**Shewanella amazonensis** sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds

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A new bacterial species belonging to the genus *Shewanella* is described on the basis of phenotypic characterization and sequence analysis of its 16S rRNA-encoding and gyrase B (gyrB) genes. This organism, isolated from shallow-water marine sediments derived from the Amazon River delta, is a Gram-negative, motile, polarly flagellated, facultatively anaerobic, rod-shaped eubacterium and has a G+C content of 51.7 mol%. Strain SB2B1 is exceptionally active in the anaerobic reduction of iron, manganese and sulfur compounds. SB2B1 grows optimally at 35°C, with 1–3% NaCl and over a pH range of 7–8. Analysis of the 16S rDNA sequence revealed a clear affiliation between strain SB2B1 and members of the gammaproteobacteria. High similarity values were found with certain members of the genus *Shewanella*, especially with *Shewanella putrefaciens*, and this was supported by cellular fatty acid profiles and phenotypic characterization. DNA-DNA hybridization between strain SB2B1 and its phylogenetically closest relatives revealed low similarity values (246–42%7%) which indicated species status for strain SB2B1. That SB2B1 represents a distinct bacterial species within the genus *Shewanella* is also supported by gyrB sequence analysis. Considering the source of the isolate, the name *Shewanella amazonensis* sp. nov. is proposed and strain SB2B1 (= ATCC 7003291) is designated as the type strain.

**Keywords:** *Shewanella amazonensis* sp. nov., metal reduction, gamma *Proteobacteria*, 16S rRNA, DNA gyrase

**INTRODUCTION**

Biogeochemical data have long suggested a significant role for iron and manganese reduction in global nutrient cycling. In recent years, a number of dissimilatory metal-reducing bacteria have been described (see review by Nealson & Safarini, 1994), and a role for such micro-organisms as catalysts of both manganese and iron reduction in natural systems has been inferred. One of the first bacteria shown to link its respiratory growth to the reduction of metals was *Shewanella putrefaciens* (Meyers & Nealson, 1988).

In this paper we describe the isolation of a new metal-reducing bacterium of the genus *Shewanella* from shallow-water marine deposits derived largely from the Amazon River delta off the Amapá coast of Brazil. The Amazon River delta is one of the major sediment depocentres on Earth (~3–6% of global riverine sediment supply), and is characterized by unusually extensive zones of sedimentary Fe and Mn cycling (Aller et al., 1986, 1996, 1997; Allison et al., 1995; Kuehl et al., 1986). The upper 1–2 m of delta topset deposits, encompassing a mass of ~20–30 × 10^9 metric tonnes of sediment, are dominated by non-sulfidic, suboxic redox conditions, with pore-water-dissolved Fe²⁺ concentrations typically ranging from ~0.1–1 mM. Samples from the seasonally mobile intertidal deposits at the initiation of this coastal system
Strain SB2B² was isolated from Amazonian shelf coastal muds, and is a highly active reducer of iron and manganese oxides, thiosulfate and elemental sulfur. Bacterial isolates showing the properties of Gram-negative motile rods with positive oxidase and catalase reactions, strict respiratory metabolism, ability to reduce a variety of electron acceptors, including trimethylamine N-oxide (TMAO), and production of hydrogen sulfide (Stenstrom & Molin, 1990) have until recently been placed under S. putrefaciens. Shewanella was established around its type species S. putrefaciens and included Shewanella hanedai (Jensen et al., 1980) and Shewanella benthica (MacDonnell & Colwell, 1985). Simidu et al. (1990) described Shewanella alga [corrected to Shewanella alga] (Trüper & de' Clari, 1997)] as mesophilic, and relatively high in G+C % content. On the basis of whole-cell protein profiles, ribotyping and 16S rRNA-encoding gene sequence analysis, S. alga was recently phylogenetically characterized (Fonnesbech-Vogel et al., 1997).

Conventional phenotypic and chemotaxonomic analyses identified strain SB2B² as S. putrefaciens. However, PCR probes designed to recognize S. putrefaciens based on gyrB (encoding the B subunit of DNA gyrase, topoisomerase II) failed to generate a specific amplicon for SB2B² (data not shown), suggesting that this strain may represent a new species. To elucidate the phylogenetic status of SB2B², its 16S rDNA and gyrB gene sequences were analysed. Both sequences differ from all known shewanellae, suggesting that the organism does indeed deserve the status of new species.

