

Antarcticibacterium arcticum sp. nov., a bacterium isolated from marine sediment of the Canadian Beaufort Sea

Yung Mi Lee^{1,*}, †, Kiwoon Baek^{2,3} †, Dong-Hun Lee⁴, Yerin Park¹, Seung Chul Shin¹ and Young Keun Jin⁵

Abstract

A Gram-stain-negative, aerobic, yellow-pigmented, flexirubin-negative, rod-shaped and non-motile bacterial strain, PAMC 28998^T, was isolated from a surface sediment sample collected from the Canadian Beaufort Sea. Strain PAMC 28998^T grew at 4–37 °C (optimum, 25 °C), at pH 7.0–9.0 (optimum, pH 7.5) and in the presence of 1.0–10.0% (w/v) NaCl. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain PAMC 28998^T belongs to the genus *Antarcticibacterium* showing the highest sequence similarity (96.8%) with *Antarcticibacterium flavum* JB01H24^T. The average nucleotide identity and genome-to-genome distance values between PAMC 28998^T and the most closely related species (*A. flavum* JB01H24^T) were 74.1 and 18.5%, respectively, indicating that strain PAMC 28998^T is clearly distinguished from *A. flavum*. The genomic DNA G+C content calculated from genome sequences was 39.8%. The major fatty acids (>10%) were iso-C_{15:0} (19.5%), anteiso-C_{15:0} (18.0%), iso-C_{16:0} (11.6%) and summed feature 3 (C_{16:1} ω6c and/or C_{16:1} ω7c; 11.4%). The major polar lipids were phosphatidylethanolamine, aminoglycolipid, two unidentified aminolipids, three unidentified phospholipids and four unidentified lipids. The major respiratory quinone was MK-6. Based on the phylogenetic, genomic and phenotypic data presented here, strain PAMC 28998^T is considered to represent a novel species of the genus *Antarcticibacterium*, for which the name *Antarcticibacterium arcticum* sp. nov. is proposed with the strain PAMC 28998^T (=KCCM 43316^T=JCM 33514^T).

The genus *Antarcticibacterium*, a member of the family *Flavobacteriaceae*, was firstly described with the species *Antarcticibacterium flavum* [1]. At the time of writing, the genus *Antarcticibacterium* encompasses one validly published species in the List of Prokaryotic Names with Standing in Nomenclature (www.bacterio.net/Antarcticibacterium.html) [2], which was isolated from marine sediment of the Ross Sea, Antarctica. The genus *Antarcticibacterium* is characterized as being Gram-stain-negative, strictly aerobic, yellow-pigmented, oval to rod-shaped and non-motile. In this study, a bacterial strain (PAMC 28998^T) was isolated from Arctic marine sediment and subjected to polyphasic taxonomic analysis. Strain PAMC 28998^T is considered to represent a

novel species of the genus *Antarcticibacterium*, for which the name *Antarcticibacterium arcticum* sp. nov. is proposed.

HABITAT AND ISOLATION

A sediment sample was collected at an active submarine mud volcano of a depth of 282 m in the Beaufort Sea (70° 38.992' N, 135° 56.811' W) in August, 2014 by using a gravity corer. The sediment core was split, subsampled on board, and stored at –80 °C until use [3]. For cultivation, 100 μl surface sediment suspension in 0.85% NaCl solution was spread on marine agar (MA; Difco) plates and incubated at 10 °C for 15 days. Strain PAMC 28998^T was isolated and subsequently streaked on MA plate three times to obtain pure cultures. PAMC 28998^T was

Author affiliations: ¹Division of Polar Life Sciences, Korea Polar Research Institute, 26 Songdomirae-ro, Yeosu-gu, Incheon 21990, Republic of Korea; ²Department of Biological Sciences, Inha University, Inha-ro 100, Incheon 22212, Republic of Korea; ³Bioresources Collection & Research Division, Nakdonggang National Institute of Biological Resources, 137 Donam 2-gil, Sangju 37242, Republic of Korea; ⁴Hanyang University ERICA Campus, Hanyangdaehak-ro, Sangrok-gu, Ansan 15588, Republic of Korea; ⁵Division of Polar Earth-System Sciences, Korea Polar Research Institute, 26 Songdomirae-ro, Yeosu-gu, Incheon 21990, Republic of Korea.

