Sediminicola arcticus sp. nov., a psychrophilic bacterium isolated from deep-sea sediment, and emended description of the genus *Sediminicola*

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A Gram-stain-negative, rod-shaped and non-motile strain, designated PAMC 27266^T, was isolated from deep-sea sediment of the Arctic Ocean. Analysis of the 16S rRNA gene sequence of strain PAMC 27266^T showed closest affiliation with the genus *Sediminicola*. Phylogenetic analyses revealed that strain PAMC 27266^T formed a robust clade with *Sediminicola luteus* CNI-3^T, with which it shared 98.9 % 16S rRNA gene sequence similarity. Genomic relatedness analyses based on the average nucleotide identity and genome-to-genome distance showed that strain PAMC 27266^T is clearly distinguished from *S. luteus*. Cells of strain PAMC 27266^T grew optimally at 15 °C and pH 6.5–7.5 in the presence of 3.5 % (w/v) sea salts. The major polar lipids were phosphatidylethanolamine, two unidentified aminophospholipids and two unidentified lipids. The only respiratory quinone was menaquinone-6. The major cellular fatty acids (>10 %) were $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$ and $C_{15:0}$. The genomic DNA G+C content was 37.9 mol%. Based on the phylogenetic, genomic, chemotaxonomic and phenotypic data presented, we propose strain PAMC 27266^T (=KCCM 43038^T=JCM 19894^T) as the type strain of a novel species, with the name *Sediminicola arcticus* sp. nov.

The genus *Sediminicola* was established by Khan *et al.* (2006), with *Sediminicola luteus* as the type species, for a non-motile bacterium isolated from sediment of the shore of the Sea of Japan. At the time of publication of Khan *et al.* (2006), the genus *Sediminicola* was found to be phylogenetically distantly related to other valid genera in the family *Flavobacteriaceae* with low 16S rRNA gene sequence similarities (approximately 88–91 %). The absence of pentadecanoic acid ($C_{15:0}$) from *S. luteus* CNI-3^T (=NBRC 100966^T) along with three sister strains corroborated the proposal of a novel genus within the family *Flavobacteriaceae* (Khan *et al.*, 2006). In this study, a new strain phylogenetically affiliated with the genus *Sediminicola* was isolated from deep-sea sediment and subjected to a polyphasic taxonomic analysis.

A sediment core, ARA03B-15(B)MUC-02, was taken using a multi-core sampler from the Mendeleev Ridge in the western Arctic Ocean (78° 6′ 35″ N, 175° 14′ 2″ W; water column depth of 1149 m) during the *Araon* expedition in August 2012. A surface sample of the sediment core was diluted approximately 50-fold with autoclaved ambient overlying seawater. An aliquot (100 µl) of the sediment slurry was spread onto a plate containing marine agar 2216 (MA; Difco) and the plate was incubated aerobically at 20 °C for 1 week. Strain PAMC 27266^T was isolated on the plate and subsequently purified four times on fresh MA at 20 °C. The strain was maintained both on MA at 4 °C and in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at −80 °C.

S. luteus NBRC 100966^T was obtained from NITE Biological Resource Center (NBRC) to compare the physiological and chemotaxonomic characteristics with strain PAMC 27266^T. In preliminary growth tests, optimal temperatures for growth were different between the two strains (see below). Thus, unless otherwise specified, all characteristics were based on cultures grown aerobically on MA for 3–5 days at 15 and 20 °C for strains PAMC 27266^T and *S. luteus* NBRC 100966^T, respectively. Under these conditions, both strains appeared to be in mid- to late-exponential phase. A sister strain, *S. luteus* NBRC 100967 (=PMAOS-27; Khan *et al.*, 2006), was also obtained from the NBRC for genomic comparison.

Abbreviations: ANI, average nucleotide identity; GGDC, genome-to-genome distance calculation.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PAMC 27266^T is KM576847.

