from possible pathogenicity. Lastly, *S. antarcticus* KOPRI 21702T and *S. keddieii* ST-74T grew well at 0 °C with prolonged lag phases of 10–14 days (Table 1).

The ability of all type strains to grow on chitin suggests a potential pathogenicity for cold-blooded arthropods, but this feature may also categorize them as primary decomposers of organic matter in the global biogeochemical cycle. The assessment of their potential pathogenicity for arthropods, and particularly insects, should be performed for biotechnological applications. Numerous articles characterizing extracellular chitinases have been published in the last decade [26, 27]. All these features should also be added to the description of the family *Sanguibacteriaceae* [28, 29] of the order *Actinomycetales* [30, 31].

The fatty acid profile of the novel isolate was quite different from that of *S. soli* but close to the profiles of thalassic species *S. marinus* and *S. antarcticus*, both of which have anteiso-C15:0 in large amounts (∼50%), and C14:0 and C16:0 in similar digns.

The draft genome sequence of strain ISLP-3T was prepared by the DOE Joint Genomes Institute (see Supplementary Materials). It comprised 4.04 Mbp in 18 scaffolds. Coding density was 91.2%, and the G+C content was 69.5%. A total of 3599 protein and 67 RNA encoding genes were identified. The genome possessed two 16S rRNA genes. It also encoded three CRISPRs (clustered regularly interspersed short palindromic repeats), nucleotide transferases and phage proteins, suggesting that horizontal gene transfer and phages were an influential component of the strain’s history. Many potentially catalytic dehydrogenases and proteases were indicative of a heterotrophic metabolism. The genome also encoded catalase, superoxide dismutase and peroxidase, confirming the facultative anaerobic physiology along with the catalase-positive test. Genes associated with ureolytic capacity of the isolates were also found, including two glutamate dehydrogenases (NADPH type) and two semi-aldehyde type, acetyl ornithine aminotransferase holoenzyme and other enzymes of the ornithine cycle. Flagellar motility proteins and type IV pilus assembly proteins with type II secretory pathway were also annotated. DNA-directed RNA polymerase and DNA-directed DNA polymerase were listed.

Consistent with the 16S rRNA gene phylogeny, the closest BLAST hits for individual genes were largely to the genome sequence of *S. suarezii*. The pairwise ANI between ISLP-3T and other *Sanguibacter* species is shown in Table 2. All ANI values were precomputed by IMG except for *S. suarezii*, whose genome sequence was not available at the IMG [32]. Instead, the ANI between ISLP-3T and *S. suarezii* was computed using the default parameters by the ANI calculator hosted by the Kostas lab at the Georgia Institute of Technology (http://enve-omics.ce.gatech.edu/ani/) [33].

Based on the phenotypic and genotypic characteristics (Tables 1 and 2), the new isolate ISLP-3T is proposed here as a separate species within the genus *Sanguibacter*. The name *Sanguibacter gelidistatuariae* sp. nov. was chosen for the novel species to reflect its unique source of isolation.

**DESCRIPTION OF SANGUIBACTER GELIDISTATUARIAE SP. NOV.**

*Sanguibacter gelidistatuariae* (ge.li.di sta.tu. a’ri.ae. L. adj. gel-idus ice-cold; L. fem. statuaria a statue, sculpture, gen. n. statuariae of ice sculpture (geological term); L. fem. gen. n. gelidistatuariae pertaining to the naturally formed ice sculpture, from which the type strain was isolated).

Cells are straight or irregularly curved ‘baseball bat’-shaped rods with pointed ends and with sizes of 0.4–0.5×1.2–3.5 μm. Cells occur singly, in pairs or in short chains, often gathering into small irregular clusters. Cells are either motile or non-motile. Not spore-forming. Facultatively anaerobic catalase-positive. Psychrotolerant mesophile with a temperature range of 3–35 °C and optimum 22–24 °C. The salinity range is 0–2% (w/v) NaCl optimum 0.25%. The pH range is 6–9.5 optimum 7.4–7.5. Growth occurs on: d-glucose, d-fructose, d-ribose, d-arabinose, maltose, sucrose, d-trehalose, d-mannose, d-cellobiose, lactose, starch, chitin, N-acetylgalactosamine and triethylamine. The best growth is on d-cellobiose, and poor growth occurs on d-fructose. Ureolytic growth is inhibited by urea. No growth is observed on: H2/CO2 (80:20), formate, acetate, pyruvate, lactate, propionate, butyrate, citrate, oxalate, methanol, ethanol, glycerol, d-mannitol, acetone, betaine, trimethylamine, peptone, yeast extract, Casamino acids or pectin. The quinone system is composed of the major menaquione MK-9(H2), moderate amounts of MK-8(H4) and MK-9(H6) and minor amounts of MK-9(H4) and MK-8(H6). Major fatty acids are: anteiso-C15:0, iso-C16:0, C16:1ω7c C14:0 and iso-C15:0. Sensitive to antibiotics: ampicillin, gentamycin, kanamycin, rifampicin, tetracycline and chloramphenicol. The genomic G+C content is 69.5 mol% (from the draft genome sequence).
Table 1. Distinguishing features among *Sanguibacter* species