*Correspondence: Yung Mi Lee, ymlee@kopri.re.kr

Keywords: *Antarcticibacterium arcticum* sp. nov.; polyphasic taxonomy; Arctic; Beaufort Sea.

Abbreviations: AAI, Average Amino acid Identity; AGL, Aminoglycolipid; AL, Aminolipid; ANI, Average Nucleotide Identity; APL, Aminophospholipid; GGDC, Genome-to Genome Distance Calculation; L, Lipid; MA, Marine Agar; MIDI, Microbial Identification; ML, Maximum-likelihood; MP, Maximum-parsimony; NJ, Neighbour-joining; PE, Phosphatidylethanolamine; PL, Phospholipid; RAST, Rapid Annotation using Subsystems Technology; TEM, Transmission Electron Microscopy.

The Genbank accession number for the 16S rRNA gene sequence of strain PAMC 28998^T is MN100311. The accession number of the genome sequence of the strain PAMC 28998^T is CP042476 under the BioProject number PRJNA551306.

†These authors contributed equally to this work

One supplementary table and three supplementary figures are available with the online version of this article.

maintained on MA at 25 °C after the determination of optimal temperature and preserved as 20% (v/v) glycerol at –80 °C. *A. flavum* JB01H24^T was purchased from the Korean Collection of Type Cultures and used as a reference strain following cultivation under the same conditions as PAMC 28998^T.

PHYLOGENY

For 16S rRNA gene amplification by PCR, the genomic DNA was extracted by using the Mini Tissue DNA kit (Cosmogenetech) according to the manufacturer's instructions. The 16S rRNA gene was amplified with two universal primers, 27F and 1492R [4]. PCR products were purified using LaboPass PCR purification kit (Cosmogenetech) and sequenced using primers, 27F, 785F, 926R and 1492R [4]. The 16S rRNA gene sequence (1489 nt) was compared with those of all type strains in the EzBioCloud database [5] and aligned with its closely related type strains of the genera of the family *Flavobacteriaceae* using the jPHYDIT [6]. Phylogenetic trees were reconstructed using three tree-making algorithms, (neighbour-joining [7], maximum-parsimony [8] and maximum-likelihood [9]) using the MEGA X program [10].

The robustness topologies were assessed by bootstrap analyses based on 1000 replications of the sequences. Comparison of 16S rRNA gene sequences showed that strain PAMC 28998^T was closely related to *A. flavum* JB01H24^T (96.8% sequence similarity), *Gillisia myxillae* UST050418-085^T (94.6% sequence similarity) and *Gillisia mitskevichiae* KMM 6034^T (94.1% sequence similarity). In phylogenetic trees inferred from three algorithms, PAMC 28998^T formed a clade with *A. flavum* JB01H24^T and was separated from strains of the other genera of the family *Flavobacteriaceae*, indicating that strain PAMC 28998^T is a member of the genus *Antarcticibacterium* (Fig. 1).

GENOMIC CHARACTERIZATION AND PHYLOGENY

Genomic DNA for whole genome sequencing of PAMC 28998^T was extracted using a MagAttract HMW DNA Kit (Qiagen) according to the manufacturer's instructions. Sequencing was performed using the PacBio RS II instrument (Pacific Biosciences) by constructing a 20 kb insert library with

