Polyphasic Taxonomic Analysis of *Snuella sedimenti* sp. nov., Isolated from Isolated from Marine Sediment

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Research Article

Keywords: *Snuella sedimenti* sp. nov., Flavobacteriaceae, marine sediment

Posted Date: June 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-578638/v1>

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Version of Record: A version of this preprint was published at Archives of Microbiology on August 17th, 2021. See the published version at <https://doi.org/10.1007/s00203-021-02528-8>.

Abstract

A Gram stain-negative, aerobic, motile by gliding, rod-shaped bacterial strain CAU 1569^T 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 1569^T formed a distinct lineage within the type strain of *Snuella*. Strain CAU 1569^T showed high similarity to *S. lapsa* JC2132^T (95.8%), *Mariniflexile gromovii* KMM 60388^T (95.5%), *Aestuariibaculum marinum* IP7^T (95.4%), *A. suncheonense* SC17^T (94.6%) and *Yeosuana aromativorans* GW1-1^T (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 isoprenoid quinone was only menaquinone 6 (MK-6). The major fatty acids were iso-C_{15:0}, C_{15:1}-iso G, and C_{17:0} iso 3-OH. Strain CAU 1569^T contained diphosphatidylglycerol, aminoglycolipid, unidentified aminolipid, and three unidentified lipids. Based on phylogenetic, genomic, physiologic, and chemotaxonomic characterizations, strain CAU 1569^T represents a novel *Snuella* species, which the name *Snuella sedimenti* sp. nov. is proposed. The type of strain is CAU 1569^T (= KCTC 82409^T = MCCC 1K05670^T).

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 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 (Gavriilidou 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. The genus comprises Gram stain-negative, aerobic, non-motile, and non-spore forming strains. The major isoprenoid quinone is MK-6 and Major fatty acids are iso-C_{15:0}, iso-C_{15:1}, and iso-C_{17:0} 3-OH. In the course of investigating novel bacteria, strain CAU 1569^T 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^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* JC2132^T (=KACC 14152^T), *Mariniflexile gromovii* KMM6038^T (=KCTC 12570^T), *Aestuariibaculum marinum* IP7^T (=KCTC 52521^T), *A. suncheonense* SC17^T (=KACC 16186^T) and *Yeosuana aromativorans* GW1-1^T (=KCCM 42019^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^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^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 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).

Whole-genome analysis

The draft genome of strain CAU 1569^T was sequenced using an Illumina HiSeq sequencer by Macrogen (Seoul, Korea). The sequence data was 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^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^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, Craaponne, France). Strain CAU 1569^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 - 15% (1 % increments, w/v). Catalase and oxidase activity were examined using bubble production in 3% (v/v) H₂O₂ 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 were examined using API ZYM kit (bioMérieux) and the results was 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^T and 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^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^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 rRNA gene sequence analysis revealed that strain CAU 1569^T was a novel strain belonging to the genus *Snuella* with 95.8%, 95.5%, and 95.4% pairwise similarities to *S. lapsa* JC2132^T, *M. gromovii* KMM 6038^T, and *A. marinum* IP7^T, respectively. The neighbour-joining phylogenetic tree indicated that strain CAU 1569^T clustered with members of the genus *Snuella* (Fig. 1). The draft genome of strain CAU 1569^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^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^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^T with RAST server are summarized in Fig. S1. Genome annotations (> 7%) of strain CAU 1569^T were included “Amino Acids and Derivatives” (214 genes), “Carbohydrates” (122 genes), “Respiration” (26 genes), and “Cofactors, Vitamins, Prosthetic Groups, Pigments” (148 genes).