One supplementary table and two supplementary figures are available with the online Supplementary Material.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony by a boiling method (Englen & Kelley, 2000). The crude extract served as the DNA template for PCRs, which included *Taq* DNA polymerase (Promega) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using shrimp alkaline phosphatase and exonuclease I (USB), with incubation at 37 °C for 30 min and subsequently at 80 °C for 10 min. Direct sequencing of the purified PCR product was performed using sequencing primers (27F, 337F, 518R, 785F and 1492R; Lane, 1991; Anzai *et al.*, 1997) with an Applied Biosystems sequencer (ABI 3730XL) at Cosmo Genetech (Seoul, Korea). The almost-complete 16S rRNA gene sequence of strain PAMC 27266^T (1416 bp) was obtained. Phylogenetic analyses were performed as described by Hwang *et al.* (2015).

The fatty acid methyl esters in whole cells of strains PAMC 27266^T and *S. luteus* NBRC 100966^T grown on MA for 5 days at 15 and 20 °C, respectively, were analysed by GC (Agilent technologies 6890) according to the instructions of the Microbial Identification System (MIDI; version 6.2) with the RTSBA6 database at the Korean Culture Center of Microorganisms (KCCM). Isoprenoid quinone composition was determined according to Minnikin *et al.* (1984) and analysed by HPLC at the KCCM as described by Collins (1985). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures described by Minnikin *et al.* (1984). The DNA G+C content was determined by HPLC analysis (Tamaoka & Komagata, 1984) carried out by the identification service of the KCCM. For genomic comparison, genomic DNA of strain PAMC 27266^T, *S. luteus* NBRC 100966^T and *S. luteus* NBRC 100967 was extracted using the DNeasy Tissue and Blood kit (Qiagen) and genome sequencing was performed using the 454 Genome Sequencer FLX+ system (Roche) at Macrogen (Seoul, Korea). The level of pairwise genome-based similarity was estimated based on both the average nucleotide identity (ANI) value following the BLAST-based ANI calculation method described by Goris *et al.* (2007) and the genome-to-genome distance calculation (GGDC) method described by Auch *et al.* (2010).

Tests for phenotypic characteristics of strain PAMC 27266^T were performed in duplicate along with the type strain of *S. luteus*, with repeat experiments on different days. Gram-staining was performed as described by Smibert & Krieg (1994). Cell motility was assessed by the hanging drop method (Skerman, 1967) with cells grown in MB for 5 days. Cellular morphology and the presence of flagella were observed using transmission electron microscopy (EX2; JEOL). Anaerobic growth was tested in an anaerobic jar containing an AnaeroPak (Mitsubishi Gas Chemical) at 15 °C for 2 weeks. The temperature range for growth was examined by assessing changes in OD_{600} with time in MB at 4, 10–30 (at increments of 5 °C), 37 and 42 °C. The pH range (pH 5.0–10.0 at intervals of 0.5 pH units) for growth was determined by assessing changes in OD_{600} in pH-buffered MB (Hwang & Cho, 2008) using citric acid-phosphate buffer for pH 5.0, MES for pH 5.5–6.5, MOPS for pH 7.0–7.5, AMPD for pH 8.0–9.5 and CAPS for pH 10.0, each at a final concentration of 50 mM for up to 10 days. Salt tolerance was determined by assessing changes in OD_{600} at 15 °C using synthetic ZoBell broth (Bacto peptone, 5 g; yeast extract, 1 g; ferric citrate, 0.1 g; distilled water, 1 litre) supplemented with 0–3.5 % (at intervals of 0.5 %), 4–10 % (at intervals of 1 %) and 12 % (w/v) sea salts (Sigma).

Catalase and oxidase tests were performed according to the methods described by Smibert & Krieg (1994) and Cappuccino & Sherman (2002), respectively. Decomposition of casein, hypoxanthine and xanthine was determined as described by Smibert & Krieg (1994). Hydrolysis of starch, Tween 40 and Tween 80 was investigated as described by Hansen & Sørheim (1991). In addition, other enzyme activities and acid production were assayed using the API ZYM, API 20NE and API 50CH kits (bioMérieux) according to the manufacturer's instructions except that the cell suspension was prepared as described by Hwang *et al.* (2009). Carbon utilization was tested according to Bruns *et al.* (2001) with a final concentration of 0.4 % carbon source. Carbon utilization was scored as negative when growth was equal to, or less than, that in the negative control with no carbon source. Growth was measured by monitoring changes in the OD_{600} for 20 days at 15 °C.

Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain PAMC 27266^T belonged to the family *Flavobacteriaceae* (Fig. 1). Strain PAMC 27266^T was closely related to *S. luteus* CNI-3^T with 98.9 % similarity, and no other species shared more than 94 % sequence similarity with strain PAMC 27266^T in the family *Flavobacteriaceae*. The tree topologies inferred from three tree-making algorithms showed that strain PAMC 27266^T formed a robust cluster with *S. luteus* CNI-3^T (Fig. 1). This grouping was supported by high bootstrap values (neighbour-joining, 100 %; minimum-evolution, 100 %; maximum-likelihood, 99 %).

Details of the genome relatedness of the draft genomes of strains PAMC 27266^T, *S. luteus* NBRC 100966^T and *S. luteus* NBRC 100967 are summarized in Table S1 (available in the online Supplementary Material). The ANI values calculated for estimation of the degree of pairwise genome-based relatedness between strain PAMC 27266^T and the two strains of *S. luteus* were 85.1–85.3 % (Table S1). This level is obviously below the proposed cut-off ANI values of 95–96 % for delineating bacterial species (Goris *et al.*, 2007; Richter & Rosselló-Móra, 2009). Consistently, DNA–DNA hybridization values estimated by GGDC were 25.9–26.2 % between strain PAMC 27266^T and the two strains of *S. luteus* (Table S1), indicating that strain PAMC 27266^T is a member of a separate species of the genus *Sediminicola* (Rosselló-Mora & Amann, 2001).

The morphological, physiological and biochemical characteristics for strain PAMC 27266^T are given in the species description and Table 1. The sole isoprenoid quinone in strain PAMC 27266^T was menaquinone-6 (MK-6), as in *S.*

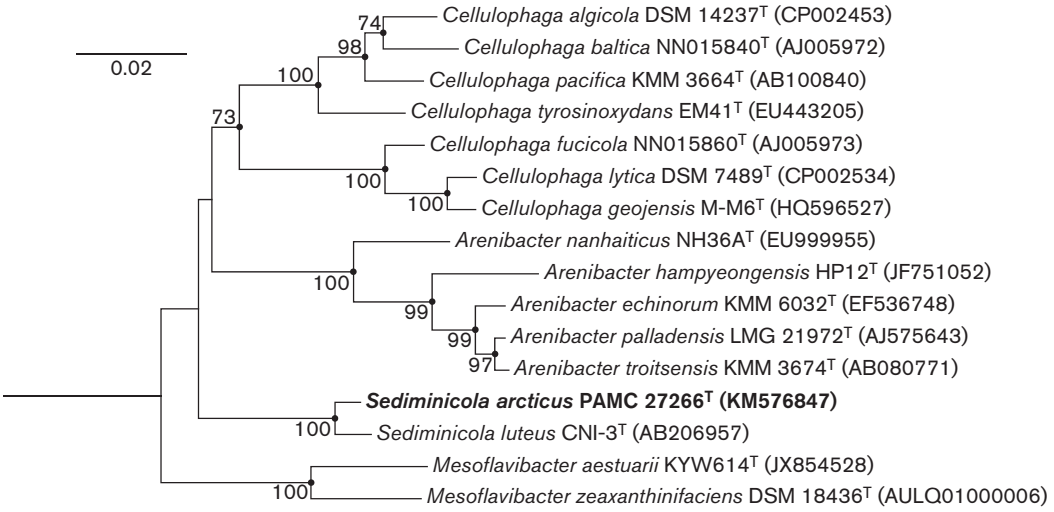

Fig. 1. Neighbour-joining tree derived from 16S rRNA gene sequences for strain PAMC 27266^T and related members in the family *Flavobacteriaceae* with *Weeksella virosa* ATCC 43767^T (accession no. M93152) as an outgroup (not shown). Only bootstrap values above 70 % are shown (1000 resamplings) at branch points. Solid circles indicate that the corresponding nodes were also recovered in the minimum-evolution and maximum-likelihood trees. Bar, 0.02 nt substitutions per site.