**METHODS**

**Sample collection.** Strain SB2B² was isolated from relatively low salinity (pore water Cl⁻ ~ 0.1–0.2 mM), mud flat sediment obtained in ~ 1 m water, a few kilometres south of Cabo Cassiporé, Amapá, Brazil, on 12 March 1996 (Station SB2B, 03° 52.59′ N, 51° 04′ 30′ W). At the time of collection, surface pore-water salinities were relatively low, Cl⁻ ~ 0.1–0.2 mM, but as indicated by the activity of the naturally occurring radionuclide ²²⁴Th (t₁/₂ = 24.1 d), these deposits frequently exchange with regions offshore, and thus experience a wide range of salinities over weekly timescales. Pore-water-transport models indicate that the upper few decimetres of sediment are physically mixed with waves and currents over timescales of ~ 1 week. Sediment cores were taken manually using CAB tubing (15.2 cm o.d.), the upper ~ 50 cm was placed in 500 ml polyethylene bottles, and then stored in larger wide-mouth glass jars filled with sediment from the same site. Intertidal surface water temperatures along the coast ranged from ~ 26–31.5 °C. Sediment was kept at ~ 28 °C except during ~ 2 d transport (4 °C).

**Bacterial strains.** S. putrefaciens ATCC 8071T, S. algae ATCC 51192T, S. hanedai ATCC 33224T, S. benthica ATCC 43992T, Shewanella woodyi ATCC 51908T, Shewanella sp. ANG-SQ1 and Shewanella sp. MR-1 were either purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) or isolated in our laboratory. Purified DNAs of newly characterized (Bowman et al., 1997) strains, Shewanella frigidimarina ACAM 591T and Shewanella gelidi-
water. Sugars and amino acids were tested on LB broth at a concentration of 1% as described elsewhere (West & Colwell, 1984). Haemolytic activity was recorded on Trypticase soy agar supplemented with 5% defibrinated sheep blood. Additional phenotypical characteristics were determined by the Biolog microbial identification system.

**Fatty acid methyl ester (FAME) analysis.** Bacteria (SB2BT, ATCC 8071T and ATCC 51192T) were cultivated in Trypticase soy broth (Difco) overnight at 37°C. Cellular fatty acids were extracted from dry cells, methylated and analysed by GC (Moss et al., 1974). FAMES were analysed on a cross-linked 5% phenyl silicone capillary column (0.2 mm i.d. x 25 m long) on a gas chromatograph (HP 5890A; Hewlett-Packard). The column temperature was programmed from 80 to 140°C at 20°C min⁻¹, then at 3°C min⁻¹ to 270°C, and finally maintained at 270°C for 10 min. Injection temperature was 250°C. The FAME peaks were identified by retention time comparison with authentic FAME standards. Quantification of samples was done by the integration of peak areas.

**DNA isolation and characterization.** DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (1977). The G+C content of the DNA was determined by HPLC as described by Mesbah et al. (1989). DNA-DNA hybridization was carried out according to the methods of De Ley et al. (1970), with modifications as described by Hu et al. (1983) and Escara & Hutton (1980), using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter. Renaturation rates were computed by the program TRANSFER.BAS (Jahnke, 1992).

Organisms included in the DNA-DNA hybridization experiments were strain SB2BT (ATCC 700329T), *S. algae* ATCC 51192T, *S. hamedai* DSM 6066T, *S. benthica* DSM 8812T, *S. putrefaciens* ATCC 8071T, *S. woodyi* ATCC 51908T and *Shewanella* sp. ANG-SQ1.

**PCR amplification and direct sequencing.** Chromosomal DNA from overnight cultures was purified by phenol/chloroform extraction and ethanol precipitation (Johnson, 1981). Purified genomic DNA was used as template for PCR amplification. PCR assays were performed in a DNA Thermal Cycler (Perkin Elmer). The 1.2 kb *gyrB* gene (Yamamoto & Harayama, 1995) and the 1.5 kb small-subunit rDNA (Ruimy et al., 1994) were amplified as per established protocols.

The identities of the fragments were verified by sequencing from both ends using the DyeDeoxy chain-termination method with the Sequenase DNA sequencing kit (United States Biochemical) and by ABI 373A automated sequencing as described by the manufacturer (Perkin-Elmer). DNA sequences were determined from both strands by extension from the N- and C-terminal ends using universal primers (Ruimy et al., 1994; Yamamoto & Harayama, 1995) and followed by primer walking.

**Phylogenetic analysis and alignment.** The 16S rDNA sequence was compared with about 500 other 16S rDNA sequences already available in GenBank for their phylogenetic relationships. The *gyrB* sequence was compared with 150 other *gyrB* sequences that were retrieved from the ICB database (http://www.mbio.co.jp). Evolutionary trees were constructed with the PAUP program for Macintosh (Swofford, 1990) and the ARB program package for the Sun Microstation (Strunk & Ludwig, 1995), using the maximum-likelihood analysis method.

**RESULTS AND DISCUSSION**

**Morphological characteristics.** Strain SB2BT T (= ATCC 700329T) is a Gram-negative, rod-shaped, non-spore-forming organism. It grows well at 35°C in standard bacteriological nutrient media such as LB or Trypticase soy broth supplemented with 1% NaCl. Cells are 2 to 3 μm in length and 0.4–0.7 μm in diameter, and are motile by a single unsheathed polar flagellum. On LB agar incubated at 35°C, young colonies are circular, with a diameter of 1–2 mm, smooth, convex, and slightly pinkish, with regular edges, similar to those of *S. putrefaciens* and *S. algae*. Neither diffusible pigments nor bioluminescence was observed.

**Physiological properties.** Biochemical characterization of SB2BT T is presented in Table 1. Type strains of *S. putrefaciens* (ATCC 8071T) and *S. algae* (ATCC 51192T) were included to compare the physiological traits of strain SB2BT T. Strain SB2BT T was positive for cytochrome oxidase, catalase and gelatinase, and negative for the production of amylase, lipase, alginase, arginine dihydrolase and deoxyribonucleases of lysine and ornithine. It reduced nitrate to nitrite and nitrogen gas was formed from nitrite. Strain SB2BT T was unable to ferment glucose but reduced TMAO and produced hydrogen sulfide. Indole and ketone were not produced by this strain. As with *S. algae* strains, sheep blood cells were haemolysed by SB2BT T. The carbon substrate profile of SB2BT T as measured by the Biolog system showed an identification match for *S. putrefaciens*. Phenotypically, SB2BT T resembles *S. algae* more than *S. putrefaciens* (Table 1). Of the 48 properties tested, nine were different from those of *S. putrefaciens* and only four were different from *S. algae*. Growth at 40°C, N₂ gas production from nitrite, gelatinase production and haemolysis of sheep blood cells were the characteristic features that could differentiate SB2BT T from *S. putrefaciens*. *S. algae* cells did not exhibit any growth when grown at 4°C for 24 h. Strain SB2BT T did not grow at NaCl concentration more than 3%. These phenotypic characters were useful to differentiate strain SB2BT T from *S. putrefaciens* and *S. algae*.

**Optimum growth conditions.** SB2BT T grew between 4 and 45°C, with optimum growth at 37°C, and over the pH range of 6–9 (optimum 7–8). Although growth was seen in the absence of NaCl, growth yield was high in 1% NaCl.
Table 1. Differential biochemical characterization of *S. amazonensis*

All strains are straight rods, Gram-negative; grow at 35 °C in marine agar, 1 or 3 % NaCl and pH 6–10; they do not grow at pH 5 or > 10; positive for production of oxidase, catalase and H₂S from thiosulfite. Reduce nitrate to nitrite; negative for production of chitinase, alginate, amylase, arginine dihydrolase, lysine and ornithine decarboxylase, indole, α-ketoine and diffusible pigment; utilizes D-mannose, D-fructose, DL-lactate, L-serine as sole carbon source; do not utilize sucrose, maltose, D-mannitol, glycerol, D-sorbitol, DL-malate, putrescine and L-histidine as sole carbon source. +, Positive reaction; −, negative reaction.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>S. amazonensis</em> ATCC 700329T</th>
<th><em>S. putrefaciens</em> ATCC 8071T</th>
<th><em>S. algae</em> ATCC 51192T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 °C</td>
<td>+</td>
<td>+</td>
<td>−*</td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0% NaCl</td>
<td>Weak</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>6% NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10% NaCl</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>NO₂ to N₂</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gelatinase production</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Haemolysis of sheep blood cells</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Sole carbon source:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</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>Citrate</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>G+C content (mol %)</td>
<td>51.7</td>
<td>47.0</td>
<td>52.4</td>
</tr>
</tbody>
</table>

* No growth in 24 h.

Table 2. Fatty acid composition of various *Shewanella* species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th><em>S. amazonensis</em> ATCC 700329T</th>
<th><em>S. putrefaciens</em> ATCC 8071T</th>
<th><em>S. algae</em> ATCC 51181</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straight-chain fatty acids:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>1.43</td>
<td>2.31</td>
<td>1.32</td>
</tr>
<tr>
<td>15:0</td>
<td>9.17</td>
<td>3.17</td>
<td>6.51</td>
</tr>
<tr>
<td>16:0</td>
<td>6.11</td>
<td>19.05</td>
<td>16.81</td>
</tr>
<tr>
<td>17:0</td>
<td>3.95</td>
<td>1.54</td>
<td>4.07</td>
</tr>
<tr>
<td>18:0</td>
<td>0.10</td>
<td>1.40</td>
<td>0.39</td>
</tr>
<tr>
<td>Unsaturated tertiary-branched fatty acids:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:0-iso</td>
<td>4.70</td>
<td>2.50</td>
<td>0.50</td>
</tr>
<tr>
<td>14:0-iso</td>
<td>1.55</td>
<td>0.25</td>
<td>1.40</td>
</tr>
<tr>
<td>15:0-iso</td>
<td>26.69</td>
<td>21.12</td>
<td>27.39</td>
</tr>
<tr>
<td>16:0-iso</td>
<td>1.41</td>
<td>0.13</td>
<td>0.51</td>
</tr>
<tr>
<td>17:0-iso</td>
<td>1.80</td>
<td>1.65</td>
<td>1.43</td>
</tr>
<tr>
<td>Monoenoic cyclopropyl fatty acids:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:1o6c</td>
<td>0.83</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>16:1o7c</td>
<td>14.65</td>
<td>29.57</td>
<td>15.28</td>
</tr>
<tr>
<td>16:1o9c</td>
<td>0.69</td>
<td>3.53</td>
<td>2.82</td>
</tr>
<tr>
<td>17:1o6c</td>
<td>2.43</td>
<td>0.95</td>
<td>0.92</td>
</tr>
<tr>
<td>17:1o8c</td>
<td>23.45</td>
<td>6.70</td>
<td>10.87</td>
</tr>
<tr>
<td>18:1o7c</td>
<td>4.46</td>
<td>5.96</td>
<td>5.16</td>
</tr>
<tr>
<td>18:1o9c</td>
<td>1.39</td>
<td>3.78</td>
<td>4.95</td>
</tr>
</tbody>
</table>
being isolated from the intertidal zone, SB2B$^T$ might be adapted to the salty environment.

**Cellular fatty acid composition**

Fatty acid compositions of strain SB2B$^T$, *S. putrefaciens* ATCC 8071$^T$ and *S. algae* ATCC 51181 are shown in Table 2. SB2B$^T$ contains straight chain, unsaturated tertiary branch, and monoenoic cyclopropyl fatty acids with a composition of 20:7, 36:1 and 47:9%, respectively. Among the fatty acids measured, SB2B$^T$ was found to contain three major fatty acids, namely 15:0-iso (26.7%), 17:1o8c (23.5%), and 16:1o7c (14.7%). Many of the fatty acids present in SB2B$^T$ are the same as that of *S. putrefaciens* and *S. algae*.

Although some key characteristics differentiate strain SB2B$^T$ from other species of *Shewanella*, conventional biochemical testing and FAME profiles place SB2B$^T$ within the *S. putrefaciens*–*S. algae* group.

**Metal reduction**

Fig. 1 illustrates the reduction of ferric oxide by strain SB2B$^T$ and its metal-reducing activity relative to that of the well-studied *Shewanella* sp. MR-1 (Nealson & Saffarini, 1994). When microcosms were incubated anaerobically at room temperature with ferric oxide as the terminal electron acceptor, a threefold increase in soluble iron was found at 8 h in the microcosm containing SB2B$^T$ compared with MR-1. The release of free iron by SB2B$^T$ was twice that of MR-1 between 25 and 75 h and 1.5-fold greater, even after 100 h.

Manganese reduction by strain SB2B$^T$ is depicted in Fig. 2. When microcosms were incubated anaerobically at room temperature with manganese oxide as the terminal electron acceptor, the initial release of soluble manganese by SB2B$^T$ was 18-fold greater than that of MR-1. The release of free manganese in SB2B$^T$ microcosms was still six- and fourfold greater than that noted for MR-1 after 2 and 4 h, respectively, and threefold after 7 h incubation.

SB2B$^T$ was streaked onto sulfur-containing plates and incubated anaerobically. Both SB2B$^T$ and *Shewanella* sp. MR-1 showed activity on solid agar plates but the zone of sulfur clearing was wider for SB2B$^T$. It was difficult to determine if SB2B$^T$ had higher sulfur reduction activity per se, as this method is purely qualitative.

**Molecular phylogenetic analysis**

As SB2B$^T$ was identified as *S. putrefaciens* by both conventional phenotypic and chemotaxonomies, we originally thought that this bacterium was *S. putrefaciens*. While we have screened hundreds of shewanellae against *S. putrefaciens*-specific gyrB probes, no PCR amplification product was observed for SB2B$^T$ (data not shown). This led us to perform a phylogenetic analysis of SB2B$^T$. In addition, this strain is a more active metal reducer than any of the other shewanellae we have studied.