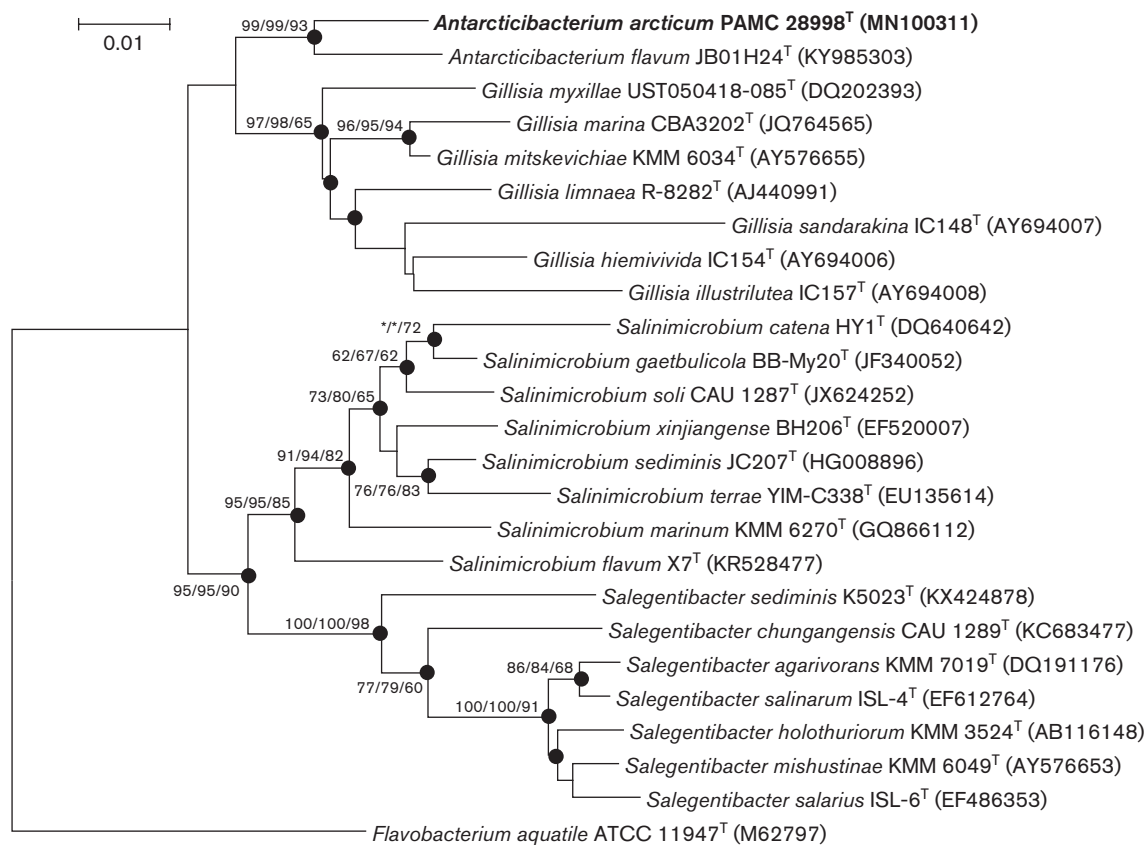


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships of *Antarcticibacterium arcticum* PAMC 28998^T with closely related members of the family *Flavobacteriaceae*. Filled circles indicate that the corresponding nodes were recovered by all treeing methods, neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP). Percent bootstrap supports (>60%) are given at each node (NJ/ML/MP). *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

SMRTbell Template Prep Kit 1.0 (Pacific Biosciences), and the Hierarchical Genome Assembly Process (HGAP.3) [11] was performed for sequence assembly [11] at Lab Genomics (Republic of Korea). Contamination of genome was checked on the basis of 16S rRNA [12] and protein-coding genes [13]. Genome annotation was performed using the Rapid Annotation using Subsystems Technology (RAST) server [14] and a complete 16S rRNA gene sequence was retrieved from the genome sequence of strain PAMC 28998^T after genome annotation. The genomic DNA G+C content was calculated from genome sequences. The 16S rRNA gene sequence of strain PAMC 28998^T determined by direct sequencing was identical to that retrieved from its genome sequence and no contamination of genome was found. The genome size of strain PAMC 28998^T was 3.41 Mbp with a DNA G+C content of 39.8% (Table 1 and S1, available in the online version of this article). A total of 3282 protein-coding sequences were predicted, and 45 tRNA genes and 3 rRNA operons were identified in the genome (Table S1). According to the minimal standards for the use of genomic data in prokaryotic taxonomy [15], other statistics for the genome of strain PAMC 28998^T are given in Table S1.

The degree of pairwise genome-based relatedness with the most close strain *A. flavum* JB01H24^T [16] was estimated by both an average nucleotide identity (ANI) value following the BLAST-based ANI calculation method [17], and the genome-to-genome distance calculation (GGDC) method [18]. The ANI values calculated for the estimation of the degree of pairwise genome-based relatedness between strain PAMC 28998^T and *A. flavum* JB01H24^T was 74.1% and this level is well below the ANI cut-off values (95–96%) to delineate bacterial species [17, 19]. The DNA–DNA hybridization value between strain PAMC 28998^T and *A. flavum* JB01H24^T estimated by GGDC was 18.5%, indicating that strain PAMC 28998^T is distinguishable from *A. flavum* JB01H24^T [20]. In addition, the average amino acid identity (AAI) value between strain PAMC 28998^T and *A. flavum* JB01H24^T was calculated with the Kostas Lab AAI Calculator (<http://enve-omics.ce.gatech.edu/aa/>) using the sequence-based comparison tools output file from RAST [14]. The AAI values ranged from 62.0 to 75.2%, supporting the proposal these strains belong to the same genus [21].

