Mizugakiibacter sediminis gen. nov., sp. nov., isolated from a freshwater lake

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A novel, moderately thermophilic, bacterial strain (skMP5T) was isolated from sediment of a freshwater lake in Japan. The cells were rod-shaped, motile and Gram-stain-negative. Growth was observed at temperatures ranging from 25 to 52 °C, with optimum growth observed at 48–50 °C. The pH range for growth was pH 5.0–8.2, with optimum growth at pH 6.0–7.0. The G+C content of genomic DNA was 72 mol%. The major components in the fatty acid profile were iso-C_{17:0} and iso-C_{17:1}ω9c. The predominant isoprenoid quinone of the strain was ubiquinone Q-8. The strain was facultatively anaerobic, and reduced nitrate to nitrite under anoxic conditions. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate was a member of the family Xanthomonadaceae within the class Gammaproteobacteria, showing highest sequence similarity with Tahibacter aquaticus RaM5-2 (93.6 %) and Metalibacterium scheffleri DK6T (93.3 %). On the basis of phylogenetic and phenotypic properties, strain skMP5T represents a novel species of a new genus, Mizugakiibacter sediminis gen. nov., sp. nov. The type strain of the type species is skMP5T (=DSM 27098T =NBRC 109608T).

Lake Mizugaki is a freshwater lake in Japan, where some culture-independent studies were performed to investigate the microbial community (Kojima et al., 2009, 2014; Tsutsumi et al., 2011). In recent years, several novel bacteria were isolated and described from water and sediment of this lake (Kojima & Fukui, 2010, 2011; Watanabe et al., 2013, 2014). In this study, a novel member of the family Xanthomonadaceae within the class Gammaproteobacteria, showing highest sequence similarity with Tahibacter aquaticus RaM5-2 (93.6 %) and Metalibacterium scheffleri DK6T (93.3 %). On the basis of phylogenetic and phenotypic properties, strain skMP5T represents a novel species of a new genus, Mizugakiibacter sediminis gen. nov., sp. nov. The type strain of the type species is skMP5T (=DSM 27098T =NBRC 109608T).

Strain skMP5T was isolated from sediment of Lake Mizugaki via enrichment culture of autotrophic sulfur-oxidizing bacteria. The enrichment culture was established and maintained at 45 °C, as described previously (Watanabe et al., 2014). The medium used for enrichment was bicarbonate-buffered, low-salt defined medium (Kojima & Fukui, 2011) with the following composition (l^{-1}): 0.2 g MgCl_{2}, 6H_{2}O, 0.1 g CaCl_{2}, 2H_{2}O, 0.1 g NH_{4}Cl, 0.1 g KH_{2}PO_{4}, 0.1 g KCl, 1 ml trace element solution, 1 ml selenite-tungstate solution, 1 ml vitamin mixture solution, 1 ml vitamin B_{12} solution, 1 ml thiamine solution, 30 ml NaHCO_{3} solution and 1.5 ml Na_{2}S_{2}O_{3} solution. All stock solutions were prepared as described previously (Widdel & Bak, 1992).

For isolation and characterization of the strain, 10-fold-diluted tryptone soy broth supplemented with glucose was used as the basal medium. The medium (henceforth designated TSG medium) was prepared by dissolving 3 g tryptone soy broth l^{-1} (Difco) and 10 g glucose l^{-1} in distilled water and adjusting the pH to 6.5 with HCl. Strain skMP5T was obtained by repeated streaking on TSG medium solidified with 1.5 % agar.

The purity of the isolate was checked by phase-contrast light microscopy and sequencing of 16S rRNA gene fragments amplified with several universal PCR primer pairs. Gram-staining and catalase and oxidase activity tests were performed as described previously (Kojima & Fukui, 2011). The genomic G+C content of the DNA was determined with HPLC methods as described previously (Katayama-Fujimura et al., 1984).

