**Desulfurispirillum indicum** sp. nov., a selenate- and selenite-respiring bacterium isolated from an estuarine canal

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Strain **S5**⁷, a novel bacterium that was isolated for its capability to respire selenate to elemental selenium, is described. In addition to selenate respiration, it was also capable of dissimilatory selenite, arsenate and nitrate reduction with short-chain organic acids such as pyruvate, lactate and acetate as the carbon sources and electron donors. The isolate was unable to grow fermentatively. Strain **S5**⁷ was isolated from sediment of an estuarine canal in Chennai, India. Phylogenetic analysis of the 16S rRNA gene of this novel isolate revealed that it belonged to the family *Chrysiogenaceae* with sequence similarities of 92 and 98%, respectively, with the type strains of *Chrysiogenes arsenatis* and *Desulfurispirillum alkaliphilum*, its closest known relatives. Strain **S5**⁷ and *D. alkaliphilum* were closely related in terms of their 16S rRNA gene phylogeny; however, they varied greatly in their genomic DNA G+C content (56 mol% versus 45 mol%) and cellular fatty acid compositions, as well as in many metabolic capabilities. **S5**⁷ represents a novel species for which the name *Desulfurispirillum indicum* sp. nov. is proposed; the type strain is **S5**²⁷ (= DSM 22899T = ATCC BAA-1389T).

Selenium (Se), a naturally occurring element, is essential for most organisms (Conde & Sanz Alaejos, 1997; Zannoni et al., 2007). Although considered to be a trace element, its toxicity is highly dependent on its oxidation state and speciation. Se occurs in a variety of oxidation states, water-soluble selenate (SeO₄²⁻) and selenite (SeO₃²⁻), insoluble elemental selenium (Se⁰) and gaseous selenide. Even though redox transformations of selenium oxyanions can occur abiotically (Myheni et al., 1997; Kessi & Hanselmann, 2004), the global selenium cycle is greatly influenced by micro-organisms (Stolz et al., 2006). Various microbial species have been found to not only reduce selenate and/or selenite to elemental Se, but to also respire selenate oxyanions in order to gain cellular energy (Oremland et al., 1989; Steinberg & Oremland, 1990; Narasingarao & Häggblom, 2007b; Stolz & Oremland, 1999; Stolz et al., 2006). These selenium oxyanion-respiring micro-organisms display a broad phylogenetic diversity and can be found among the phyla *Proteobacteria*, *Deferribacteres*, *Firmicutes* and *Actinobacteria* (Narasingarao & Häggblom, 2006, 2007a, b; Switzer Blum et al., 2001; von Wintzingerode et al., 2001). The ability of bacteria to use selenate and selenite and other alternative terminal electron acceptors, such as arsenate, nitrate, sulfate and/or iron, plays a major role in the oxidation of organic carbon in sediments and contributes to the biogeochemical cycling of these elements in nature. In this study, strain **S5**⁷, a novel member of the family *Chrysiogenetes*, is described.

Strain **S5**⁷ is a strictly anaerobic, dissimilatory selenate- and selenite-respiring bacterium isolated from an enrichment culture of sediments collected in an estuarine channel in Chepauk in Chennai, located in southern India (Narasingarao & Häggblom, 2007a). **S5**⁷ was isolated after sequential transfers into fresh anaerobic medium with selenate as electron acceptor and pyruvate as carbon source and electron donor. Purity of the culture was verified by microscopic analysis and by terminal restriction fragment length polymorphism analysis as described by Fennell et al. (2004). **S5**⁷ was maintained in an anaerobic minimal salts medium (Fennell et al., 2004) with 20 mM pyruvate as the carbon source and 10 mM selenate as the electron donor under a headspace of N₂.

Genomic DNA was extracted by phenol/chloroform extraction as described by Kerkhof & Ward (1993). The 16S rRNA gene was selectively amplified by PCR from the genomic DNA as described by Narasingarao & Häggblom (2006). PCR products were sequenced by Genewiz Inc, South Plainfield, NJ, USA. Sequence data were compiled...
using Contig Express (Vector NTI Suite; Informax). The
16S rRNA gene sequences of related micro-organisms were
identified by a BLAST search (Altschul et al., 1997) and
downloaded from GenBank. Phylogenetic and molecular
evolutionary analyses of all sequences were conducted
using MEGA4 (Tamura et al., 2007), which utilizes CLUSTAL
w (Chenna et al., 2003) to align sequences. Neighbour-
joining and maximum-parsimony phylogenetic trees with
1000 bootstrap replications were reconstructed using the
program package MEGA4 with the maximum composite
likelihood model for nucleotide substitutions. Comparative
16S rRNA gene sequence analysis placed strain S5T within
the family Chrysioegenaceae, which, at the time of writing,
has only two known members. Chrysioegenes arsenatis, the
first species to be identified, was isolated for its ability to
respire arsenate to arsenite coupled to oxidation of acetate
and other short-chain organic acids (Macy et al., 1996). Desulfurispirillum alkaliphilum, the second organism, is
capable of reducing elemental sulfur to sulfide and nitrate
to ammonium (Sorokin et al., 2007). The type strain of D.
alkaliphilum only shared 91% 16S rRNA gene similarity
with that of C. arsenatis and so it was placed in a new
genus, Desulfurispirillum (Sorokin et al., 2007). The 16S
rRNA gene sequence of strain S5T shared similarities of 94
and 98%, respectively, with those of the type strains of C.
arsenatis and D. alkaliphilum. Phylogenetic analysis with
unambiguously aligned 16S rRNA gene sequences of strain
S5T and other related strains (Fig. 1) indicated that strain
S5T belongs to the family Chrysioegenaceae and is most
closely related to D. alkaliphilum and C. arsenatis. This
was supported by high bootstrap values in both the neighbour-
joining and maximum-parsimony analyses. All further
physiological and molecular characterizations of strain S5T
were made in comparison to the two above-named
organisms.

