**Snuella sedimenti** sp. nov., isolated from marine sediment

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**Abstract**

A Gram stain-negative, aerobic, motile by gliding, rod-shaped bacterial strain CAU 1569T was isolated from marine sediment on Shido Island in Incheon. It grew at 20–37 °C (optimum, 30 °C), pH 6.0–9.0 (optimum, 7.0), 2–6% NaCl (w/v) (optimum, 2%). Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain CAU 1569T formed a distinct lineage with only the type strain of *Snuella*. Strain CAU 1569T showed high similarity to *S. lapsa* KACC 14152T (95.8%), *Mariniflexile gromovii* KMM KCTC 12570T, *Aestuariibaculum marinum* KCTC 52521T (95.4%), *A. suncheonense* KACC 16186T (94.6%) and *Yeosuana aromativorans* KCCM 42019T (94.4%). The genome contained 57 contigs, 3,437 protein-coding gene, 3 rRNAs (5, 16, and 23S), 43 tRNAs, and with a 35.7 mol% G + C content. The DDH value between strain CAU 1569T and *S. lapsa* KACC 14152T was 39.4 ± 0.6%. The only isoprenoid quinone was menaquinone 6 (MK-6). The major fatty acids were iso-C15:0, C15:1-iso G, and C17:0 iso 3-OH. Strain CAU 1569T contained diphosphatidylglycerol, aminoglycolipid, unidentified aminolipid, and three unidentified lipids. Based on phylogenetic, genomic, physiologic, and chemotaxonomic characterizations, strain CAU 1569T represents a novel *Snuella* species, which the name *Snuella sedimenti* sp. nov. is proposed. The type of strain is CAU 1569T (= KCTC 82409T = MCCC 1K05670T).

**Keywords** *Snuella sedimenti* sp. nov. · *Flavobacteriaceae* · Marine sediment

**Introduction**

The family *Flavobacteriaceae* was described by Reichenbach (1991) and emended by Bernardet et al. (1996, 2002). At the time of writing, the family contains 150 genera with the validly published and correct name (https://lpsn.dsmz.de/family/flavobacteriaceae). Members of the family *Flavobacteriaceae* are isolated from various environments, especially in marine such as tidal flat sediment (Kim et al. 2008), mud (Nedashkovskaya et al. 2004), and deep-sea seamount (Wang et al. 2020), coastal surface seawater (Bhumika et al. 2013), surface of algae, sponges and corals (Gavriliidou et al. 2020). The genus *Snuella*, a member of the family *Flavobacteriaceae*, was first proposed by Yi and Chun (2011) and comprises only one species, *Snuella lapsa*, which was isolated from a tidal flat sediment. In the course of investigating novel bacteria, strain CAU 1569T was isolated from a marine sediment of Shido Island sample collected in the Republic of Korea, was identified as a novel bacterium based on its unique taxonomic position using a polyphasic approach, including the determination of phenotypic, genomic, and chemotaxonomic characteristics.

**Materials and methods**

**Bacterial isolation and ecology**

A sediment sample was collected from Shido Island, Incheon (37° 32' 11.0" N 126° 25'09.0" E) in South Korea. A yellow colony was isolated from 100-µl aliquots of serial
dilutions of the sediment sample spread on marine agar (MA) 2216 plates (BD Difco, Sparks, MD, USA). Strain CAU 1569\textsuperscript{T} was purified by routine re-streaking on MA plates and the single strain was preserved in marine broth (MB) 2216 (BD Difco) supplemented with 25% (v/v) glycerol stocks at −80 °C. *S. lapsa* KACC 14152\textsuperscript{T}, *Mariniflexile gromovii* KCTC 12570\textsuperscript{T}, *Aestuariibaculum marinus* KCTC 52521\textsuperscript{T}, *A. suncheonense* KACC 16186\textsuperscript{T} and *Yeosuana aromativorans* KCCM 42019\textsuperscript{T} were obtained from Korean Collection for Type Cultures (Jeongeup, Korea), Korean Culture Center of Microorganisms (Seodaemun, Korea) and Korean Agricultural Culture Collection (Jeonju, Korea) and evaluated for comparative analysis as reference strains.