Table 1

Differential properties of strain CAU 1569^T and the type strains of the most closely related taxa

Characteristic	1	2	3	4	5	6
Temperature (°C):						
Range	4-37	10-40 ^a	4-37 ^b	4-37 ^c	5-40 ^d	23-39 ^e
Optimum	25-30	30 ^a	23-25 ^b	30 ^c	25-30 ^d	33-36 ^e
pH:						
Range	4.5-12.0	6.0-9.0 ^a	5.5-10.0 ^b	6.0-11.0 ^c	6.0-8.5 ^d	5.0-8.0 ^e
Optimum	7.0-7.5	7.0 ^a	7.5/8.3 ^b	7.0-9.0 ^c	7.0 ^d	7.0 ^e
NaCl (% w/v)						
Range	0.0-15.0	1.0-7.0 ^a	0.5–9.0 ^a	0.5–9.0 ^b	1.0-8.0 ^c	0.5-3.0 ^e
Optimum	0.0-4.0	3.0-4.0 ^a	0.0-6.0 ^a	2.0-4.0 ^b	1.0-2.0 ^c	1.0 ^e
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 ^a	35.7 ^b	37.4 ^c	46.4 ^d	51.4 ^e

Strains: 1, CAU 1569^T; 2, *S. lapsa* KACC 14152^T (this study and Yi et al. (2011)^a); 3, *M. gromovii* KCTC 12570^T (this study and Nedashkovskaya et al. (2006)^b); 4, *A. marinum* KCTC 52521^T (this study and Choi et al. (2018)^c); 5, *A. suncheonense* KACC 16186^T (this study and Jeong et al. (2013)^d); 6 *Y. aromativorans* KCCM 42019^T (this study and Kwon et al. (2006)^e). 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.

+ Positive, – Negative.

Physiological characterization

Strain CAU 1569^T were gram-stain-negative, aerobic, motile by gliding and rod-shaped (0.6 – 0.8 μm in width and 1.8 - 2.5 μm in length). Colonies were observed to be yellow, circular, smooth, opaque and convex with entire margins with 1.0 – 3.0 mm in diameter. Cells have no flagella (Fig. S2). Gliding motility, which does not require flagella or pili, is common characterization among the family *Flavobacteriaceae* (Gavriilidou et al., 2020). Strain CAU 1569^T grew at temperature range of 20–37°C, NaCl concentration of 2–6% (w/v) and pH 6.0–9.0. The characteristics between strain CAU 1569^T and closely related reference strains of the genus *Snuella* were summarized in Table 1. Strain CAU 1569^T was positive for catalase, esculin hydrolysis, citrate utilization, acetoin production, cystine arylamidase, α-glucosidase, β-glucosidase, α-fucosidase and potassium 5-keto-gluconate, but negative for oxidase production, hydrolysis of casein and starch, urease, gelatinase, nitrate reduction, indole production, arginine dihydrolase, lysine decarboxylase, and tryptophane deaminase. Strain CAU 1569^T differed from closely related species, namely, *S. lapsa* JC2132^T, based on negative reactions for oxidase production. In addition, strain CAU 1569^T differed from *M. gromovii* KMM 6038^T, *A. marinum* IP7^T, *A. suncheonense* SC17^T and *Y. aromativorans* GW1-1^T based on positive reactions for α-fucosidase. Based on the comparison of phenotypic characteristics, strain CAU 1569^T could be distinguished from recognized species of the genus *Snuella*.

Chemotaxonomic characterization

The isoprenoid quinone detected from strain CAU 1569^T was only MK-6, which is typical feature of the genus *Snuella* (Yi and Chun, 2011) as well as the family *Flavobacteriaceae* (Bernardet et al., 1992). The major fatty acids (> 10% of the total fatty acids) of strain CAU 1569^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^T was similar with those of *S. lapsa* JC2132^T, *A. marinum* IP7^T, *A. suncheonense* SC17^T and *Y. aromativorans* GW1-1^T, 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^T were diposphatidylglycerol and aminoglycolipid, and minor components were unidentified

aminolipid, and three unidentified lipids (Fig. S3). The major polar lipids of strain CAU 1569^T were similar with those of *S. lapsa* JC2132^T in that diphosphatidylglycerol and aminoglycolipid are major components.