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<th>Characteristic</th>
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<td><strong>Cell morphology</strong></td>
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<td>Cell size (µm)</td>
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<td>0.4–0.6×4.5</td>
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<td>pH range (optimum)</td>
<td>6–9.5 (7.5)</td>
<td>7.2</td>
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<td>4–9 (5–6)</td>
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<td>Temperature range (°C)</td>
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<td>22–24</td>
<td>25–30</td>
<td>23–26</td>
<td>25–30</td>
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<td>Aerobic growth at 37°C</td>
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<td>Growth at 0°C</td>
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<td>NaCl range (optimum) (%)</td>
<td>0–2 (0.25)</td>
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<td>0–7 (2–5)</td>
<td>0–7 (3)</td>
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<td>d-Arabinose</td>
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<tr>
<td>d-Ribose</td>
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<tr>
<td>d-Mannose</td>
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<td>d-Mannitol</td>
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<td>+</td>
<td>(+)</td>
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<td>Lactose</td>
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<td><strong>Sensitivity to antibiotics</strong></td>
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<tr>
<td>Ampicillin, kanamycin, gentamycin,</td>
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<td>ND</td>
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<td>rifampin, tetracycline, chloramphenicol</td>
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<td>Vancomycin, rifampin, minocycline,</td>
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<td>ND</td>
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<td>imipenem, clindamycin, clarithromycin</td>
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<td><strong>DNA G+C content (mol%)</strong></td>
<td>69.5 (genome)</td>
<td>70.5 (Tm)</td>
<td>70 (Tm)</td>
<td>69.5 (Tm)</td>
<td>73.4 (Tm)</td>
<td>69.8 (Tm)</td>
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<td><strong>Menaquinones</strong></td>
<td>MK-9H₄, MK-8H₄, H₂</td>
<td>ND</td>
<td>MK-9H₄</td>
<td>ND</td>
<td>MK-9H₄</td>
<td>MK-9H₄</td>
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<tr>
<td><strong>Isolation source</strong></td>
<td>Ice sculpture, Antarctica</td>
<td>Blood of healthy cow</td>
<td>Blood of healthy cow</td>
<td>Blood of healthy cow</td>
<td>Sea sand, Antarctica</td>
<td>Coastal sediments, China</td>
<td>Coastal sediments, Korea</td>
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<td><strong>Type strain/collections</strong></td>
<td>ISLP-3⁣¹, ATCC ST26⁣², JCM 30887⁣³, DSM 100501⁣³</td>
<td>ST 26⁣², ATCC 51766, CCUG 36691, CECT5439, CIP106322, DSM 10543, JCM 11442, NCIMB 703024</td>
<td>ST 74⁣², CCUG 36689, CIP104915, JCM 11442, NCIMB 703023</td>
<td>ST 74⁣²</td>
<td>KOPRI 21702⁴, DSM 18896, JCM 14623, KCTC 13143</td>
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<td>GenBank accession number</td>
<td>KM406784</td>
<td>X79452</td>
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<td>CP001819.1</td>
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</table>

*Our data: aerobic growth of *S. inulinus* and *S. keddieii* was negative at 37°C; *S. inulinus* survived at 37°C and grew well only after return to 24°C; *S. suarezii* after long lag phase grew (weakly) at 37°C in anaerobic medium, but growth was better with a shorter lag phase at 24°C.

†S. DCY22⁴ is a facultative anaerobe (aerobe in original description).

‡Our data for substrate utilization (tests were performed in AM medium).
The type strain, ISLP-3T (=ATCC TSD-17T=DSM 100501T =JCM 30887T), was isolated from ice of a naturally formed ice sculpture on the shore of Lake Podprudnoye in East Antarctica.

Based on the experimental data of our comparative analysis of the novel isolate with all Sanguibacter species, we provide emended descriptions of the family Sanguibacteraceae, the genus Sanguibacter and species of this genus.