luteus NBRC 100966^T (Khan *et al.*, 2006). The fatty acid profiles and the polar lipid profiles were generally similar between strain PAMC 27266^T and *S. luteus* NBRC 100966^T (Table 2, Fig. S1). The dominant fatty acids of strain PAMC 27266^T were summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; 11.4 %), $C_{15:0}$ (10.0 %), iso-$C_{16:0}$ 3-OH (9.3 %), iso-$C_{15:1}$ G (8.8 %) and iso-$C_{17:0}$ 3-OH (9.1 %). Pentadecanoic acid ($C_{15:0}$) was detected in both strain PAMC 27266^T and *S. luteus* NBRC 100966^T (Fig. S2), while the absence of $C_{15:0}$ was given as a characteristic feature of the genus *Sediminicola* by Khan *et al.* (2006). The major polar lipids of strain PAMC 27266^T were phosphatidylethanolamine, two unidentified aminophospholipids and two unidentified lipids (Fig. S1). The genomic DNA G+C

Table 1. Differential characteristics between strain PAMC 27266^T and *Sediminicola luteus* NBRC 100966^T

Data were obtained from this study unless otherwise indicated. +, Positive; −, negative.

Characteristic	PAMC 27266^T	*S. luteus* NBRC 100966^T
Isolation source	Deep-sea sediment	Coastal sediment*
Temperature range for growth (optimum) (°C)	4–20 (15)	4–37 (20)
pH range for growth (optimum)	6.0–8.0 (6.5–7.5)	6.0–8.5 (7.0–7.5)
Salt tolerance range for growth (optimum) (%, w/v)	0.5–8.0 (3.5)	0.5–10.0 (3.5)
Acid production (API 50CH) from:		
L-Arabinose	+	−
D-Fucose	−	+
Glycerol	−	+
Glycogen	−	+
D-Mannose	−	+
L-Sorbose	+	−
DL-Xylose	+	−
Utilization of sole carbon source:		
N-Acetyl-D-galactosamine	+	−
L-Glutamic acid	−	+
Succinate	+	−
DNA G+C content (mol%)	37.9	38–40*

*Data from Khan *et al.* (2006).

content of strain PAMC 27266^T was 37.9 mol%, which is similar to that of *S. luteus* NBRC 100966^T (Table 1).

Strain PAMC 27266^T can be distinguished from *S. luteus* NBRC 100966^T by the inability to grow at 25–37 °C (Table 1). According to the definition given by Morita (1975), strain PAMC 27266^T is a psychrophilic bacterium. Other phenotypic characteristics also differentiate strain PAMC 27266^T from *S. luteus* NBRC 100966^T, namely the inability to grow in the presence of 9–10 % sea salts, ability to produce acid from L-arabinose, L-sorbose and DL-xylose, inability to produce acid from D-fucose, glycerol, glycogen or D-mannose, and inability to utilize L-glutamic acid and ability to utilize *N*-acetyl-D-galactosamine and succinate as a sole carbon source (Table 1).

Overall, the phylogenetic, genomic, chemotaxonomic and phenotypic data obtained in this study indicate that strain PAMC 27266^T should be assigned to a novel species in the genus *Sediminicola*, for which the name *Sediminicola arcticus* sp. nov. is proposed.

Table 2. Cellular fatty acid contents of strain PAMC 27266^T and *Sediminicola luteus* NBRC 100966^T

Fatty acids were analysed for cells grown on MA for 5 days. Incubation temperatures were 15 and 20 °C for strain PAMC 27266^T and *S. luteus* NBRC 100966^T, respectively. Values are percentages of total fatty acids. TR, Trace amount (<1 %), −, not detected.