Cultures were incubated at 45 °C without shaking unless otherwise specified, and each experiment was performed in duplicate. Effects of salt concentrations [0, 1, 2, 3, 4 and 5 % (w/v) NaCl] and temperature (10, 15, 18, 22, 25, 28, 32, 37, 40, 42, 45, 48, 50, 52, and 55 °C) on growth of the strain were tested under aerobic conditions using TSG medium. The effect of pH on growth of strain skMP5T was tested with modified TSG media buffered with 20 mM citrate (for pH 4.5, 4.9, 5.4), MES (pH 5.0, 5.2, 5.4, 5.6, 5.7, 6.1, 6.2, 6.4, 6.6, 6.8, 7.0), PIPES (pH 6.4, 6.8, 7.1), MOPS (pH 6.6, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.2), or Tricine (pH 7.7, 8.1, 8.2, 8.4, 8.7, 8.8, 9.0).

Anaerobic growth was tested in anoxic R2A broth (Diago) prepared by bubbling with N_{2} gas. Fermentative growth was tested in the medium without additional electron
acceptors. Growth dependent on anaerobic respiration was
tested in the medium supplemented with nitrate (10 mM)
or poorly crystalline Fe(III) oxide (10 mM). The stock
slurry of poorly crystalline Fe(III) oxide was prepared as
described previously (Lovley, 2013). Changes in concentra-
tions of nitrate and nitrite were determined with ion
chromatography.

Tests with the API 20E, API 20NE and API ZYM kits
(bioMérieux) were performed with 14 h-old cultures grown
in R2A broth (Daigo), generally according to the manu-
facturer’s instructions. Cells were harvested by centri-
fugation, and incubation was performed at 42 °C. API 20E
and API 20NE strips were read after 48 h incubation, and
the API ZYM strip was read after 4 h incubation.

The fatty acid profile of the isolate was analysed from cells
grown under two conditions: in TSG liquid medium and
on R2A agar (Daigo). Fatty acid analysis was performed by
using the Sherlock Microbial Identification System (version
6.0; database, TSBA40; MIDI). Isoprenoid quinones were
extracted from cells grown in R2A broth with shaking, and
analysed by HPLC as described previously (Nishijima
et al., 1997). Analysis of polar lipids was carried out by the
Identification Service of the Leibniz-Institut Deutsche
Sammlung von Mikroorganismen und Zellkulturen
(DSMZ), Braunschweig, Germany.

The 16S rRNA gene fragment was amplified with the primer
pair 27F and 1492R (Lane, 1991), and the resulting PCR
product was directly sequenced. The obtained sequence was
aligned with related sequences retrieved from the DDBJ/
EMBL/GenBank databases using the program CLUSTAL_X
(Tamura et al., 1997). Phylogenetic trees were recon-
structed with the program MEGA version 5.05 (Tamura et al.,
2011).

Cells of isolate skMP5T were motile, Gram-stain-negative
rods (1.5–8.0 μm long and 0.4–0.6 μm wide), occurring
singly or in pairs (Fig. S1, available in the online Supple-
mental Material). Spore formation was not observed. On
the agar-solidified TSG medium, the strain formed almost
colourless transparent colonies after 2 days incubation at
45 °C. Colonies on R2A agar were slightly yellowish and
translucent, with a diameter of approximately 1.2 mm. The
strain was catalase-negative and oxidase-positive. The
G+C content of the genomic DNA of strain skMP5T was
72 mol%. Ubiquinone Q-8 was the only major respiratory
quinone detected.