**EMENDED DESCRIPTION OF SANGUIBACTERACEAE STACKEBRANDT AND SCHUMANN 2000**

The description of the family is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. Facultative anaerobes. Saccharolytic, urease- and chitinase-positive. Some species grow on proteolysis products and sugar alcohols. In cytology, compartmentalization of the nucleoid by membranous formation is observed. Motility by flagella and gliding by IV type pili. Possess type II secretion system. Predominant cellular fatty acid profile includes: branched and saturated pentadecanoic, heptadecanoic, hexadecanoic, tetradecanoic and tridecanoic acids. The respiratory system possesses MK 8 and nitrates are used as electron acceptors. Urease extract, triethylamine and sugar alcohols. Acetogens. Oxy-

**EMENDED DESCRIPTION OF SANGUIBACTER FERNANDEZ-GARAYZABAL, DOMINGUEZ, PASCUAL, JONES AND COLLINS 1995**

The description of the genus is as given by Ramos and Fernández-Garayzabal [29], with the following modifications. Mesophilic and some species are psychrotolerant with poor/or no growth at 35–37°C. Includes thalassic and athalassic species. Neutrophilic and alkalitolerant. All species are facultative anaerobes and catalase-positive with preferred growth under aerobic conditions, and able to grow with chitin. Some species grow on peptone, yeast extract, triethylamine and sugar alcohols. Acetogens. Oxygen and nitrates are used as electron acceptors. Urease activity has been reported.

**Table 2.** Pairwise average nucleotide identity (ANI) between strain ISLP-3T and other Sanguibacter species

<table>
<thead>
<tr>
<th>Strain</th>
<th>ANI (%)</th>
<th>Accession number*</th>
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</thead>
<tbody>
<tr>
<td>ISLP-3T</td>
<td>100</td>
<td>2657245272</td>
</tr>
<tr>
<td>S. suarezii ST26T</td>
<td>85.1</td>
<td>1054700590</td>
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<td>S. keddiei DSM 10542T</td>
<td>80.6</td>
<td>646564565</td>
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<td>S. antarcticus KOPRI 21702T</td>
<td>78.8</td>
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<td>S. soli DCY 22T</td>
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<td>S. marinus 1-19T</td>
<td>77.3</td>
<td>2622736429</td>
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*Accession numbers are IMG Genome IDs except for S. suarezii, for which a GI number is presented. S. inulinus does not have a draft genome sequence, and therefore it is not included in the table.

**EMENDED DESCRIPTION OF SANGUIBACTER ANTARCTICUS HONG, LEE, CHUN AND LEE 2008**

The description of the species is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. The minimum temperature for growth is 0°C; no growth at −3°C. Grows on starch, yeast extract chitin. No growth on peptone or triethylamine.

**EMENDED DESCRIPTION OF SANGUIBACTER INULINUS PASCUAL, COLLINS, GRIMONT, DOMINGUEZ AND FERNÁNDEZ-GARAYZÁBAL 1996**

The description of the species is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. No aerobic growth at 37°C (survives at this temperature, and afterwards grows well at 24°C). Minimum growth temperature is 0°C no growth at −3°C. Grows on starch and chitin, but no growth on triethylamine, peptone or yeast extract.

**EMENDED DESCRIPTION OF SANGUIBACTER KEDDIEII FERNÁNDEZ-GARAYZÁBAL, DOMINGUEZ, PASCUAL, JONES AND COLLINS 1995**

The description of the species is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. No aerobic growth at 37°C. Grows well at 24°C and with 0.5% (w/v) NaCl. Minimum growth temperature is 0°C no growth at −3°C. Grows on sucrose or chitin. No growth on: D-fructose, D-celllobiose, arabinose, D-trehalose, maltose, starch, triethylamine, peptone or yeast extract.

**EMENDED DESCRIPTION OF SANGUIBACTER MARINUS HUANG, DAI, HE, WANG, LII AND LIU 2005**

The description of the species is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. Minimum growth temperature is 4°C no growth at 0°C. Grows on peptone chitin, but no growth on triethylamine or yeast extract.

**EMENDED DESCRIPTION OF SANGUIBACTER SUAREZZI FERNÁNDEZ-GARAYZÁBAL, DOMINGUEZ, PASCUAL, JONES AND COLLINS 1995**

The description of the species is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. Weak growth at 37°C (under anaerobic conditions with a prolonged 72h lag phase); better growth at room temperature [24°C and 0.5% (w/v) NaCl]. Minimum growth temperature is 0°C; no growth at −3°C. Grows on chitin, celllobiose and sucrose. No growth on: D-fructose, D-
arabinose, D-trehalose, maltose, triethylamine, peptone or yeast extract.

**EMENDED DESCRIPTION OF SANGUIBACTER SOLI KIM, PULLA, KIM, YI, SOUNG AND YANG 2008**

The description of the species is as given by Pascual Ramos and Fernández-Garayzabal [29], with the following modifications. Facultative anaerobe.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


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