Multiple sequence alignment of the concatenated 120 ubiquitous single-copy proteins [22] for the available sequences of the genera of *Antarcticibacterium*, *Gillisia*, *Salinimicrobium* and *Salegentibacter* in the family *Flavobacteriaceae* was performed by GTDB-Tk [23]. A phylogenomic tree using the neighbour-joining algorithm was reconstructed based on 1000 sets of sequence replication using MEGA X [10]. The phylogenomic tree showed that strain PAMC 28998^T formed a distinct clade from other genera in the family *Flavobacteriaceae* with the exception of *Gillisia limnaea* and *Antarcticibacterium flavum* (Fig. S1). PAMC 28998^T formed a monophyletic clade with *Gillisia limnaea*. However, the ANI values and GGDC between PAMC 28998^T and *Gillisia limnaea* were 73.8% and 18.2%, respectively, and this clade was distinct from other strains in the genus *Gillisia*

Table 1. Major characteristics that differentiate strain PAMC 28998^T from closely related type strain *Antarcticibacterium flavum* JB01H24^T

Strains: 1, PAMC 28998^T; 2, *Antarcticibacterium flavum* JB01H24^T. All data were obtained in this study unless otherwise indicated.

Characteristics	1	2
Growth conditions:		
Temperature (°C) range (optimum)	4–37 (25)	4–42* (25–30)
Sea salt (%) tolerance (optimum)	1.0–10.0 (3–4)	1.0–12.5 (3)
pH range (optimum) [†]	7.0–9.0 (7.5)	7.0–9.5 (7.5–8.0)
Reduction of nitrate	–	+
Enzyme activity:		
α-Galactosidase	+	–
β-Glucuronidase	+	–
Acid production from: [‡]		
D-Galactose, methyl α-D-mannopyranoside, melibiose, potassium 5-ketogluconate	+	–
D-Fructose, inulin, melezitose, raffinose, turanose, D-lyxose	–	+
Polar lipids [§]	PE, AGL, AL, PL, L	PE, APL, AL, L
Genome size (bp)	3409178	4319074
DNA G+C content (%)	39.8	40.9 (42.4)

*Growth temperature range was 4–40 °C [1].

[†]pH range (optimal pH) was 7.0–9.0 for *A. flavum* JB01H24^T according to the results from Li et al. [1].

[‡]Acid production from D-galactose and melibiose of *A. flavum* JB01H24^T determined using the Biolog GEN III MicroPlates was positive [1].

[§]Abbreviations of polar lipids are as follows: PE, phosphatidylethanolamine; AGL, aminoglycolipid; AL, aminolipid; PL, phospholipid; L, lipid; APL, aminophospholipid.

^{||}DNA G+C content data from genome sequences [11] and determined using high-performance liquid chromatography [1], respectively.

supporting that strain PAMC 28998^T belongs to the genus *Antarcticibacterium* (Fig. S1).

Genomic analysis revealed that strain PAMC 28998^T does not possess genes for ABC transporters of amino acids, oligosaccharide or polysaccharide. Instead, genes encoding starch utilization system such as TonB-dependent transporter, starch binding protein, α-glucosidase and neopullulanase were found, implying that starch can be utilized as an energy source [24]. Cold shock proteins were identified which may support the growth of PAMC 28998^T at low temperature (4 °C) consistently with *A. flavum* JB01H24^T [16]. The difference in the genome size between PAMC 28998^T and *A. flavum* JB01H24^T, 0.91 Mbp, may be related with the environmental conditions

that those strains were isolated. However, it is still difficult to predict the ecological functions of the strains of the genus *Antarcticibacterium* and, thus, further study on proteins with unknown function will provide clues as to the role and adaptation mechanism of the strains of the genus *Antarcticibacterium* in marine sediments of polar seas.

PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

The temperature range and optimal temperature for growth were determined by culturing strain PAMC 28998^T and *A. flavum* JB01H24^T on MA at different temperatures (0, 4, 10, 15, 20, 25, 30, 37 and 42 °C) for 14 days. PAMC 28998^T grew at 4–37 °C (optimum, 20–30 °C) and *A. flavum* JB01H24^T grew at 4–42 °C. Thus, all tests were performed using cultures grown on MA at 25 °C. Salt tolerance test was carried on ZoBell agar medium supplemented with 0–20% (0, 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15 and 20%) of NaCl (w/v). The pH range and optimal pH for growth were determined in marine broth medium at pH 5.5–10.0 (at intervals of 0.5 pH unit). The pH was adjusted using the following buffering systems; MES pH 5.5–6.5, MOPS pH 7.0–7.5, AMPD pH 8.0–9.5 and CAPS pH 10.0. The growth of each culture was assessed by measuring optical density at 600 nm (EnVision plate reader, PerkinElmer) every day for up to 14 days. Gram staining was carried out using Gram-stain kit (Sigma) according to the manufacturer's instructions. Motility was determined by the observation of growth after inoculation in the MA liquid medium with 0.4% agar. The presence of flexirubin-type pigments was determined by the bathochromic shift test with 20% KOH solution. Catalase activity was tested with 3% H₂O₂ and oxidase activity was determined using tetramethyl-*p*-phenylenediamine [25]. Hydrolysis was tested on MA supplemented with Tweens 20, 40, 60 and 80, skim milk, xanthine, and hypoxanthine (each 1%, w/v). Other biochemical activities of PAMC 28998^T were determined by using API 20NE, API ZYM and API 50CH kits (bioMérieux) according to manufacturer's instructions except that bacterial strains were suspended in artificial seawater medium [26]. Anaerobic growth was tested on MA in a jar containing an AnaeroPak (Mitsubishi Gas Chemical) for up to 14 days at 25 °C. Morphology of cells was examined by using transmission electron microscopy (TEM) (JEM1010, JEOL). For TEM, cells were negatively stained with 2.0% uranyl acetate on a carbon-coated copper grid. The physiological, morphological, and biochemical characteristics of strain PAMC 28998^T are listed in Table 1, Fig. S2 and the species description. Among the number of physiological characteristics, especially temperature range, salt requirement and pH range for growth of PAMC 28998^T were narrower than those of *A. flavum* JB01H24^T. In addition, acid production from a variety of substrates and enzyme activities differentiated strain PAMC 28998^T from *A. flavum* JB01H24^T (Table 1).

For cellular fatty acids analysis, strains PAMC 28998^T and *A. flavum* JB01H24^T were grown on MA at 25 °C for 5 days. Cellular fatty acids were extracted as described by Sasser [27] and were analysed according to the method described

by the Sherlock Microbial Identification System version 6.1 (MIDI) using the TSBA6.1 database. The isoprenoid quinones extracted according to the method described in Minnikin *et al.* [28] were analysed by using high-performance liquid chromatography [29] at the Korean Culture Collection of Microorganisms (Republic of Korea). Polar lipids of strain PAMC 28998^T were extracted from lyophilized bacterial cells, separated using two-dimensional thin layer chromatography, and detected by spraying the reagents molybdatophosphoric acid, ninhydrin, molybdenum blue, α -naphthol, Dragendorff's solution and Schiff's solution [28]. The major fatty acids (>10%) of strain PAMC 28998^T were iso-C_{15:0} (19.5%), anteiso-C_{15:0} (18.0%), iso-C_{16:0} (11.6%) and summed feature 3 (C_{16:1} ω 6c and/or C_{16:1} ω 7c; 11.4%) (Table 2). MK-6 was the menaquinone present in strain PAMC 28998^T. The polar lipids of strain PAMC 28998^T comprised phosphatidylethanolamine, aminoglycolipid, two unidentified aminolipids, three unidentified phospholipids and four unidentified lipids (Fig. S3). This composition was similar to that of the closely related type strain, *A. flavum* JB01H24^T, with respect to the presence of phosphatidylethanolamine, unidentified aminolipids and unidentified lipids [1]. However, the presence of aminoglycolipid and three unidentified phospholipids in PAMC 28998^T distinguished it from *A. flavum* JB01H24^T.

Overall, strain PAMC 28998^T formed a monophyletic clade with *A. flavum* JB01H24^T (Fig. 1 and S1). However, physiological characteristics such as temperature range, salt tolerance, acid production from a variety of substrates, enzyme activities and polar lipid profiles differentiated strain PAMC 28998^T from closely related type strain *A. flavum* JB01H24^T. Therefore, strain PAMC 28998^T represents a novel species of the genus *Antarcticibacterium* for which the name *Antarcticibacterium arcticum* sp. nov. is proposed.