The phylogenetic position of SB2B$^T$ was examined by comparing its 16S rDNA sequence with those of various eubacterial phyla (Woese, 1987). All phylogenetic analyses of its 16S rDNA sequence un-
ambiguously demonstrated that SB2BT belonged to the gamma subclass of the class Proteobacteria. The 16S rDNA sequences of all known gamma Proteobacteria were compared with that of SB2BT. Their phylogenetic relationships were then analysed, and this study was repeated with several different subdomains of the 16S rDNA sequence and bootstrapping analysis was performed to avoid sampling artifacts. Both 16S rDNA and gyrb nucleotide sequences of SB2BT indicate that it shares a close phylogenetic relationship with the species of Shewanella, Alteromonas and Vibrio.

Neighbour-joining, parsimony and maximum-likelihood analyses were then undertaken on this subset of bacteria, using several subdomains of the 16S rDNA. The results of these analyses are summarized in Fig. 3. In all analyses, SB2BT was most closely associated with members of the genus Shewanella. When we were preparing this manuscript, only five Shewanella species had been formally described, namely S. putrefaciens, S. hanedai, S. benthica, S. algae and S. colwelliana (MacDonell & Colwell, 1985; Fonnesbech-Vogel et al., 1997). However, GenBank contains 16S rDNA sequences for eight Shewanella species. This includes the above four well-described species (ATCC 8071T, 33224T, 43992T, 51908T; S. colwelliana sequence is not available), two recently described Antarctic sea ice isolates (ACAM 456T, 591T; Bowman et al., 1997), one squid isolate (ANG-SQ1), and one new luminous isolate (ATCC 51908T; Makemson et al., 1997). The 16S rDNA sequence of SB2BT was compared with the eight other Shewanella species. Variation of 16S rDNA nucleotide sequences of SB2BT and type strains of S. algae, S. benthica, S. frigidimarina, S. gelidimarina, S. hanedai, S. putrefaciens, S. woodyi and Shewanella sp. ANG-SQ1 was 7-1, 8-8, 8-1, 7-9, 8-7, 6-3, 9-0 and 7-9%, respectively.

Nucleotide sequences of the gyrb genes of eight Shewanella strains described above along with SB2BT were determined. A phylogenetic tree based on gyrb nucleotide sequences is shown in Fig. 4. Variation of gyrb nucleotide sequences of SB2BT and type strains of S. algae, S. benthica, S. frigidimarina, S. gelidimarina, S. hanedai, S. putrefaciens, S. woodyi and Shewanella sp. ANG-SQ1 was 19-5, 22-1, 23-7, 23-9, 22-6, 20-9, 25-8 and 24-7%, respectively. Unlike the situation with the 16S rDNA, variation between gyrb genes was very high (>20%). A very high 25-8% variation was noted between strain SB2BT and S. woodyi ATCC 51908T. The conclusions drawn from
**Shewanella amazonensis sp. nov.**

The **Shewanella amazonensis** sp. nov. was isolated from intertidal sediments. Strain SB2B\textsuperscript{T} is a facultatively anaerobic, polarly flagellated bacterium. No endospores nor capsules are formed. Peritrichous flagellation is not observed when the organism is cultivated on solid media. Colonies on LB agar medium are circular, smooth and convex with an entire edge, and beige to pinkish depending on the age of the colonies. Cells are able to grow at mesophilic temperatures. Optimal growth is observed at 37°C. Denitrifies nitrate to nitrite and nitrite to N\textsubscript{2}. Exhibits cytochrome oxidase, catalase and gelatinase activity, and produces hydrogen sulfide from thiosulfate. Haemolysates sheep blood cells and does not grow at NaCl concentrations above 3%. Utilizes acetate, succinate, fumarate and citrate as sole carbon sources as well as a few carbohydrates and amino acids. Very active in the reduction of iron, manganese and sulfur compounds. Strain SB2B\textsuperscript{T} was isolated from intertidal sediments. The G+C content of the DNA is 51.7 mol\%. The type strain, SB2B\textsuperscript{T}, has been deposited with the American Type Culture Collection as ATCC 700329\textsuperscript{T}.

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**REFERENCES**


American Society for Microbiology.

measurement of DNA hybridisation from renaturation rates.

K.

renaturation of DNA in dimethylsulphoxide solutions

celeration of renaturation rate.


&

Fonnesbech-Vogel, B., Jorgensen, K., Christensen, H., Olsen, J. E.


