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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 SB2B^T is exceptionally active in the anaerobic reduction of iron, manganese and sulfur compounds. SB2B^T 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 SB2B^T and members of the gamma subclass of the class *Proteobacteria*. 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 SB2B^T and its phylogenetically closest relatives revealed low similarity values (24·6–42·7%) which indicated species status for strain SB2B^T. That SB2B^T 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 SB2B^T (= ATCC 700329^T) 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 unusally 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 Fe^{2+} concentrations typically ranging from ~ 0·1–1 mM. Samples from the seasonally mobile intertidal deposits at the initiation of this coastal system

Abbreviation: FAME, fatty acid methyl ester.

The GenBank accession numbers for the nucleotide sequences in this paper are: *S. amazonensis* ATCC 700329^T – AF005257 (*gyrB*) and AF005248 (16S rDNA); *S. algae* ATCC 51192^T – AF005686 (*gyrB*) and AF005249 (16S rDNA); *S. benthica* ATCC 43992^T *gyrB*, AF014949; *S. hanedai* ATCC 33224^T *gyrB*, AF005693; *S. frigidimarina* ACAM 591^T *gyrB*, AF014947; *S. gelidimarina* ACAM 456^T *gyrB*, AF014946; *S. putrefaciens* ATCC 8071^T *gyrB*, AF005669; *S. woodyi* ATCC 51908^T *gyrB*, AF014944; *Shewanella* sp. ANG-SQ1 *gyrB*, AF014945.

south of Cabo Cassiporé were obtained for the present study.

Strain SB2B^T 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 algae* (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. algae* was recently phylogenetically characterized (Fonnesbech-Vogel *et al.*, 1997).

Conventional phenotypic and chemotaxonomic analyses identified strain SB2B^T 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 SB2B^T (data not shown), suggesting that this strain may represent a new species. To elucidate the phylogenetic status of SB2B^T, 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 SB2B^T 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 ^{234}Th ($t_{1/2}$ = 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 by 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 ~ 26·4–31·5 °C. Sediment was kept at ~ 28 °C except during ~ 2 d transport (4 °C).

Bacterial strains. *S. putrefaciens* ATCC 8071^T, *S. algae* ATCC 51192^T, *S. hanedai* ATCC 33224^T, *S. benthica* ATCC 43992^T, *Shewanella woodyi* ATCC 51908^T, *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 591^T and *Shewanella gelidimarina* ACAM 456^T, were received from the University of Tasmania, Australia. All strains were maintained in semi-solid nutrient agar (Difco) and bench cultures were made in either LB liquid or agar media (Sambrook *et al.*, 1989).

Growth conditions. Strain SB2B^T was cultured aerobically in LB liquid medium, and either 10 M HCl or 10% (w/v) NaOH were used to obtain a pH range from 5 to 10. Cultures inoculated in LB liquid medium (pH 7) were incubated at various temperatures under aerobic conditions. The effects of various concentrations of NaCl (0–10%) were studied using 1% Bacto-tryptone (Difco) as the basal medium. Growth was monitored at appropriate intervals by OD_{600} measurements with a visible-light spectrophotometer (LKM Biochrom Ultrospec 4050). Anaerobic growth was accomplished in an anaerobic chamber (Coy Laboratory Products) maintained at 2% hydrogen, the balance in nitrogen.

For visualizing cell shape and flagella, cells were negatively stained with osmium chloride according to the methods of Cole & Popkin (1981) and then observed with an Hitachi H-600 transmission electron microscope.