16S rRNA gene sequence and phylogeny

Genomic DNA of strain CAU 1569\textsuperscript{T} was extracted using a genomic DNA extraction kit (iNtRON, Seongnam, Korea) according to the manufacturer’s instructions. The amplification of 16S rRNA gene was performed as described previously (Nam et al. 2004). Identification and calculation of pairwise identity of 16S rRNA gene sequence similarity between strain CAU 1569\textsuperscript{T} and the most closely related strains were performed from the EzBioCloud server (http://www.ezbiocloud.net/eztaxon) (Kim et al. 2012) and GenBank database. Multiple alignment and phylogenetic analysis were carried out using the MEGA7 program (Kumar et al. 2016). Phylogenetic trees were constructed using algorithms, including neighbour-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981), and maximum-parsimony (MP) (Fitch 1971) approaches for distance analysis. The topology of the phylogenetic tree was estimated by bootstrap values with 1000 replicates (Felsenstein 1985). The DNA-DNA pairing between strain CAU 1569\textsuperscript{T} and the closest strain *S. lapsa* KACC 14152\textsuperscript{T} was performed using the fluorometric microplate method (Ezaki et al. 1989).

Whole-genome analysis

The draft genome of strain CAU 1569\textsuperscript{T} was sequenced using an Illumina Hiseq sequencer by Macrogen (Seoul, Korea). The sequence data were assembled using de novo assembly method with SPAdes version 3.13.0 (http://cab.spbu.ru/software/spades). The whole-genome sequence deposited at GenBank database under the accession number is NZ_JAELVQ000000000. K-mer analysis was performed with GenomeScope (http://qb.cshl.edu/genomescope) and Jellyfish (version 2.2.3) (http://www.genome.umd.edu/jellyfish.html). Genome annotation was performed through Rapid Annotation using Subsystem Technology (https://rast.nmpdr.org) (Aziz et al. 2008).

Physiological characterization

For morphological analysis of strain CAU 1569\textsuperscript{T}, cells were grown on MA for 2 days at 30 °C. Cell morphology was observed under a DM 1000 light microscope (Leica, Wetzlar, Germany) and the JEM 1010 transmission electron microscope (JEOL, Tokyo, Japan) using cells growing at the exponential phase. Motility of strain CAU 1569\textsuperscript{T} was determined by a semisolid agar tube (Wolfe and Berg 1989) and gliding motility was performed using hanging-drop method as described by Bernardet et al. (2002). Gram staining was carried out using a Gram staining kit (bioMérieux, Craponne, France). Strain CAU 1569\textsuperscript{T} was inoculated at various temperatures (4, 10, 20, 25, 30, 37, and 45 °C) in MA, pH (4.5–12.0 at 0.5 pH unit intervals; adjusted with 1 M HCl or 1 M NaOH), and NaCl concentrations in NaCl-free MB supplemented with 0 and 15% (1% increments, w/v). Catalase and oxidase activity were examined using bubble production in 3% (v/v) H\textsubscript{2}O\textsubscript{2} solution and oxidase reagent (bioMérieux), respectively. Hydrolysis of casein and starch were determined as described previously (Smibert and Krieg 1994). Identification of gram-negative bacteria and carbon sources assimilation were evaluated using API 20E, API 20NE and API 50CH kits (bioMérieux), respectively, and the results were recorded after 48 h of incubation at 30 °C. Enzyme activity was examined using API ZYM kit (bioMérieux) and the results were analyzed after 24 h of incubation at 37 °C.

