**Desulfatitalea tepidiphila** gen. nov., sp. nov., a sulfate-reducing bacterium isolated from tidal flat sediment

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A novel sulfate-reducing bacterium, strain S28bF\textsuperscript{T}, was isolated from tidal flat sediment from Tokyo Bay, Japan. Cells of strain S28bF\textsuperscript{T} were rod-shaped (0.5–0.6×1.7–3.8 μm), motile and Gram-stain-negative. For growth, the optimum pH was pH 6.8–7.3 and the optimum temperature was 34–42 °C. Strain S28bF\textsuperscript{T} used sulfate and thiosulfate as electron acceptors, but not nitrate. The G+C content of the genomic DNA was 56.6 mol%. The fatty acid profile of strain S28bF\textsuperscript{T} was characterized by the presence of anteiso-C\textsubscript{15:0} and C\textsubscript{16:0} as the major components. Phylogenetic analyses based on genes for 16S rRNA, the alpha subunit of dissimilatory sulfite reductase (dsrA) and adenosine-5'-phosphosulfate reductase (aprA) revealed that the isolated strain belonged to the class Deltaproteobacteria. Its closest relative was *Desulftosarcina cetonica* DSM 7267\textsuperscript{T} with a 16S rRNA gene sequence similarity of 93.3%. Two other strains, S28OL1 and S28OL2 were also isolated from the same sediment. These strains were closely related to S28bF\textsuperscript{T} with 16S rRNA gene sequence similarities of 99%, and the same physiological characteristics were shared with strain S28bF\textsuperscript{T}. On the basis of phylogenetic and phenotypic characterization, a novel species in a new genus, *Desulfatitalea tepidiphila* gen. nov., sp. nov., is proposed to accommodate the strains obtained in this study. The type strain is S28bF\textsuperscript{T} (=NBRC 107166\textsuperscript{T} = DSM 23472\textsuperscript{T}).

Sulfate-reducing bacteria are anaerobes which utilize organic compounds and hydrogen as electron donors. They usually prefer relatively low-molecular-mass organic substrate such as short-chain fatty acids. Some strains of sulfate-reducing bacteria can also degrade hydrocarbons. Recently, a novel strain of toluene-degrading sulfate reducer, 28bB2T, was isolated from an enrichment culture established with marine sediments as inoculum (Higashioka et al., 2011). In the process of isolation of the strain 28bB2T, another bacterium with characteristic 16S rRNA gene sequences was also enriched. In the present study, the bacterium, designated strain S28bF\textsuperscript{T}, was isolated in a pure culture along with two closely related strains, and was investigated in more detail.

Strain S28bF\textsuperscript{T} was isolated from marine sediment obtained from Sanban-ze, situated in Tokyo Bay, Japan (Tabuchi et al., 2010) (39°39.0' N, 139°55.5' E). Throughout the present study, a defined saltwater medium was used for cultivation under sulfate-reducing conditions (Widdel & Bak, 1992). The majority of the isolation procedures were performed as described previously for strain 28bB2T (Higashioka et al., 2011). These strains were obtained from the same enrichment culture established after several changes of substrate for growth, and they were separated in the final step of isolation. As described previously, strain 28bB2T was isolated by serial dilution with medium supplemented with toluene. From the same culture, strain S28bF\textsuperscript{T} was isolated by repeated serial dilution using 10 mM fumarate as the substrate.

Two additional strains were isolated from the crude-oil-degrading enrichment culture described in the previous study (Higashioka et al., 2011). The enrichment culture, established at 28 °C, was subjected to agar shake dilution using sodium lactate as the substrate (Widdel & Bak 1992). Two isolated colonies were picked and inoculated into a liquid medium supplemented with 20 mM sodium lactate. The resulting pure cultures were designated strain S28OL1 and strain S28OL2.