Components of the fatty acid profile of the strain cultivated
under two different conditions are shown in Table 1. The
predominant fatty acid was iso-C17:0 in both cases,
accounting for more than one-third of the total. The
second most predominant component was consistently
iso-C17:1ω9c. Other major fatty acids (>5 % of total in both)
were iso-C15:0, iso-C11:0 3-OH, iso-C16:0 and an unknown
fatty acid (ECL 11.798). Among these, the contents of iso-
C15:0 and iso-C16:0 differed markedly between cells
cultured under the different conditions. The polar lipids

![Table 1. Cellular fatty acid contents (% of total) of strain skMP5T grown in TSG medium and on R2A agar](https://example.com/table1.png)

- Not detected.

of strain skMP5T included phosphatidylethanolamine,
phosphatidylglycerol and several unidentified phospholi-
pids (Fig. S2).

Table 1. Cellular fatty acid contents (% of total) of strain skMP5T grown in TSG medium and on R2A agar

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>TSG</th>
<th>R2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-C11:0</td>
<td>1.44</td>
<td>0.69</td>
</tr>
<tr>
<td>iso-C11:0 3-OH</td>
<td>8.43</td>
<td>8.89</td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>0.28</td>
<td>–</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>0.25</td>
<td>–</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.25</td>
<td>–</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>17.47</td>
<td>9.93</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>0.9</td>
<td>0.66</td>
</tr>
<tr>
<td>C16:0 N alcohol</td>
<td>0.27</td>
<td>–</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>5.44</td>
<td>16.88</td>
</tr>
<tr>
<td>C16:0</td>
<td>2.91</td>
<td>2.36</td>
</tr>
<tr>
<td>iso-C17:1ω9c</td>
<td>19.79</td>
<td>16.97</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>34.8</td>
<td>33.69</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>1.33</td>
<td>1.85</td>
</tr>
<tr>
<td>iso-C18:0</td>
<td>0.44</td>
<td>2.81</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>0.72</td>
<td>–</td>
</tr>
<tr>
<td>Unknown, ECL 11.798</td>
<td>5.27</td>
<td>5.27</td>
</tr>
</tbody>
</table>

Growing of strain skMP5T was observed at a temperature
range between 25 °C and 52 °C, and the optimum
temperature for growth was 48–50 °C. The pH range for
growth was pH 5.0–8.2, and the optimum pH for growth
was pH 6.0–7.0. No growth was observed in the medium
containing 3 % or higher NaCl, and 2 % NaCl exhibited a
negative effect on growth.

Growth in the defined medium used for the enrichment
was tested with various substrates, but no stable growth
was observed under nitrate-reducing conditions. A modi-
ified version of the medium buffered with 20 mM MOPS/
NaOH was used to test aerobic growth, but none of the
tested substrates supported growth in the defined medium.

Under anoxic conditions, the strain did not grow in R2A
media without an additional electron acceptor. Anaerobic
growth was observed in the medium supplemented with
nitrate, along with nitrate consumption and nitrite
production. Poorly crystalline Fe(III) did not serve as an
electron acceptor to support growth of the strain.

In the API ZYM test, activities of alkaline phosphatase,
esterase (C4), esterase lipase (C8), leucine arylamidase, acid phospho-
tase, naphthol-AS-BI-phosphohydrolase, and β-glucosidase
(2-naphthyl-β-D-galactopyranosidase) were detected. Weak activity of valine arylamidase was also observed, but activities of the other enzymes were not detected. In the API 20NE tests, reduction of nitrates to nitrite and hydrolysis of gelatin were detected. Weakly positive signals were observed for hydrolysis of aesculin and β-galactosidase (p-nitrophenyl-β-D-galactopyranosidase) activity. No enzymic activities were detected in the API 20E tests except for gelatin hydrolysis. No growth or acid production was observed in substrate assimilation tests in the API 20E and API 20NE strips. Glucose assimilation was