Chemotaxonomic characterization

Isoprenoid quinones were extracted and analyzed by reverse-phase high-performance liquid chromatography (HPLC) as previously described (Komagata and Suzuki 1987). For analysis of fatty acids, strain CAU 1569\textsuperscript{T} and the five reference strains were cultured on MA at 30 °C and cell mass was harvested after 3 days (exponential growth phase, optical density at 600 nm = 0.8). Fatty acids of strain CAU 1569\textsuperscript{T} were saponified, methylated and extracted using standard Microbial Identification System (MIDI, version 6.2B) methods. Fatty acids were analyzed by gas chromatography (Hewlett Packard 6890) and identified using the TSBA6 database of the Microbial
Identification System (Sasser 2006). Polar lipids were extracted from cell mass harvested at exponential growth phase as described by Minnikin et al. (1984) and separated by two-dimensional TLC as described by Embley and Wait (1994). The polar lipids were identified using specific spraying reagents as described Kim et al. (Kim et al. 2015).

**Results and discussion**

### Phylogenetic and genome characterization

The nearly complete the 16S rRNA gene sequence of CAU 1569\(^\text{T}\) (1,511 bp) was investigated for similarity among sequences of related bacterial strains in the GenBank databases (access May 2021) and submitted to the GenBank/EMBL/DDBJ under the accession number MN544292. The 16S RNA gene sequences analysis revealed that strain CAU 1569\(^\text{T}\) showed the highest pairwise similarity to *S. lapsa* KACC 14152\(^\text{T}\) (95.8%), followed by *M. gromovii* KCTC 12570\(^\text{T}\) (95.5%) and *A. marinus* KCTC 52521\(^\text{T}\) (95.4%). The neighbour-joining phylogenetic tree indicated that strain CAU 1569\(^\text{T}\) clustered in a single branch of *S. lapsa* KACC 14152\(^\text{T}\) (Fig. 1). The DDH value between strain CAU 1569\(^\text{T}\) and *S. lapsa* KACC 14152\(^\text{T}\) was 39.4 ± 0.6%, which is clearly < 70% value was recommended by Wayne et al. (1987) for species differentiation. The draft genome of strain CAU 1569\(^\text{T}\) was composed 57 contigs (average length 76,651 bp) and a total genome size of 4.4 Mb. DNA G + C content of CAU 1569\(^\text{T}\) was 35.7 mol%, this value is lower than that of closely related members of the genus *Snuella* (Table 1). The K-mer coverage was 272.8, and the N50 value was 147,344 bp. The genome of strain CAU 1569\(^\text{T}\) was composed contained 3,437 protein-coding genes with 3,462 coding sequences, 3 rRNAs (5, 16, and 23S), and 43 tRNAs. The overall genome-related index (OGRI) was not calculated according to minimal standards for using genome data (Chun et al. 2018), which is the threshold value of 16S sequence similarity (< 98.5%) of a novel species. The subsystem genome features of strain CAU 1569\(^\text{T}\) with RAST server are summarized in Supplementary Fig. 1. Genome annotations (> 7%) of strain CAU 1569\(^\text{T}\) were included “Amino Acids and Derivatives” (214 genes), “Carbohydrates” (122 genes), “Respiration” (26 genes), and “Cofactors, Vitamins, Prosthetic Groups, Pigments” (148 genes).

### Physiological characterization

Strain CAU 1569\(^\text{T}\) was motile by gliding and rod shaped (0.6–0.8 μm in width and 1.8–2.5 μm in length). Cells have no flagella (Supplementary Fig. 2). Gliding motility, which does not require flagella or pili, is a common characterization among the family *Flavobacteriaceae* (Gavriilidou et al. 2020). The characteristics between strain CAU 1569\(^\text{T}\) and closely related reference strains of the genus *Snuella* were summarized in Table 1. Strain CAU 1569\(^\text{T}\) differed from closely related species, *S. lapsa* KACC 14152\(^\text{T}\), based on negative reactions for oxidase production, arginine dihydrolase, acid production from D-ribose and positive reactions for cystine arylamidase and α-glucosidase enzyme activity. Strain CAU 1569\(^\text{T}\) differed from *M. gromovii* KCTC 12570\(^\text{T}\) based on negative reaction for α-chymotrypsin and positive reaction for α-glucosidase. In addition, strain CAU 1569\(^\text{T}\) differed from *A. marinus* KCTC 52521\(^\text{T}\), *A. suncheonense* KACC 16186\(^\text{T}\) based on negative reaction at α-galactosidase. Compared with *Y. aromativorans* KCCM 42019\(^\text{T}\), strain CAU 1569\(^\text{T}\) showed differences at the negative reaction for β-galactosidase and arginine dihydrolase. Based on the comparison of phenotypic characteristics, strain CAU 1569\(^\text{T}\) could be distinguished from recognized species of the genus *Snuella*. The list of all negative reactions of strain CAU 1569\(^\text{T}\) from API test strips are shown in Supplementary Table 1.