Fatty acid	PAMC 27266^T	*S. luteus* NBRC 100966^T
Saturated		
$C_{15:0}$*	10.0	16.3
$C_{16:0}$	1.9	4.4
$C_{18:0}$	1.3	TR
Unsaturated		
$C_{15:1}\omega6c$	2.9	1.6
$C_{17:1}\omega6c$	2.6	1.1
$C_{17:1}\omega8c$	1.3	1.7
$C_{18:1}\omega9c$	1.0	TR
Branched		
iso-$C_{14:0}$	−	1.8
iso-$C_{15:1}$ G	8.8	5.9
anteiso-$C_{15:1}$ A	1.3	TR
iso-$C_{15:0}$	3.8	11.6
anteiso-$C_{15:0}$	4.3	4.7
iso-$C_{16:0}$	1.8	4.3
iso-$C_{16:1}$ H	1.9	TR
Hydroxy		
$C_{15:0}$ 3-OH	2.4	TR
iso-$C_{15:0}$ 3-OH	7.3	3.4
$C_{16:0}$ 3-OH	1.8	1.9
iso-$C_{16:0}$ 3-OH	9.3	4.7
iso-$C_{17:0}$ 3-OH	9.1	11.8
Summed features†		
3	11.4	9.8
5	1.0	−
9	3.6	3.9

*Although $C_{15:0}$ was not listed in the MIDI version 6.2, an obvious peak with equivalent chain-length of 15.00 was detected by GC in each strain, as shown in Fig. S2.

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprised $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; summed feature 5 comprised $C_{18:2}\omega6,9c$ and/or anteiso-$C_{18:0}$; summed feature 9 comprised 10-methyl $C_{16:0}$ and/or iso-$C_{17:1}\omega9c$.

Emended description of the genus *Sediminicola* Khan *et al.*, 2006

The description is as given by Khan *et al.* (2006) with the following amendment. The major polar lipids are phosphatidylethanolamine, two unidentified aminophospholipids and two unidentified lipids.

Description of *Sediminicola arcticus* sp. nov.

Sediminicola arcticus (arc′tic.us. L. masc. adj. *arcticus* of the Arctic, the environment from where the type strain was isolated).

Gram-stain-negative, aerobic, non-motile rods approximately 0.3–0.5 μm wide and 0.8–2.9 μm long. After 7 days on MA plates at 15 °C, colonies are red–orange, circular and convex, and approximately 1–2 mm in diameter. Grows at 4–20 °C (optimum 15 °C) and pH 6.0–8.0 (optimum pH 6.5–7.5). Growth occurs with 0.5–8.0 % (w/v) sea salts (optimum 3.5 %). Positive for oxidase and catalase. Starch, Tween 40 and Tween 80 are hydrolysed, but casein, hypoxanthine and xanthine are not. According to the API ZYM test, positive for acid phosphatase, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, esterase (C4), esterase lipase (C8) and α-mannosidase, but negative for α-fucosidase, β-glucuronidase and lipase (C14). According to the API 20NE test, positive for gelatinase, aesculin hydrolysis, β-galactosidase (PNPG) and nitrate reductase, but negative for arginine dihydrolase, glucose fermentation, indole production and urease. According to the API 50CH test, acid is produced from *N*-acetylglucosamine, aesculin, amygdalin, L-arabinose, arbutin, cellobiose, D-galactose, D-glucose, lactose, maltose, D-mannitol, melezitose, melibiose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, raffinose, salicin, L-sorbose, sucrose, trehalose and DL-xylose, but not from D-adonitol, D-arabinose, DL-arabitol, dulcitol, erythritol, D-fructose, DL-fucose, gentiobiose, glycogen, glycerol, inositol, inulin, D-lyxose, D-mannose, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-rhamnose, D-ribose, D-sorbitol, starch, D-tagatose, turanose or xylitol. *N*-Acetyl-D-galactosamine, L-aspartic acid, cellobiose, D-galactose,

glycogen, maltose, mannitol, D-mannose, melibiose, L-ornithine, raffinose, succinate, sucrose and L-threonine are utilized as a sole carbon source, but acetate, citrate, formic acid, glycerol and L-rhamnose are not. The major cellular fatty acids are $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$ and $C_{15:0}$. The major polar lipids are phosphatidylethanolamine, two unidentified aminophospholipids and two unidentified lipids. The major quinone is MK-6.

The type strain, PAMC 27266^T (=KCCM 43038^T=JCM 19894^T), was isolated from Arctic deep-sea sediment. The DNA G+C content of the type strain is 37.9 mol%.

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