DESCRIPTION OF ANTARCTICIBACTERIUM ARCTICUM SP. NOV.

Antarcticibacterium arcticum (arc'ti.cum. L. neut. adj. *arcticum* northern, arctic, referring to the site where the type strain was isolated).

Cells are Gram-stain-negative, aerobic, yellow-pigmented, oxidase- and catalase- positive, non-motile, flexirubin-negative and rod-shaped (0.4–0.9 μ m wide and 1.1–2.2 μ m long). Colonies are circular, convex and glittering on MA plates after 7 days incubation at 25 °C. Growth occurs at 4–37 °C (optimum, 25 °C), pH 7.0–9.0 (optimum, pH 7.5) and in the presence of 1–10% (w/v) NaCl (optimum, 3.0–7.5%) after 14 days of incubation on MA at 25 °C. Tween 80 is hydrolysed. In the API 20NE system, positive for aesculin and gelatin hydrolysis, glucose fermentation and β -galactosidase, but negative for nitrate reduction (nitrate to nitrite or nitrogen), indole production, activity of arginine dihydrolyase and urease, and assimilation of adipate, caprate, citrate, D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malate, N-acetylglucosamine, phenyl acetate and potassium gluconate. In the API ZYM system, positive for the activity

Table 2. Cellular fatty acid composition of strain PAMC 28998^T and *Antarcticibacterium flavum* JB01H24^T

Strains: 1, PAMC 28998^T; 2, *Antarcticibacterium flavum* JB01H24^T. All data were taken from this study. –, Not detected. Results are presented as percentages of the total fatty acids. Fatty acids amounting to 10% or more are highlighted in bold.

Fatty acid	1	2
Saturated:		
C _{14:0}	0.6	–
C _{15:0}	4.1	6.9
C _{16:0}	4.1	1.0
C _{18:0}	1.0	–
Unsaturated:		
C _{15:1} ω6c	0.9	1.5
C _{17:1} ω6c	1.8	1.7
Branched:		
iso-C _{13:0}	–	0.5
iso-C _{14:0}	0.6	–
iso-C _{15:0}	19.5	24.7
iso-C _{16:0}	11.6	5.7
iso-C _{17:0}	1.1	0.6
anteiso-C _{15:0}	18.0	10.1
anteiso-C _{17:0}	1.6	–
Branched mono unsaturated:		
iso-C _{15:1} G	6.1	7.8
iso-C _{16:1} h	2.4	1.7
anteiso-C _{15:1} A	0.5	0.9
anteiso-C _{17:1} ω9c	3.6	4.3
Hydroxy:		
C _{15:0} 2-OH	3.3	3.5
C _{17:0} 2-OH	1.5	2.1
Iso-C _{15:0} 3-OH	1.0	3.4
Iso-C _{16:0} 3-OH	–	0.7
Iso-C _{17:0} 3-OH	1.5	4.4
Summed features:*		
3	11.4	10.1
9	3.8	8.2

*Summed features represent fatty acids that could not be separated by the GLC with the MIDI system. Summed feature 3 comprises C_{16:1} ω6c and/or C_{16:1} ω7c and summed feature 9 comprises iso C_{17:1} ω9c and/or 10-methyl C_{16:0}. MIDI, Microbial Identification .

of acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase, esterase lipase, leucine arylamidase, *N*-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase, α-chymotrypsin, α-galactosidase, α-glucosidase, β-glucosidase and β-glucuronidase, but negative for the activity of lipase, α-fucosidase, α-mannosidase and β-galactosidase. In the API 50CH system, acid is produced from starch, amygdalin, arbutin, cellobiose, D-galactose, D-glucose, lactose, maltose, melibiose, sucrose, trehalose, aesculin ferric citrate, gentiobiose, glycogen, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, *N*-acetylglucosamine, potassium 5-ketogluconate and salicin, but negative for acid production from D-adonitol, D-arabinose, D-arabitol, D-fructose, D-fucose, D-lyxose, D-mannitol, D-mannose, melezitose, raffinose, D-ribose, D-sorbitol, D-tagatose, turanose, D-xylose, dulcitol, erythritol, glycerol, inositol, inulin, L-arabinose, L-arabitol, L-fucose, L-rhamnose, L-sorbose, L-xylose, methyl β-D-xylopyranoside, potassium 2-ketogluconate, potassium gluconate and xylitol. The major cellular fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} and summed feature 3 (C_{16:1} ω6c and/or C_{16:1} ω7c). The major isoprenoid quinone is MK-6. The polar lipids are phosphatidylethanolamine, aminoglycolipid, two unidentified aminolipids, three unidentified phospholipids and four unidentified lipids. The type strain, PAMC 28998^T (=KCCM 43316^T = JCM 33514^T), was isolated from a surface sediment sample collected from the Canadian Beaufort Sea.