Strain S5T was grown with selenate (10 mM) or nitrate
(10 mM) as electron acceptors and pyruvate (20 mM) as
carbon source to examine cell morphology via transmis-
sion electron microscopy (Narasingarao & Håggblom,
2007) and phase-contrast microscopy. Strain S5T was a
Gram-negative, slender, spiral-shaped bacterium, approxi-
ately 2–7 μm long and 0.10–0.15 μm in diameter (Table
1, Fig. 2). Cells grown in the presence of selenate produced
a bright red precipitate that accumulated in the bottom of
the culture tube. The precipitate was further evaluated
and confirmed as elemental selenium by X-ray absorption
near edge structure analysis (XANES; Narasingarao &
Håggblom, 2007a). Strain S5T grew in both liquid medium
and soft agar shake tubes. It did not form distinct colonies
when grown anaerobically in soft agar (0.4% Noble agar;
Difco) shake tubes with 10 mM selenate or 10 mM
selenite. The agar first turned completely red and then
black over a period of 14 days as the selenium oxyanions
were reduced to elemental selenium.

Rapid motility was observed by microscopy; therefore, a
flagella stain was applied to visualize the presence of flagella
(Kodaka et al., 1982). One drop of liquid culture of strain
S5T was applied to a clean glass slide and allowed to air dry
completely. The flagella stain was then applied for 10 min
before being washed off with deionized water. Bipolar
flagella were observed that extended about 4–6 μm at
either end of the cell and allowed for rapid movement of
strain S5T (Fig. 2b).

A wide range of electron donors and acceptors were used to
characterize the metabolic capabilities of strain S5T (Table
1). Selenium oxyanions, nitrate and arsenate were analysed
using ion chromatography (DX 120; Dionex) as described
previously (Knight et al., 2002). The organic acids
pyruvate, lactate and acetate were measured using HPLC
(Shimadzu) equipped with a C18 column (250 × 4.6 mm,
5 μm particle size, Sphereclon; Phenomenex) as described
by Narasingarao & Håggblom (2007a). Three consecutive
transfers with no growth were scored as negative for
substrate utilization.

In addition to respiring selenate to elemental selenium with
pyruvate as the carbon source, strain S5T also respired
selenite, arsenate and nitrate (to ammonium). Pyruvate,
acetate and lactate were its preferred carbon sources.
Neither C. arsenatis nor D. alkaliphilum respired selenate
and selenite and D. alkaliphilum did not respire arsenate
(Table 1). In addition to pyruvate, lactate and acetate, both
C. arsenatis and D. alkaliphilum were also able to use
fumarate as a carbon source (Macy et al., 1996; Sorokin

![Fig. 1. Phylogenetic tree showing strain S5T with closely related strains. The tree was reconstructed with aligned 16S rRNA gene sequences using the neighbour-joining method with global gap removal algorithm. The evolutionary distances were computed using the maximum composite likelihood method (1251 positions in analysis). Bootstrap values above 50% are indicated. The 16S rRNA gene sequence of Thermotoga maritima FJSS3-B1 was used as outgroup. Bar, 0.02 substitutions per nucleotide position.](http://ijs.sgmjournals.org)
In addition, *D. alkaliphilum* was also capable of using HS$^-_2$, H$_2$ and propionate as electron donors and carbon sources (Table 1) (Sorokin et al., 2007).

To determine optimum pH, strain S5$^T$ was grown with 10 mM nitrate as electron acceptor and 20 mM pyruvate as carbon source in anaerobic growth medium. The pH of the medium was adjusted with HCl and NaOH (pH 5–9). Strain S5$^T$ grew at pH 6.8–7.6, with optimum growth at pH 7.4, in contrast to *D. alkaliphilum*, which grew at pH 8.0–10.0 (Table 1). Growth of strain S5$^T$ at different NaCl concentrations (0–2.5 M) and temperatures (4–37 °C) was determined by growing cells in anaerobic medium (pH 7.4) with 10 mM nitrate and 20 mM pyruvate. The isolate grew between 20 and 37 °C, with optimum growth at 28 °C. It grew in NaCl concentrations of 0.10–0.75 M, with optimum growth at 0.40 M NaCl.