Table 2. Differential physiological properties of strain skMP5T and strains representing related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum temperature for growth (°C)</td>
<td>48–50</td>
<td>25–30</td>
<td>25–30</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>45 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH for growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.0–8.2</td>
<td>2.6–6.5</td>
<td>5.6–8.0</td>
<td>NR*</td>
<td>6.0–11.0</td>
</tr>
<tr>
<td>Optimum</td>
<td>6.0–7.0</td>
<td>5.5</td>
<td>6.5–7.2</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>72</td>
<td>66.6</td>
<td>63.4</td>
<td>71.9</td>
<td>75</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>–</td>
<td>VW</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis/liquefaction of gelatin</td>
<td>+</td>
<td>NR</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Predominant fatty acids (&gt;20%)</td>
<td>iso-C17:0</td>
<td>iso-C17:0</td>
<td>iso-C17:109c</td>
<td>iso-C17:0</td>
<td>iso-C17:109c</td>
</tr>
</tbody>
</table>

*Enrichment, isolation and characterization were performed at pH 6.8–7.0.
also tested with API OF medium (bioMérieux), and no growth or acid production was observed after 48 h incubation at 42 °C.

In the 16S rRNA gene sequence analysis, characterized strains which showed the highest sequence similarities to strain skMP5\(^T\) were *Tahibacter aquaticus* RaM5-2 (93.6 %) and *Metallibacterium scheffleri* DKE6\(^T\) (93.3 %), belonging to the family *Xanthomonadaceae* within the class *Gammaproteobacteria*. Affiliation of the novel isolate to the family *Xanthomonadaceae* was confirmed with the maximum-likelihood phylogenetic tree (Fig. 1), in which the novel strain formed a cluster with species of the genera *Metallibacterium*, *Dyella*, *Fulvimonas* and *Rhodanobacter*. This cluster was consistently observed in the trees reconstructed with minimum-evolution (Fig. S3) and neighbour-joining (data not shown) methods. These analyses also indicated that none of the existing genera could accommodate the novel strain without loss of monophyletic (Figs 1 and S3). In addition to the independent phylogenetic position and low sequence similarities (<94%) to the relatives, physiological properties of the novel strain were distinct from members of other genera in this lineage (Table 2). On the basis of these phylogenetic and phenotypic properties, strain skMP5\(^T\) is assigned to a novel species of a new genus in the family *Xanthomonadaceae*, for which the name *Mizugakiibacter sediminis* gen. nov., sp. nov. is proposed.

**Description of Mizugakiibacter gen. nov.**

*Mizugakiibacter* (Mi.zu.ga.ki.i.bac’ter. N.L. masc. n. bacter a rod; N.L. masc. n. Mizugakiibacter a rod isolated from Lake Mizugaki).

Cells are Gram-stain-negative, catalase-negative and oxidase-positive. Phylogenetically affiliated to the family *Xanthomonadaceae* within the class *Gammaproteobacteria* based on 16S rRNA gene sequence analysis. Major fatty acids are iso-C\(_{17:0}\) and iso-C\(_{17:1}\)ω9c. The predominant quinone is Q-8. The G+C content of the genomic DNA is around 72 mol%.

The type species is *Mizugakiibacter sediminis*.

**Description of Mizugakiibacter sediminis** sp. nov.

*Mizugakiibacter sediminis* (se.di’mi.nis. L. gen. n. sediminis of sediment).

Cells are rod-shaped, 1.5–8.0 μm in length and 0.4–0.6 μm in width. Colonies on R2A agar are round, slightly yellowish and translucent. Growth occurs at temperatures between 25 and 52 °C, with optimum growth at 48–50 °C. The pH range for growth is pH 5.0–8.2, and optimum growth occurs at pH 6.0–7.0. Anaerobic growth occurs in the presence of nitrate. Reduces nitrate to nitrite and hydrolyses gelatin. In API ZYM tests, positive result for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase (2-naphthyl-β-D-galactopyranosidase) activities, and weakly positive result for valine arylamidase activity.

The type strain skMP5\(^T\) (=DSM 27098\(^T\)=NBRC 109608\(^T\)) was isolated from sediment of a freshwater lake in Japan. The G+C content of genomic DNA of the type strain is 72 mol%.

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**References**


