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 27266T, was isolated from deep-sea sediment of the Arctic Ocean. Analysis of the 16S rRNA gene sequence of strain PAMC 27266T showed closest affiliation with the genus Sediminicola. Phylogenetic analyses revealed that strain PAMC 27266T formed a robust clade with Sediminicola luteus CNI-3T, 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 27266T is clearly distinguished from S. luteus. Cells of strain PAMC 27266T 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 C16 : 1ω7c and/or C16 : 1ω5c and C15 : 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 27266T (=KCCM 43038T =JCM 19894T) 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 (C15 : 0) from S. luteus CNI-3T (=NBRC 100966T) 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 Mendeeledge 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 27266T 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 100966T was obtained from NITE Biological Resource Center (NBRC) to compare the physiological and chemotaxonomic characteristics with strain PAMC 27266T. 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 27266T and S. luteus NBRC 100966T, 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.
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\textsuperscript{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\textsuperscript{T} and S. luteus NBRC 100966\textsuperscript{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 RTSSBA6 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\textsuperscript{T}, S. luteus NBRC 100966\textsuperscript{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\textsuperscript{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 culture tube (Becto 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\textsubscript{600} for 20 days at 15 °C.

Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain PAMC 27266\textsuperscript{T} belonged to the family Flavobacteriaceae (Fig. 1). Strain PAMC 27266\textsuperscript{T} was closely related to S. luteus CNI-3\textsuperscript{T} with 98.9 % similarity, and no other species shared more than 94 % sequence similarity with strain PAMC 27266\textsuperscript{T} in the family Flavobacteriaceae. The tree topologies inferred from three tree-making algorithms showed that strain PAMC 27266\textsuperscript{T} formed a robust cluster with S. luteus CNI-3\textsuperscript{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\textsuperscript{T}, S. luteus NBRC 100966\textsuperscript{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\textsuperscript{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 & Rossello-Móra, 2009). Consistently, DNA–DNA hybridization values estimated by GGDC were 25.9–26.2 % between strain PAMC 27266\textsuperscript{T} and the two strains of S. luteus (Table S1), indicating that strain PAMC 27266\textsuperscript{T} is a member of a separate species of the genus Sediminicola (Rossello-Móra & Amann, 2001).

The morphological, physiological and biochemical characteristics for strain PAMC 27266\textsuperscript{T} are given in the species description and Table 1. The sole isoprenoid quinone in strain PAMC 27266\textsuperscript{T} was menaquinone-6 (MK-6), as in S. luteus.
Sediminicola arcticus sp. nov. (Khan et al., 2006). The fatty acid profiles and the polar lipid profiles were generally similar between strain PAMC 27266<sup>T</sup> and *S. luteus* NBRC 100966<sup>T</sup> (Table 2, Fig. S1). The dominant fatty acids of strain PAMC 27266<sup>T</sup> were summed feature 3 (C<sub>16:1</sub><i>ω6c</i> and/or C<sub>16:1</sub><i>ω7c</i>; 11.4 %), C<sub>15:0</sub> (10.0 %), iso-C<sub>16:0</sub> 3-OH (9.3 %), iso-C<sub>15:1</sub> G (8.8 %) and iso-C<sub>17:0</sub> 3-OH (9.1 %). Pentadecanoic acid (C<sub>15:0</sub>) was detected in both strain PAMC 27266<sup>T</sup> and *S. luteus* NBRC 100966<sup>T</sup> (Fig. S2), while the absence of C<sub>15:0</sub> was given as a characteristic feature of the genus *Sediminicola* by Khan et al. (2006). The major polar lipids of strain PAMC 27266<sup>T</sup> were phosphatidylethanolamine, two unidentified aminophospholipids and two unidentified lipids (Fig. S1). The genomic DNA G+C content (mol%) was 37.9 for PAMC 27266<sup>T</sup> and 38–40 for *S. luteus* NBRC 100966<sup>T</sup>.

**Table 1.** Differential characteristics between strain PAMC 27266<sup>T</sup> and *Sediminicola luteus* NBRC 100966<sup>T</sup>

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

*Data from Khan et al. (2006).
content of strain PAMC 27266T was 37.9 mol%, which is similar to that of S. luteus NBRC 100966T (Table 1).

Strain PAMC 27266T can be distinguished from S. luteus NBRC 100966T by the inability to grow at 25–37 °C (Table 1). According to the definition given by Morita (1975), strain PAMC 27266T is a psychrophilic bacterium. Other phenotypic characteristics also differentiate strain PAMC 27266T from S. luteus NBRC 100966T, namely the inability to grow in the presence of 9–10% sea salts, ability to produce acid from L-arabinose, L-sorbose and D,L-xylose, inability to produce acid from D-fucose, glycerol, glycerogen 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 27266T should be assigned to a novel species in the genus Sediminicola, for which the name Sediminicola arcticus sp. nov. is proposed.

**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, x-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 x-fucosidase, β-gluconucidase and lipase (C14). According to the API 50CH test, acid is produced from N-acetylgalactosamine, 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 D,L-xylose, but not from D-adonitol, D-arabinose, D,L-arabitol, dulcitol, erythritol, D-fructose, D,L-fucose, gentiobiose, glycerol, glycerogen, inositol, inulin, D-lyxose, D-mannose, methyl β-D-xylpyranoside, 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 C16:1ω6c and/or C16:1ω7c and C15:0. The major polar lipids are phosphatidylethanolamine, two unidentified aminophospholipids and two unidentified lipids. The major quinone is MK-6.

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

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