Isolation of metal-reducing bacteria. A population of metal-reducing bacteria was enriched as described previously (Nealson *et al.*, 1991). Briefly, sediment was mixed with an equal volume of 1·5% agar containing LM medium supplemented with carbon substrate {0·02% yeast extract, 0·01% peptone, 0·6% NaCl, 10 mM sodium bicarbonate, 10 mM HEPES, 5 mM lactate, 5 mM succinate, 5 mM glycerol, 1 mM acetate, 0·5 mM ferric chloride, 5 mM sodium molybdate and ferrozine [3-(2-pyridyl)-5,6 bis (4-phenylsulfonic acid)-1,2,4 triazine], pH 7·2}. The vials were sealed off from oxygen and incubated at room temperature; they were monitored daily and scored qualitatively for iron reduction. After a secondary enrichment, samples that showed zones of strong metal reduction were streaked onto plates with similar media, substituting 50 mM ferric citrate for the ferric chloride to isolate single colonies. Appropriate positive (*Shewanella* sp. MR-1) and negative (*Escherichia coli* ATCC 25922) controls were performed. Since anaerobic sulfur reduction appears to be a trait associated with the *Shewanella* species (Moser & Nealson, 1996), analysis was carried out as per the protocols delineated by Moser & Nealson (1996).

Measurement of metal reduction. LM growth medium containing 0·6% NaCl and 20 mM lactate (Meyers & Nealson, 1988) was used for metal reduction experiments. Amorphous manganese oxide (Lovely & Phillips, 1988) and FeOOH (Atkinson *et al.*, 1967) were prepared as described elsewhere. Inocula were grown aerobically in LB liquid medium at 30 °C, harvested by centrifugation, and adjusted to an inoculum size of approximately $2·0 \times 10^7$ (equivalent to OD_{600} = 0·2) bacterial cells per ml in LM medium containing either ferric or manganese oxides. All media and solutions were de-aerated by purging with nitrogen prior to the experiment. Samples (500 µl) were drawn at 30 min intervals, passed through a 0·2 µm filter (Millipore) and the resulting soluble (reduced) metal was measured by atomic absorption spectrometry (Burdige & Nealson, 1986). To determine total Fe and Mn concentrations, nitric acid (1% final concentration) was added to the unfiltered samples prior to measurement.

Phenotypic analysis. Routine biochemical tests were carried out according to established procedures (Venkateswaran *et al.*, 1989; West & Colwell, 1984). The ability to grow at a NaCl concentration of 1–10% was determined in T_1N_1 liquid medium (Venkateswaran *et al.*, 1989), and the ability to grow without NaCl was determined in 1% sterile tryptone

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 (SB2B^T, ATCC 8071^T and ATCC 51192^T) 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. × 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 Huá *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 SB2B^T (ATCC 700329^T), *S. algae* ATCC 51192^T, *S. hanedai* DSM 6066^T, *S. benthica* DSM 8812^T, *S. putrefaciens* ATCC 8071^T, *S. woodyi* ATCC 51908^T 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 automatic 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 SB2B^T (=ATCC 700329^T) 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 SB2B^T is presented in Table 1. Type strains of *S. putrefaciens* (ATCC 8071^T) and *S. algae* (ATCC 51192^T) were included to compare the physiological traits of strain SB2B^T. Strain SB2B^T was positive for cytochrome oxidase, catalase and gelatinase, and negative for the production of amylase, lipase, alginase, arginine dihydrolase and decarboxylases of lysine and ornithine. It reduced nitrate to nitrite and nitrogen gas was formed from nitrite. Strain SB2B^T was unable to ferment glucose but reduced TMAO and produced hydrogen sufide. Indole and ketoine were not produced by this strain. As with *S. algae* strains, sheep blood cells were haemolysed by SB2B^T. The carbon substrate profile of SB2B^T as measured by the Biolog system showed an identification match for *S. putrefaciens*. Phenotypically, SB2B^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, N_2 gas production from nitrite, gelatinase production and haemolysis of sheep blood cells were the characteristic features that could differentiate SB2B^T from *S. putrefaciens*. *S. algae* cells did not exhibit any growth when grown at 4 °C for 24 h. Strain SB2B^T did not grow at NaCl concentration more than 3%. These phenotypic characters were useful to differentiate strain SB2B^T from *S. putrefaciens* and *S. algae*.