### Chemotaxonomic characterization

The only isoprenoid quinone detected from strain CAU 1569\(^\text{T}\) was MK-6, which is a typical feature of the genus *Snuella* (Yi and Chun, 2011) as well as that of other members of the family (Bernardet et al. 1992). The major fatty acids (> 10% of the total fatty acids) of strain CAU 1569\(^\text{T}\) were iso-C\(_{15:0}\) (22.3%), C\(_{17:0}\) iso 3-OH (15.7%) and C\(_{15:1}\)-iso G (12.0%). The fatty acids profile of strain CAU 1569\(^\text{T}\) was similar to those of reference strains, especially iso-C\(_{15:0}\), C\(_{15:1}\)-iso G, and C\(_{17:0}\) iso 3-OH were major fatty acids (Table 2). The major polar lipids of strain CAU 1569\(^\text{T}\) were diphasphatidylglycerol and aminoglycolipid, and minor components were unidentified aminolipid, and three unidentified lipids (Supplementary Fig. 3). The major polar lipids of strain CAU 1569\(^\text{T}\) were similar with those of *S. lapsa* KACC 14152\(^\text{T}\) in that diphasphatidylglycerol and aminoglycolipid are major components.

### Conclusion

The integrated results of phylogenetic, genome feature, physiological and chemotaxonomic characterization supported that strain CAU 1569\(^\text{T}\) represents novel species of the genus *Snuella*, for which the name *Snuella sedimenti* sp. nov. is proposed.
Fig. 1 NJ phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain CAU 1569$^T$ and other related taxa. Filled circles indicate that the corresponding nodes were also recovered in the trees created with the maximum-likelihood and maximum-parsimony algorithms. Bootstrap values (> 70%) are indicated as percentages of 1000 resampled datasets of maximum-parsimony, neighbour-joining, and maximum-parsimony analysis, respectively. Bars represent 0.1 substitutions per nucleotide position. *Flavobacterium aquatile* ATCC 11947$^T$ (M62797) was used as the outgroup strain.
Description of Snuella sedimenti sp. nov.

*Snuella sedimenti* sp. nov. (se.dii’men’ti. L. gen. n. sediment of sediment, isolated from marine sediments).

Cells are gram-stain-negative, aerobic and rod-shaped bacterial strain. Gliding motility, flagella are absent. Growth occurred at temperature range of 20–37 °C (optimum, 30 °C), NaCl concentration of 2–6% (w/v) (optimum, 2%) and pH 6.0–9.0 (optimum, 7.0). Colonies are yellow, circular, smooth, opaque and convex with entire margins on MA. Catalase-positive, but oxidase-negative. Esculin is hydrolysed, but casein and starch are not hydrolysed. Acetoin production was observed. Citrate utilization was positive. Acids are produced from 5-keto-gluconate. Enzyme activities were positive for acid phosphatase, alkaline phosphatase, α-glucosidase, α-fucosidase, β-glucosidase and β-galactosidase, cystine arylamidase, esterase (C4), naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase and 5-keto-gluconate.

The major fatty acids (>10% of total fatty acids) are iso-C<sub>15</sub>:0, iso-C<sub>15</sub>:1-iso G and C<sub>17</sub>:0 iso 3-OH. The major polar lipids are diphosphatidylglycerol and aminoglycolipid. The DNA G + C content of type strain is 35.7 mol% (estimated from genome sequence).