The type strain is PAMC 28998^T (=KCCM 43316^T=JCM 33514^T), isolated from the surface sediment of the Canadian Beaufort Sea. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the complete genome sequence of strain PAMC 28998^T are MN100311 and CP042476, respectively.

Funding information

This study was supported by the Korea Polar Research Institute (grant no. PM19050), the Korea Institute of Marine Science and Technology Promotion (grant no. 20160247), and the Nakdonggang National Institute of Biological Resources funded by the Ministry of Environment of the Republic of Korea (grant no. NNIBR201902110).

Acknowledgements

We thank the crew of the *R/V ARAON* for their support at sea. We thank Dr Chung Yeon Hwang at Seoul National University for correcting the etymologies in nomenclature.

Conflicts of interest

The authors declare that there are no conflicts of interest

Ethical statement

The authors declare that there are no conflicts of interest

References

- Li A-Z, Lin L-Z, Zhang M-X, Zhu H-H. *Antarcticibacterium flavum* gen. nov., sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2018;68:254–259.
- Parte AC. LPSN - List of Prokaryotic names with Standing in Nomenclature (bacterio.net), 20 years on. *Int J Syst Evol Microbiol* 2018;68:1825–1829.
- Lee D-H, Kim J-H, Lee YM, Stadnitskaia A, Jin YK et al. Biogeochemical evidence of anaerobic methane oxidation on active

- submarine mud volcanoes on the continental slope of the Canadian Beaufort sea. *Biogeosciences* 2018;15:7419–7433.
4. Lane DJ, Stackebrandt E, Goodfellow M. *Nucleic Acid Techniques in Bacterial Systematics*; 1991. pp. 115–175.
 5. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y et al. Introducing EzBioCloud: a taxonomically United database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
 6. Jeon Y-S, Chung H, Park S, Hur I, Lee J-H et al. jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* 2005;21:3171–3173.
 7. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
 8. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Biol* 1971;20:406–416.
 9. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512–526.
 10. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
 11. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 2013;10:563–569.
 12. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H et al. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int J Syst Evol Microbiol* 2017;67:2053–2057.
 13. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
 14. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T et al. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
 15. Chun J, Oren A, Ventosa A, Christensen H, Arahall DR et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
 16. Lee YM, Jin YK, Shin SC. Complete genome sequence of *Antarcticibacterium flavum* JB01H24^T from an Antarctic marine sediment. *Mar Genom* 2019;100695.
 17. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P et al. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
 18. Auch AF, von Jan M, Klenk H-P, Göker M. Digital DNA–DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2010;2:117–134.
 19. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.
 20. Rosselló-Mora R, Amann R. The species concept for prokaryotes. *FEMS Microbiol Rev* 2001;25:39–67.
 21. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* 2005;102:2567–2572.
 22. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018;36:996–1004.
 23. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 2018.
 24. Foley MH, Cockburn DW, Koropatkin NM. The Sus operon: a model system for starch uptake by the human gut Bacteroidetes. *Cell Mol Life Sci* 2016;73:2603–2617.
 25. Kovacs N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 1956;178:703.
 26. Choo Y-J, Lee K, Song J, Cho J-C. *Puniceicoccus vermicola* gen. nov., sp. nov., a novel marine bacterium, and description of *Puniceicoccaceae* fam. nov., *Puniceicoccales* ord. nov., *Opiritaceae* fam. nov., *Opiritales* ord. nov. and *Opiritae* classis nov. in the phylum 'Verrucomicrobia'. *Int J Syst Evol Microbiol* 2007;57:532–537.
 27. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids 1990.
 28. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M et al. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
 29. Collins MD. Analysis of isoprenoid quinones. *Method Microbiol* 1985:329–366.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.