Cell morphology of the strains was observed by a phase-contrast microscope (Axioplan 2; Zeiss). Gram-staining was performed for strain S28bF\textsuperscript{T} by using a Gram staining kit (Fluka) as described in the manufacturer’s instructions. The fatty acid profile of the strain S28bF\textsuperscript{T} was analysed from biomass grown on fumarate. Fatty acid analysis was performed at Techno Suruga (Shizuoka, Japan) by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions.

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The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene, dsrA and aprA sequences of strain S28bF\textsuperscript{T} are AB614135, AB614136 and AB719405, respectively.

A supplementary table is available with the online version of this paper.
Each culturing experiment to characterize the isolates was carried out in triplicate at 28 °C except for the test of temperature for growth. Growth under different conditions was assessed by monitoring turbidity and concentrations of sulfide determined by a spectrophotometric method (Cord-Ruwwisch, 1985). Growth at various temperatures was examined for the three strains using the basal medium supplemented with 10 mM fumarate. The temperature range for growth was determined by culture incubation at 15 different temperatures ranging from 8 to 48 °C (8, 13, 18, 20, 22, 25, 28, 32, 34, 37, 40, 42, 43, 44 and 45 °C). To determine the pH range for growth of strain S28bF^T, NaHCO_3 in the basal medium was replaced with (per litre) 10 g MOPS and 11 g N-cyclohexyl-2-hydroxy-3-amino-propanesulfonic acid (CAPSO), and the pH was adjusted with HCl or NaOH solution. Growth was tested at eight different pH values ranging from 6.0 to 7.9 (6.0, 6.2, 6.5, 6.8, 7.0, 7.3, 7.5 and 7.9), using 10 mM fumarate as substrate.

The utilization of electron donors was tested in media containing one of the substrates: acetate, propionate, succinate, pyruvate, lactate, formate, fumarate, n-butyrate, benzoate, phenol, glucose, ethanol and yeast extract and was evaluated by monitoring the growth. The capacity for lithoautotrophic growth with H_2 and CO_2 was assessed under a gas mixture (H2 :N 2 :C O2, 50 : 40 : 10; 2 atm total pressure). In addition to these substrates, utilization of several hydrocarbons was tested for strain S28bF^T. The hydrocarbon solutions were prepared by diluting toluene, benzene, o-xylene, m-xylene, p-xylene, ethylbenzene, n-hexane, or o-decane to 2% (v/v) in 2,2,4,4,6,8,8-heptadecane. Each hydrocarbon solution was added to a 25-fold volume of basal medium. For strains S28OL1 and S28OL2, only toluene and n-hexane were tested. Utilization of electron acceptors was tested using sulfate-free basal medium containing 10 mM acetate as electron donor.

The DNA G+C contents of the isolates were determined by using a Yamasa GC kit (Yamasa Shoyu) as described previously (Katayama-Fujimura et al., 1984).

16S rRNA gene fragments were amplified with primers 27f and 1492r (Lane, 1991). Fragments of the gene for alpha subunit of dissimilatory sulfite reductase (dsrA) were amplified with primers DSR1Fdeg (Klein et al., 2001) and DSR1334R (Santillano et al., 2010). Fragments of the aprA gene encoding adenosine-5’-phosphosulfate reductase were amplified with the primer pair Apr-1-FW/Apr-5-RV (Meyer & Kuever, 2007). Nucleotide sequences of the PCR products were determined by cycle sequencing with a dye terminator (BigDye Terminator v3.1 Cycle Sequencing kit; Applied Biosystems). The nucleotide sequence of the 16S rRNA gene and the amino acid sequences deduced from the dsrA and aprA genes were aligned with reference sequences from the public database using the CLUSTAL_X program. Phylogenetic trees were constructed by the neighbour-joining method with the MEGA version 3.1 program (Kumar et al., 2004). Bootstrap analysis was performed for 1000 replicates.

Cells of strain S28bF^T were rod-shaped (0.5–0.6 × 1.7–3.8 μm), motile and Gram-stain-negative (Fig. 1). The temperature ranges for growth of the three strains were 25–43 °C (strain S28bF^T, optimum at 34–42 °C, 18–45 °C (strain S28OL1) and 13–42 °C (strain S28OL2). S28bF^T grew at a pH range of 6.2–7.9, with optimum growth at pH 6.8–7.3.