The type strain is CAU 1569<sup>T</sup> (= KCTC 82409<sup>T</sup> = MCCC 1K05670<sup>T</sup>), which was isolated from marine sediment from Shido Island, Incheon, South Korea.

Table 1 Differential properties of strain CAU 1569<sup>T</sup> and the type strains of the most closely related taxa

| Characteristic | 1          | 2          | 3          | 4          | 5          | 6          |
|---------------|------------|------------|------------|------------|------------|------------|
| Temperature (°C): | | | | | | |
| Range | 4–37 | 10–40<sup>a</sup> | 4–37<sup>b</sup> | 4–37<sup>c</sup> | 5–40<sup>d</sup> | 23–39<sup>e</sup> |
| Optimum | 25–30 | 30<sup>a</sup> | 23–25<sup>b</sup> | 30<sup>c</sup> | 25–30<sup>d</sup> | 33–36<sup>e</sup> |
| pH: | | | | | | |
| Range | 4.5–12.0 | 6.0–9.0<sup>a</sup> | 5.5–10.0<sup>b</sup> | 6.0–11.0<sup>c</sup> | 6.0–8.5<sup>d</sup> | 5.0–8.0<sup>e</sup> |
| Optimum | 7.0–7.5 | 7.0<sup>a</sup> | 7.5/8.3<sup>b</sup> | 7.0<sup>c</sup> | 7.0<sup>d</sup> | 7.0<sup>e</sup> |
| NaCl (%, w/v) | | | | | | |
| Range | 0.0–15.0 | 1.0–7.0<sup>a</sup> | 0.5–9.0<sup>b</sup> | 0.5–9.0<sup>c</sup> | 1.0–8.0<sup>d</sup> | 0.5–3.0<sup>e</sup> |
| Optimum | 0.0–4.0 | 3.0–4.0<sup>a</sup> | 0.0–6.0<sup>a</sup> | 2.0–4.0<sup>b</sup> | 1.0–2.0<sup>c</sup> | 1.0<sup>e</sup> |
| Nitrate reduction | – | – | – | + | – | – |
| Enzyme activities: | | | | | | |
| Cystine arylamidase | + | – | + | + | + | + |
| Trypsin | – | – | + | + | – | – |
| α-chymotrypsin | – | – | + | – | – | – |
| α-galactosidase | – | – | – | + | – | + |
| β-galactosidase | – | – | – | + | – | + |
| α-glucosidase | + | – | – | + | + | – |
| α-fucosidase | + | + | – | + | – | – |
| Assimilation: | | | | | | |
| Glucose | – | – | + | – | – | – |
| d-Mannose | – | – | + | – | – | – |
| Acid production: | | | | | | |
| Arbutin | – | – | – | – | + | – |
| Cellobiose | – | – | – | – | + | – |
| DNA G + C content (mol %) | 35.7 | 35.0<sup>a</sup> | 35.7<sup>b</sup> | 37.4<sup>c</sup> | 46.4<sup>d</sup> | 51.4<sup>e</sup> |

Strains: 1, CAU 1569<sup>T</sup>; 2, *S. lapsa* KACC 14152<sup>T</sup> (this study and Yi et al. (2011)<sup>a</sup>); 3, *M. gromovii* KCTC 12570<sup>T</sup> (this study and Nedashkovskaya et al. (2006)<sup>b</sup>); 4, *A. marinum* KCTC 52521<sup>T</sup> (this study and Choi et al. (2018)<sup>c</sup>); 5, *A. suncheonense* KACC 16186<sup>T</sup> (this study and Jeong et al. (2013)<sup>d</sup>); 6, *Y. aromativorans* KCCM 42019<sup>T</sup> (this study and Kwon et al. (2006)<sup>e</sup>). All data were obtained in the present study unless otherwise indicated. All strains were positive for the esculin hydrolysis, alkaline phosphate, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase and 5-keto-gluconate.
Supplementary Information  
The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02528-8.

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Data availability  
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1569T is MN544292 and the whole genome accession number is NZ_JAELVQ000000000.

Table 2  
Cellular fatty acid compositions (%) of strain CAU 1569T and the type strains of the most closely related taxa

| Fatty acid                  | 1   | 2   | 3   | 4   | 5   | 6   |
|-----------------------------|-----|-----|-----|-----|-----|-----|
| Saturated                   |     |     |     |     |     |     |
| C13:0 iso                   | 0.7 | 1.0 | 0.9 | TR  | 0.7 | 0.7 |
| C14:0                       | TR  | TR  | 0.5 | TR  | 1.7 | 0.7 |
| C14:0 iso                   | 0.8 | 1.1 | 1.2 | 0.9 | 0.8 | 2.2 |
| C15:0 anteiso               | 22.3| 20.0| –   | 26.7| 28.3| 20.3|
| C15:1 anteiso A             | 5.4 | 4.3 | 6.8 | 4.9 | 5.7 | 7.4 |
| C15:1 iso                   | 1.0 | 0.9 | 1.1 | 0.8 | TR  | TR  |
| C15:1 iso G                 | 12.0| 12.5| 18.3| 11.7| 5.8 | 6.8 |
| C16:0                       | 1.9 | 1.6 | 1.8 | 1.7 | 8.2 | 2.2 |
| C16:1 iso                   | 0.5 | 1.0 | 1.3 | 2.1 | 1.4 | 3.6 |
| C16:1 iso H                 | 0.8 | 0.8 | 0.5 | 0.9 | 1.9 | 1.5 |
| C17:0 iso                   | TR  | TR  | –   | TR  | 0.6 |   |
| C17:0 anteiso               | –   | –   | –   | –   | –   | 0.2 |
| C17:1 anteiso ω9c           | –   | –   | –   | –   | 2.0 |   |
| Unsaturated                 |     |     |     |     |     |     |
| C15:1 ω6c                   | 1.6 | 2.3 | 4.9 | 1.1 | TR  | 1.0 |
| C17:1 ω6c                   | 1.1 | 1.2 | 1.1 | 1.0 | TR  | 1.0 |
| C17:1 ω8c                   | 0.6 | 0.7 | 0.9 | TR  | TR  | 0.8 |
| C18:1 ω5c                   | 0.6 | TR  | –   | TR  | 0.9 | TR  |
| Hydroxy                     |     |     |     |     |     |     |
| C15:0 2-OH                  | 1.3 | 1.6 | 2.2 | 1.1 | 0.9 | 1.7 |
| C15:0 3-OH                  | 2.8 | 3.0 | 2.9 | 1.5 | –   | 1.7 |
| C16:0 3-OH                  | 2.0 | 1.5 | 1.2 | 1.5 | 3.4 | 1.4 |
| C17:0 2-OH                  | 2.2 | 1.9 | 1.8 | 1.6 | 1.7 | 2.6 |
| C17:0 3-OH                  | 0.9 | 0.9 | 1.0 | –   | –   | –   |
| C15:0 iso 3-OH              | 8.9 | 8.8 | 6.7 | 5.8 | 5.2 | 4.4 |
| C16:0 iso 3-OH              | 5.2 | 7.1 | 6.1 | 5.4 | 3.9 | 10.6|
| C17:0 iso 3-OH              | 15.7| 16.1| 12.2| 18.1| 14.6| 12.6|
| Summed feature*             |     |     |     |     |     |     |
| 3; C16:1 ω6c/C16:1 ω7c      | 7.1 | 7.5 | 7.0 | 9.7 | 6.1 | 10.0|
| 9; (iso-C17:1 ω9c/C16:1 10-methyl | 3.0 | 2.0 | 0.6 | 1.4 | 5.1 | 4.5 |

Strains: 1. CAU 1569T; 2. S. lapsa KACC 14152T; 3. M. gromovii KCTC 12570T; 4. A. marinum KCTC 52521T; 5. A. suncheonense KACC 16186T; 6. Y. aromativorans KCCM 42019T. All data were obtained from this study.

– not detected

TR Trace amount (<0.5%)

Declarations

Conflict of interest  
The authors declare no competing interests.

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