The genomic DNA G+C content of strain S5$^T$ was determined using a modified method of Mesbah et al. (1989). A Synergy 4U Fusion-RP 80A C18 reverse-phase column (Phenomenex) was used in an Agilent HPLC 1100 system. The mobile phase consisted of two eluents, 20 mM ammonium acetate (pH 4.5, eluent A) and acetonitrile (eluent B), at a flow rate of 1 ml min$^{-1}$. A gradient of eluent A from 95 to 60 % was established over a period of

### Table 1. Differential physiological and metabolic characteristics of strain S5$^T$ and closely related species

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Spirillum</td>
<td>Spirillum</td>
<td>Vibrio</td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>Highly motile</td>
<td>Highly motile</td>
<td>Motile</td>
</tr>
<tr>
<td><strong>Cell size (µm):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>2–7</td>
<td>2–5</td>
<td>1–2</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.10–0.15</td>
<td>0.15–0.20</td>
<td>0.50–0.75</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>56.1</td>
<td>44.8 ± 0.5*</td>
<td>49</td>
</tr>
<tr>
<td>Fermentation</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Optimum growth temperature (°C):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6.8–7.6</td>
<td>8.0–10.0</td>
<td>7.4–7.8</td>
</tr>
<tr>
<td><strong>Growth pH:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.10–0.75</td>
<td>0.1–2.5*</td>
<td>ND</td>
</tr>
<tr>
<td><strong>NaCl concentration (M):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.10–0.75</td>
<td>0.1–2.5*</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Electron donors:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Fumarate</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HS$^-_2$</td>
<td>–</td>
<td>+*</td>
<td>ND</td>
</tr>
<tr>
<td>H$_2$</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Malate</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Propionate</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Electron acceptors:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenate</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Chromate</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DMSO</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrite</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Selenite</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Selenate</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sulfur</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*Data from Sorokin et al. (2007).
†Calculated from Macy et al. (1989).
The fatty acid content of strain S5 was analyzed using cells grown at 28 °C with 20 mM pyruvate as electron donor and 10 mM nitrate. D. alkaliphilum was grown at 28 °C with 20 mM acetate as electron donor and 10 mM nitrate as electron acceptor. ND, Not detected.

Table 2. Cellular fatty acid compositions (%) of strain S5 and Desulfurispirillum alkaliphilum SR 1T.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td>C12:0</td>
<td>1.4 ± 0.8</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>C12:1 3-OH</td>
<td>4.1 ± 1.8</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>1.4 ± 0.7</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>C14:0</td>
<td>ND</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>0.5 ± 0.7</td>
<td>ND</td>
</tr>
<tr>
<td>C15:0 2-OH</td>
<td>0.5 ± 0.7</td>
<td>ND</td>
</tr>
<tr>
<td>C16:13-OH</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>C16:1107c</td>
<td>6.7 ± 0.8</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>C16:1105c</td>
<td>4.3 ± 0.6</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>C16:0</td>
<td>20.6 ± 3.6</td>
<td>14.9 ± 0.9</td>
</tr>
<tr>
<td>iso-C17:105c</td>
<td>ND</td>
<td>8.9 ± 0.6</td>
</tr>
<tr>
<td>C17:108c</td>
<td>0.9 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>C17:106c</td>
<td>2.1 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>7.8 ± 11</td>
<td>ND</td>
</tr>
<tr>
<td>C18:13-OH</td>
<td>ND</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>C18:1107c</td>
<td>38.5 ± 10.1</td>
<td>39.6 ± 3.3</td>
</tr>
<tr>
<td>C18:1105c</td>
<td>2.9 ± 0.7</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.2 ± 1.5</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>iso-C18:1 H</td>
<td>1.0 ± 0</td>
<td>ND</td>
</tr>
<tr>
<td>iso-C18:1108c H</td>
<td>3.9 ± 1.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

Description of Desulfurispirillum indicum sp. nov.

Desulfurispirillum indicum (in’di.cum. L. neut. adj. indicum of India, Indian). gram-negative, highly motile, spiral-shaped bacterium, approximately 2–7 μm long and 0.10–0.15 μm in diameter. Strictly anaerobic. Non-fermenting. Respires selenate to elemental selenium coupled to utilization of short-chain organic acids. Capable of selenite, nitrate and arsenate respiration. Mesophilic, with a maximum growth temper-
ature of 37 °C (optimum at 28 °C). Tolerates NaCl concentrations of 0.10–0.75 M. The predominant cellular fatty acids are C12:1 3-OH, C16:0, C16:1ω7c, iso-C17:0 3-OH and C18:1ω7c. Groups within the family Chrysiogenaceae.

The type strain is S5T (=DSM 22839T =ATCC BAA-1389T), isolated for its ability to respire selenate to elemental selenium from an estuarine canal in Chepauk in Chennai, located in southern India. The genomic DNA G+C content of the type strain is 56 mol%.

Acknowledgements

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References