All three strains used the following substrates as electron donor and carbon source (mM, except where stated): acetate (10), propionate (5), succinate (5), pyruvate (10), lactate (20), formate (10), fumarate (10), n-butyrate (5) and yeast extract (0.5 g l^-1). Autotrophic growth with H_2 was also observed in all strains. None of the isolates could grow on the following substrates (mM): benzoate (2.5), phenol (1), glucose (10) and ethanol (5). Growth on hydrocarbon was not observed in all tested cases. The three strains were able to use sulfate (28 mM) and thiosulfate (10 mM) as an electron acceptor, but not nitrate (10 mM).

The G+C contents (mol%) of the genomic DNA of three strains were 56.6 (strain S28bF^T), 58.2 strain S28OL1) and 56.4 (strain S28OL2).

The fatty acid profile of strain S28bF^T was characterized by high concentrations of anteiso-C_{15:0} (52.3 %), C_{16:0} (12.8 %) and anteiso-C_{17:0} (9.3 %). Other fatty acids detected are summarized in Table S1 available in IJSEM Online, along with those of Desulfosarcina variabilis DSM 2060^T, one of the species most closely related to strain S28bF^T (Rütters et al., 2001). Differences in these two strains were apparent in the major fatty acids mentioned above, as well as other minor ones. In comparison to Desulfosarcina variabilis DSM 2060^T, the fatty acid profile of strain S28bF^T had some distinct features, i.e. more varied and abundant anteiso-fatty acids, fewer variations and lower content of unsaturated fatty acids and occurrence of longer chain fatty acids with 19–20 carbons.

Analysis of almost full-length 16S rRNA gene sequences revealed that the three novel isolates were closely related to each other (99 % or higher sequence similarities), and

![Fig. 1. Phase-contrast micrographs of cells of strain S28bF^T grown with 10 mM fumarate as an electron donor. Bar, 3 μm.](image-url)
belonged to the family *Desulfobacteraceae* within the class *Deltaproteobacteria*. They were phylogenetically distinct from other members of this family with validly published names (Fig. 2). The characterized strain which showed highest 16S rRNA gene sequence similarities to the novel names (Fig. 2). The characterized strain which showed from other members of this family with validly published *Deltaproteobacteria*, belonged to the family *Desulfobacteraceae* within the family *Desulfosarcina* with *Desulfobacillum* *desulfuricans* Essex 6 and as an outgroup. Bootstrap values (% of 1000 replications) >50 are shown. Bar, 0.02 substitutions per site.

Characteristics of strain S28bF are given in Table 1 as representative of the novel isolates, along with phylogenetically related sulfate-reducing bacteria. The optimum temperatures for growth of *Desulfosarcina cetonica* DSM 7267 and *Desulfococcus multivorans* DSM 2059 were 30 °C and 35 °C, respectively. The novel strains grew well at temperatures higher than the optimum temperatures for previously described species. Species of the genus *Desulfosarcina* oxidize aromatic compounds such as benzoate and phenol, and utilization of benzoate is also a typical physiological feature of species of the genus *Desulfococcus*. In contrast, the novel strains represented by S28bF could not grow on benzoate.

Among existing genera, the genus *Desulfosarcina* is the sole candidate to accommodate the novel strains without loss of monophyleticity, as shown in the 16S rRNA gene-based phylogeny (Fig. 2). In the analyses of two functional genes, however, the novel strains were phylogenetically separated.

![Fig. 2. Neighbour-joining phylogenetic tree showing the affiliation of the 16S rRNA gene sequences of the isolated strains within the family *Desulfobacteraceae*, with *Desulfobacillum desulfuricans* Essex 6 as an outgroup. Bootstrap values (% of 1000 replications) >50 are shown. Bar, 0.02 substitutions per site.](http://ijs.sgmjournals.org)

![Fig. 3. Neighbour-joining trees showing the phylogenetic positions of the deduced DsrA (a) and AprA (b) amino acid sequences of the novel strains obtained in this study, within the class *Deltaproteobacteria*. Bootstrap values (% of 1000 replications) >50 are shown. Bars, 0.05 substitutions per site.](http://ijs.sgmjournals.org)
Table 1. Differential physiological properties of S28bF<sup>T</sup> and closely related strains

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
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<td><strong>Morphology</strong></td>
<td>Rod</td>
<td>Oval</td>
<td>Spherical</td>
<td>Oval to rod</td>
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<td><strong>Cell size (μm)</strong></td>
<td>0.5–0.6 × 1.7–3.8</td>
<td>0.8–1.2 × 1.8–2.7</td>
<td>1.5–2.2</td>
<td>0.7–1.5 × 2–2.5</td>
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<td><strong>DNA G+C content (mol%)</strong></td>
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<td>59</td>
<td>57</td>
<td>47.4</td>
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<tr>
<td><strong>Optimum temperature (°C)</strong></td>
<td>34–42</td>
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<td>35</td>
<td>NR</td>
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<td><strong>Electron donors and carbon sources</strong></td>
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<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>+</td>
<td>+</td>
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<td>Ethanol</td>
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<td>+</td>
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<td>−</td>
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<td>NR</td>
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</table>

from species of the genus *Desulfosarcina*, suggesting a distinct evolutionary history (Fig. 3a, b). Differences from members of the genus *Desulfosarcina* were also apparent in phenotypic characteristics represented by the fatty acids profile. On the basis of these results, a novel species in a new genus, *Desulfatitalea tepidiphila* gen. nov., sp. nov., is proposed to accommodate the strains obtained in this study, with the type strain S28bF<sup>T</sup> (=NBRC 107166<sup>T</sup>=DSM 23472<sup>T</sup>).

**Description of Desulfatitalea gen. nov.**

*Desulfatitalea* (De.sul.ta.i.ta’le.a. L. pref. de from; N.L. n. sulfas -atis sulfate; L. fem. n. talea a slender staff, rod, stick; N.L. fem. n. Desulfatitalea a rod-shaped sulfate-reducer).

Mesophilic, sulfate-reducing bacteria that stain Gram-negative. Rod-shaped cells. Sulfate and thiosulfate are used as electron acceptors. The major fatty acids are anteiso-C<sub>15:0</sub> and C<sub>16:0</sub>. Phylogenetically, the genus *Desulfatitalea* belongs to the family *Desulfobacteraceae* in the class *Deltaproteobacteria*. The type species is *Desulfatitalea tepidiphila*.

**Description of Desulfatitalea tepidiphila sp. nov.**

*Desulfatitalea tepidiphila* (te.pi.di’phi.la. L. adj. tepidus moderately warm; Gr. adj. philos loving; N.L. fem. adj. tepidiphila loving warmth).

Shows the following characteristics in addition to those given in the genus description. Cells are motile and rod-shaped (0.5–0.6 × 1.7–3.8 μm). The temperature range for growth is 13–45°C (optimum 34–42°C). The pH range for growth is 6.2–7.9 (optimum pH 6.8–7.3). Nitrate is not used as an electron acceptor. In the presence of sulfate as an electron acceptor, acetate, succinate, formate, propionate, n-butyrate, pyruvate, fumarate, yeast extract and lactate are utilized as electron donors and carbon sources. Benzoate, glucose, phenol, ethanol, toluene, benzene, o-xylene, m-xylene, p-xylene, ethylbenzene, n-hexane and n-decane are not utilized.

The type strain, S28bF<sup>T</sup> (=NBRC 107166<sup>T</sup>=DSM 23472<sup>T</sup>), was isolated from tidal flat sediment from Tokyo Bay, Japan. The DNA G+C content of the type strain is 56.6 mol%.

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**References**