Phenotypic characteristics, such as positive oxidase and catalase reactions, the absence of glucose fermentation, a strict respiratory metabolism, the ability to reduce a variety of electron acceptors, including TMAO, and the production of hydrogen sulfide seem to be sufficient to warrant the inclusion of SB2B^T in the genus *Shewanella*.

Optimum growth conditions

SB2B^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 H_2S from thiosulfite. Reduce nitrate to nitrite; negative for production of chitinase, alginase, 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.

Characteristic	*S. amazonensis* ATCC 700329^T	*S. putrefaciens* ATCC 8071^T	*S. algae* ATCC 51192^T
Growth:			
4 °C	+	+	−*
40 °C	+	−	+
0% NaCl	Weak	+	−
6% NaCl	−	+	+
10% NaCl	−	−	+
NO_2 to N_2	+	−	+
Gelatinase production	+	−	+
Haemolysis of sheep blood cells	+	−	+
Sole carbon source:			
Lactose	−	+	−
Succinate	+	−	+
Fumarate	+	−	−
Citrate	+	−	+/−
G+C content (mol%)	51·7	47·0	52·4

* No growth in 24 h.

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

Fatty acid	Percentage composition of fatty acids in:		
	S. amazonensis ATCC 700329^T	*S. putrefaciens* ATCC 8071^T	*S. algae* ATCC 51181
Straight-chain fatty acids:			
14:0	1·43	2·31	1·32
15:0	9·17	3·17	6·51
16:0	6·11	19·05	16·81
17:0	3·95	1·54	4·07
18:0	0·10	2·10	0·39
Unsaturated tertiary-branched fatty acids:			
13:0-iso	4·70	2·50	0·50
14:0-iso	1·55	0·25	1·40
15:0-iso	26·69	21·12	27·39
16:0-iso	1·41	0·13	0·51
17:0-iso	1·80	1·65	1·43
Monoenoic cyclopropyl fatty acids:			
15:1ω6*c*	0·83	0·19	0·16
16:1ω7*c*	14·65	29·57	15·28
16:1ω9*c*	0·69	3·53	2·82
17:1ω6*c*	2·43	0·95	0·92
17:1ω8*c*	23·45	6·70	10·87
18:1ω7*c*	4·46	5·96	5·16
18:1ω9*c*	1·39	3·78	4·95

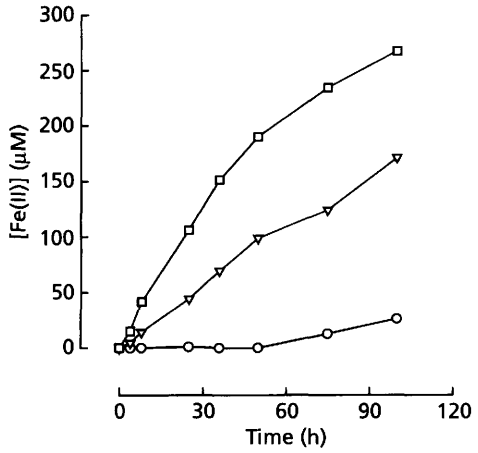

Fig. 1. Amorphous Fe(III) oxide reduction by *S. amazonensis* ATCC 700329^T (□), *Shewanella* sp. MR-1 (▽) and a control with no cells (○). Experiments were conducted at room temperature (23 °C) in LM medium supplemented with 0·6% NaCl at pH 7·2 under anaerobic conditions (98% N_2 and 2% H_2); 400 μM FeOOH with 20 mM lactate (Atkinson *et al.*, 1967) was used as the sole electron acceptor. Approximately $2·0 \times 10^7$ (equivalent to $OD_{600} = 0·2$) bacterial cells ml^{-1} were added. All media and solutions were de-aerated prior to starting the experiment. Samples (500 μl) were drawn at appropriate intervals in triplicate and passed through a 0·2 μm filter, and then added to 4·5 μl 1% nitric acid. Atomic absorption spectroscopy was used to measure the reduced soluble Fe(II).

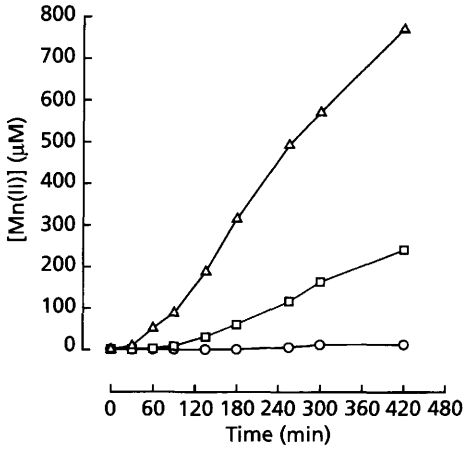

Fig. 2. Amorphous manganese oxide reduction by *S. amazonensis* ATCC 700329^T (□), *Shewanella* sp. MR-1 (▽) and a control with no cells (○). Experimental and analytical conditions were as described in Fig. 1 where Fe(III) oxide was replaced with 1000 μM manganese oxide.

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%), 17:1ω8*c* (23·5%), and 16:1ω7*c* (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 lead 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-

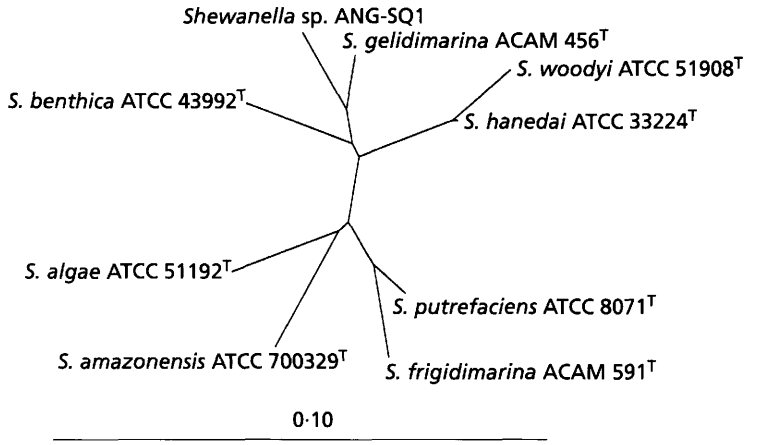

Fig. 3. Phylogenetic position of *S. amazonensis* ATCC 700329^T based on 16S rDNA nucleotide sequences. An unrooted phylogenetic tree was obtained by performing a maximum-likelihood analysis.

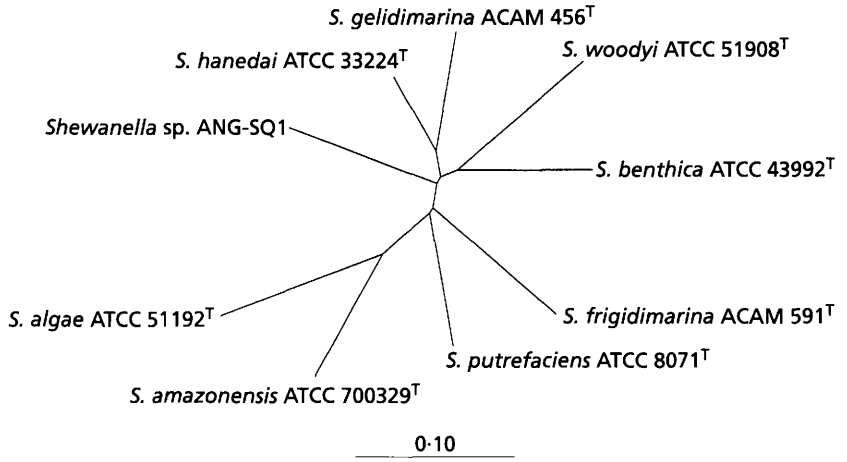

Fig. 4. Phylogenetic tree of *S. amazonensis* ATCC 700329^T based on *gyrB* nucleotide sequences by maximum-likelihood analysis.

ambiguously demonstrated that SB2B^T belonged to the gamma subclass of the class *Proteobacteria*. The 16S rDNA sequences of all known gamma *Proteobacteria* were compared with that of SB2B^T. 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 SB2B^T indicate that it shares a close phylogenic 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, SB2B^T 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. colewelliana* (MacDonnell & 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 8071^T, 33224^T, 43992^T, 51192^T; *S. colwelliana* sequence is not available), two recently described Antarctic sea ice isolates (ACAM 456^T, 591^T; Bowman *et al.*, 1997), one squid isolate (ANG-SQ1), and one new luminous isolate (ATCC 51908^T; Makemson *et al.*, 1997). The 16S rDNA sequence of SB2B^T was compared with the eight other *Shewanella* species. Variation of 16S rDNA nucleotide sequences of SB2B^T 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 SB2B^T were determined. A phylogenetic tree based on *gyrB* nucleotide sequences is shown in Fig. 4. Variation of *gyrB* nucleotide sequences of SB2B^T 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 SB2B^T and *S. woodyi* ATCC 51908^T. The conclusions drawn from

gyrB sequence analysis are consistent with those drawn from 16S rDNA analysis. Hence, strain SB2B^T is distinct and deserves the status of a new species.

Analysis of both 16S rDNA and *gyrB* sequences results in compelling information about the taxonomic position of SB2B^T. This bacterium displays a non-ambiguous affiliation with the gamma subclass of the *Proteobacteria*. The closest relatives for which a 16S rDNA and *gyrB* sequence data are presently available are *S. putrefaciens* and *S. algae*, but it is not possible to determine a significant match to any known bacterium.

Strain SB2B^T has a G+C content of 51·7 mol%, which does not fall within the range that is typical of *S. putrefaciens* (43–47 mol%) whereas the G+C content of *S. algae* is in this range (Fonnesbech-Vogel *et al.*, 1997). Strain SB2B^T shares 94% and less similarity with any of the *Shewanella* species for which 16S rDNA sequences have been deposited. At this level of relatedness, affiliation of this strain to any of the *Shewanella* species is unlikely as demonstrated by Stackebrandt & Goebel (1994). Nevertheless, to confirm the separate species status of strain SB2B^T, DNA–DNA hybridization studies were performed (Table 3) between this strain and *Shewanella* species: *S. algae* ATCC 51192^T, *S. benthica* DSM 8812^T, *S. hanedai* DSM 6066^T, *S. putrefaciens* ATCC 8071^T, *S. woodyi* ATCC 51908^T and *Shewanella* sp. ANG-SQ1. The highest value of 42·7% was determined for the pair SB2B^T and *S. putrefaciens* ATCC 8071^T. This value is significantly below the 70% similarity level, recommended as the threshold value for species delineation (Wayne *et al.*, 1987). Similarity values for strain SB2B^T and the other species ranged between 25 and 40%. It can thus be concluded on the basis of moderate 16S rDNA and DNA–DNA similarity values that strain SB2B^T represents a novel species within the genus *Shewanella*.

Though many *Shewanella* isolates share common phenotypic traits, the 16S rDNA (Fonnesbech-Vogel *et al.*, 1997) and DNA–DNA hybridization studies (Owen *et al.*, 1978; Semple & Westlake, 1987) have revealed the obvious heterogeneity of various strains that have been grouped within the *S. putrefaciens* group. This paper also supports the fact that without nucleotide sequence analysis, strain SB2B^T would have been placed within *S. putrefaciens*.

Description of the *Shewanella amazonensis* sp. nov.

Shewanella amazonensis (ama.zo.nen.sis. M.L. n. *amazonensis* named after the area from which the bacterium was collected).

Cells of the type strain are rod-shaped, 2–3 μm in length and 0·4–0·7 μm in diameter, