Bacillus selenatarsenatis sp. nov., a selenate- and arsenate-reducing bacterium isolated from the effluent drain of a glass-manufacturing plant

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A facultatively anaerobic, selenate- and arsenate-reducing bacterium, designated strain SF-1T, was isolated from a selenium-contaminated sediment obtained from an effluent drain of a glass-manufacturing plant in Japan. The bacterium stained Gram-positive and was a motile, spore-forming rod capable of respiring with selenate, arsenate and nitrate as terminal electron acceptors. The major cellular fatty acids of the strain were iso-C15 : 0, iso-C17 : 1v10c and C16 : 1v7c alcohol. The G+C content of the genomic DNA was 42.8 mol%. Though the nearest phylogenetic neighbour was Bacillus jeotgali JCM 10885T, with a 16S rRNA gene sequence similarity of 99.6 %, DNA–DNA hybridization studies showed only 14 % relatedness between these strains, a level that is clearly below the value recommended to delimit different species. This, together with the phenotypic differences (utilization of electron acceptors, NaCl tolerance), suggests that strain SF-1T represents a novel species of the genus Bacillus, for which the name Bacillus selenatarsenatis sp. nov. is proposed. The type strain is SF-1T (=JCM 14380T =DSM 18680T).
Yamamura et al. (2003). For aerobic growth, 10 g glucose L⁻¹ was used as the electron donor, whereas sodium lactate (20 mM) was used instead of glucose for anaerobic growth with one of selenate, arsenate or nitrate (1 mM) as the electron acceptor. Bacillus jeotgalii JCM 10885ᵀ, obtained from the RIKEN BRC-JCM, was cultivated on an appropriate medium (Yoon et al., 2001) or under the same condition as strain SF-1ᵀ, except that the pH was adjusted to 7.5, for phenotypic comparison. Biochemical tests were carried out using API 20E and API 50CHB kits according to the instructions of the manufacturer (bioMérieux). To determine sensitivity to antibiotics, a culture of strain SF-1ᵀ was inoculated onto LB agar plates (pH 8.0) containing kanamycin, tetracycline, chloramphenicol, ampicillin or erythromycin at 1–30 μg ml⁻¹ and incubated at 37 °C for 4 days. Fatty acid methyl esters were extracted and analysed following the standard protocol of the Sherlock Microbial Identification System (MIDI).

For genomic DNA isolation, strain SF-1ᵀ and B. jeotgalii JCM 10885ᵀ were grown aerobically in 1 litre batches to mid-exponential phase and cells were harvested by centrifugation at 8000 g (10 min, 4 °C). Genomic DNA was prepared using the DNeasy Tissue kit or DNeasy Plant kit (Qiagen) according to the manufacturer’s instructions.

The G + C content of the DNA was determined by HPLC as described previously (Katayama-Fujimura et al., 1984). DNA–DNA hybridization of strain SF-1ᵀ and B. jeotgalii JCM 10885ᵀ was carried out by the method of Ezaki et al. (1989).

Gene fragments specific to the 16S rRNA-encoding regions were amplified by PCR using primers 20F (5'-GAGTTACCTTGTTACGACTT-3') and 1500R (5'-GAGTTACCTTGTTACGACTT-3'); positions 9–27) and 1500R (5'-GAGTTACCTTGTTACGACTT-3'); positions 1509–1492) (Kawasaki et al., 1993). Positions in the 16S rRNA gene numbering system are based on the Escherichia coli numbering system (GenBank accession no. V00348) of Brosius et al. (1981).

Amplified 16S rRNA genes were sequenced directly using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit and an ABI PRISM model 310 Genetic Analyzer (Applied Biosystems). The following primers were used for sequencing: 20F, 1500R, 5'-CAGCAGCGCGTGTAATAC-3'; positions 519–536), 520R (5'-GTATTACCGCGGCTGCTG-3'; positions 536–519), 920F (5'-AAACTCAATGAATTGACGG-3'; positions 907–926) and 920R (5'-CCGTCATTCTATGGT-3'; positions 926–907).

The 16S rRNA gene sequence of strain SF-1ᵀ determined was compared with reference sequences using BLAST similarity searches (Altschul et al., 1997) and the closely related sequences were obtained from GenBank. Multiple alignments were generated and the calculation of distance matrices for the aligned sequences (Kimura, 1980) was carried out using CLUSTAL X (Thompson et al., 1997) and MEGA version 3.1 (Kumar et al., 2004). Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. The phylogenetic tree topology was evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985). Sequence similarity values were calculated using GENETYX version 8 (Genetyx Corporation).

Strain SF-1ᵀ is a Gram-positive, oxidase-negative, catalase-positive, motile, spore-forming, facultatively anaerobic and rod-shaped bacterium (Fujita et al., 1997). Phylogenetic analysis based on the 16S rRNA gene sequence (1413 bp) of strain SF-1ᵀ indicated that it fell within the low-G + C-content, Gram-positive, aerobic, spore-forming bacilli (Fig. 1 and Supplementary Fig. S1 available in IJSEM Online). The closest phylogenetic relative of strain SF-1ᵀ was B. jeotgalii JCM 10885ᵀ, with a sequence similarity of 99.6%; lower similarity was observed with some phylogenetically related Bacillus type strains, including Bacillus vireti LMG 21834ᵀ (96.7 %), Bacillus novalis LMG 21837ᵀ (96.6 %), Bacillus drentensis LMG 21831ᵀ (96.5 %), Bacillus selenatarsenatis JCM 10885T, with a sequence similarity of 96.7 %, and Bacillus drentensis JCM 10885T, with a sequence similarity of 96.7 %.

![Fig. 1. Neighbour-joining tree derived from 16S rRNA gene sequences showing the relationships between strain SF-1ᵀ and related Bacillus species. Bootstrap percentages (based on 1000 replications) greater than 50 % are given at branching points. Bar, 0.01 substitutions per nucleotide position. Paenibacillus polymyxa DSM 36ᵀ was used as the outgroup to root the tree. Accession numbers are given in parentheses. A maximum-parsimony tree is available as Supplementary Fig. S1 in IJSEM Online.](http://dx.doi.org/10.1099/ijsem.0.0000000)
*Bacillus niacini* NBRC 15566T (96.1%), *Bacillus soli* LMG 21838T (95.9%), *Bacillus bataviensis* LMG 21833T (95.9%) and *Bacillus fumarioli* LMG 17489T (95.5%).

The optimal pH for aerobic growth of strain SF-1T is 8.0 and the optimal temperature is 40°C. Strain SF-1T was able to grow on LB agar plates containing kanamycin (3 μg ml⁻¹), tetracycline (3 μg ml⁻¹) and chloramphenicol (5 μg ml⁻¹), whereas it was highly sensitive to ampicillin and erythromycin. Several other biochemical and physiological characteristics of strain SF-1T were compared with those of its nearest phylogenetic relative on the basis of 16S rRNA gene sequence analysis, *B. jeotgali* JCM 10885T (Table 1). Strain SF-1T can grow not only aerobically but also anaerobically using selenate, arsenate and nitrate as terminal electron acceptors, although nitrate supported weak growth. *B. jeotgali* JCM 10885T can grow in the presence of 13% (w/v) NaCl, whereas strain SF-1T did not grow in the presence of more than 7% (w/v) NaCl. Additionally, strain SF-1T was positive for acid production from D-xylose, arbutin, salicin and amygdalin, whereas *B. jeotgali* JCM 10885T could not produce acid from these carbon compounds. Production of H₂S was also observed only in strain SF-1T. These phenotypic differences seemed to distinguish SF-1T from its phylogenetic relative. The cellular fatty acid profile of strain SF-1T, in comparison with that of *B. jeotgali* JCM 10885T, is shown in Table 2. The major cellular fatty acid of strain SF-1T was found to be iso-C₁₅:₀ (47.3 mol%), as is the case for *B. jeotgali* JCM 10885T.

Table 1. Comparison of phenotypic characteristics between strain SF-1T and *B. jeotgali* JCM 10885T

Unless otherwise specified, data for strain SF-1T were obtained from Fujita et al. (1997) and Yamamura et al. (2003) and data for *B. jeotgali* JCM 10885T were obtained from Yoon et al. (2001) and Imada et al. (2005). +, Positive; −, negative; w, weakly positive. Both strain SF-1T and *B. jeotgali* JCM 10885T are positive for growth in the presence of 2 and 5% NaCl, use of oxygen as an electron acceptor, catalase, β-galactosidase, nitrate reduction, decomposition of starch and acid production from glucose, fructose, ascorcin, cellobiose, maltose, sucrose, trehalose, starch, glyco- gen, gentiobiose and 5-ketogluconate. Both strains are negative for oxidase, formation of indole and acid production from glycerol, erythritol, D-arabinose, L-arabinose, ribose, L-xylose, adonitol, methyl β-D-xyllose, galactose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl 2-D-mannoside, methyl 2-D-glucoside, N-acetylgalactosamine, lactose, melibiose, inulin, melezitose, raffinose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate and 2-ketogluconate.

Table 2. Cellular fatty acid compositions (%) of strain SF-1T and *B. jeotgali* JCM 10885T

Data for strain SF-1T were obtained in this study, whereas data for *B. jeotgali* JCM 10885T were taken from Yoon et al. (2001).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain SF-1T</th>
<th><em>B. jeotgali</em> JCM 10885T</th>
</tr>
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<tbody>
<tr>
<td>Gram stain</td>
<td>Positive</td>
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<td>Temperature range for growth (°C)</td>
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<td>10−45</td>
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<tr>
<td>pH range for growth</td>
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<td>7.0−8.0</td>
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<tr>
<td>H₂S production</td>
<td>+ *</td>
<td>−</td>
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<td>Acid production from:</td>
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</tr>
<tr>
<td>D-Xylose</td>
<td>+ *</td>
<td>−</td>
</tr>
<tr>
<td>Arbutin</td>
<td>+ *</td>
<td>−</td>
</tr>
<tr>
<td>Salicin</td>
<td>+ *</td>
<td>−</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>+ *</td>
<td>−</td>
</tr>
<tr>
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</tr>
<tr>
<td>7%</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>13%</td>
<td>−</td>
<td>+</td>
</tr>
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<td>Electron acceptors:</td>
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<tr>
<td>Selenate</td>
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<td>−</td>
</tr>
<tr>
<td>Arsenate</td>
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<td>−</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+</td>
<td>w*</td>
</tr>
</tbody>
</table>

*Results obtained in this study.

*Summed features represent groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 1 contained iso-C₁₅:₀ H and/or C₁₃:₀ 3-OH; summed feature 4 contained anteiso-C₁₇:₀ B and/or iso-C₁₇:₁ I.
The results from biochemical characterizations and DNA–DNA hybridization strongly indicated that strain SF-1T can be distinguished from its closest phylogenetic relative, although they were not differentiated on the basis of cellular fatty acid profiles or 16S rRNA gene sequence analysis. Based on the evidence presented above, we describe a novel species within the genus *Bacillus*, *Bacillus selenatarsenatis* sp. nov.

**Description of Bacillus selenatarsenatis sp. nov.**


The following description is based on data from this study and from Fujita et al. (1997) and Yamamura et al. (2003). Cells stain Gram-positive and are spore-forming, motile rods (1 x 3–6 μm). Colonies are round and white. Growth occurs at 25–40 °C and at pH 7.5–9.0. Growth occurs in the presence of 2–5% NaCl, but not in the presence of 7% NaCl. Positive results are obtained for catalase, β-galactosidase, H₂S production and nitrate reduction and negative results are obtained for oxidase, the Voges–Proskauer test, indole production and phenylalanine deamination. Gelatin and starch are hydrolysed. Acid is produced from D-xylose, indole production and phenylalanine deamination. Gelatin lactose, melibiose, inulin, melezitose, raffinose, xylitol, D-glucose, fructose, amygdalin, arbutin, aesculin, salicin, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-D-glucoside, galactose, mannose, sorbose, β-D-xyllose, β-D-galactose, galactose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, lactose, melibiose, inulin, melezitose, raffinose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-ketogluconate. Cells are resistant to 3 μg kanamycin ml⁻¹, 3 μg tetracycline ml⁻¹ and 5 μg chloramphenicol ml⁻¹, but highly sensitive to ampicillin and erythromycin. The bacterium is a facultative anaerobe that respires oxygen, selenate, arsenate and nitrate as terminal electron acceptors. Selenate is reduced to elemental selenium via the intermediate selenite, arsenate as terminal electron acceptors. Positive results are obtained for catalase, β-galactosidase, H₂S production and nitrate reduction and negative results are obtained for oxidase, the Voges–Proskauer test, indole production and phenylalanine deamination. Gelatin and starch are hydrolysed. Acid is produced from D-xylose, indole production and phenylalanine deamination. Gelatin lactose, melibiose, inulin, melezitose, raffinose, xylitol, D-glucose, fructose, amygdalin, arbutin, aesculin, salicin, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-D-glucoside, galactose, mannose, sorbose, β-D-xyllose, β-D-galactose, galactose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, lactose, melibiose, inulin, melezitose, raffinose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-ketogluconate. Cells are resistant to 3 μg kanamycin ml⁻¹, 3 μg tetracycline ml⁻¹ and 5 μg chloramphenicol ml⁻¹, but highly sensitive to ampicillin and erythromycin. The bacterium is a facultative anaerobe that respires oxygen, selenate, arsenate and nitrate as terminal electron acceptors. Selenate is reduced to elemental selenium via the intermediate selenite, arsenate as terminal electron acceptors. Selenate is reduced to elemental selenium via the intermediate selenite, arsenate as terminal electron acceptors. Selenate is reduced to elemental selenium via the intermediate selenite, arsenate as terminal electron acceptors.

The type strain, SF-1T (=JCM 14380T =DSM 18680T), was isolated from an effluent drain in a glass-manufacturing plant in Japan.

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**References**