Table 2

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

Fatty acid	1	2	3	4	5	6
Saturated						
C _{13:0} -iso	0.7	1.0	0.9	TR	0.7	0.7
C _{14:0}	TR	TR	0.5	TR	1.7	0.7
C _{14:0} -iso	0.8	1.1	1.2	0.9	0.8	2.2
C _{15:0} -iso	22.3	20.0	-	26.7	28.3	20.3
C _{15:0} anteiso	5.4	4.3	6.8	4.9	5.7	7.4
C _{15:1} anteiso A	1.0	0.9	1.1	0.8	TR	TR
C _{15:1} -iso	-	-	18.3	-	-	-
C _{15:1} -iso G	12.0	12.5	18.3	11.7	5.8	6.8
C _{16:0}	1.9	1.6	1.8	1.7	8.2	2.2
C _{16:0} -iso	0.5	1.0	1.3	2.1	1.4	3.6
C _{16:1} -iso H	0.8	0.8	0.5	0.9	1.9	1.5
C _{17:0} -iso	TR	TR	-	TR	TR	0.6
C _{17:0} anteiso	-	-	-	-	-	0.2
C _{17:1} anteiso <i>ω</i> 9 <i>c</i>	-	-	-	-	2.0	-
Unsaturated						
C _{15:1} <i>ω</i> 6 <i>c</i>	1.6	2.3	4.9	1.1	TR	1.0
C _{17:1} <i>ω</i> 6 <i>c</i>	1.1	1.2	1.1	1.0	TR	1.0
C _{17:1} <i>ω</i> 8 <i>c</i>	0.6	0.7	0.9	TR	TR	0.8
C _{18:1} <i>ω</i> 5 <i>c</i>	0.6	TR	-	TR	0.9	TR
Hydroxy						
C _{15:0} 2-OH	1.3	1.6	2.2	1.1	0.9	1.7
	2.8	3.0	2.9	1.5	-	1.7

C _{15:0} 3-OH						
C _{16:0} 3-OH	2.0	1.5	1.2	1.5	3.4	1.4
C _{17:0} 2-OH	2.2	1.9	1.8	1.6	1.7	2.6
C _{17:0} 3-OH	0.9	0.9	1.0	-	-	-
C _{15:0} iso 3-OH	8.9	8.8	6.7	5.8	5.2	4.4
C _{16:0} iso 3-OH	5.2	7.1	6.1	5.4	3.9	10.6
C _{17:0} iso 3-OH	15.7	16.1	12.2	18.1	14.6	12.6
Summed feature*						
3; C _{16:1} ω 6c/C _{16:1} ω 7c	7.1	7.5	7.0	9.7	6.1	10.0
9; (iso-C _{17:1} ω 9c/C _{16:0} 10-methyl	3.0	2.0	0.6	1.4	5.1	4.5
Strains: 1, CAU 1569 ^T ; 2, <i>S. lapsa</i> KACC 14152 ^T ; 3, <i>M. gromovii</i> KCTC 12570 ^T ; 4, <i>A. marinum</i> KCTC 52521 ^T ; 5, <i>A. suncheonense</i> KACC 16186 ^T ; 6 <i>Y. aromativorans</i> KCCM 42019 ^T . All data were obtained from this study. –, not detected, TR, Trace amount (<0.5%).						

Conclusion

The integrated results of phylogenetic, genome feature, physiological and chemotaxonomic characterization supported that strain CAU 1569^T represents novel species of the genus *Snuella*, for which the name *Snuella sedimenti* sp. nov. is proposed.

Description of *Snuella sedimenti* sp. nov.

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

Cells are gram-stain-negative, aerobic and rod-shaped bacterial strain. Gliding motility was observed without flagella. 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. 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, leucine arylamidase, and

valine arylamidase. The isoprenoid quinone is only MK-6. The major fatty acids (>10% of total fatty acids) are iso-C_{15:0}, C_{15:1}-iso G and C_{17:0} 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^T (=KCTC 82409^T =MCCC 1K05670^T), which was isolated from marine sediment from Shido Island, Incheon, South Korea.

Declarations

Acknowledgments:

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201902203), and the Chung-Ang University Research Grants in 2019.

Conflicts of interest/Competing Interests

The authors declare no competing interests.

Availability of data and material

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

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Figures

Figure 1

NJ phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain CAU 1569T 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 1,000 resampled datasets of maximum-parsimony, neighbour-

joining, and maximum-parsimony analysis, respectively. Bars represent 0.1 substitutions per nucleotide position. *Flavobacterium aquatile* ATCC 11947T (M62797) was used as the outgroup strain.

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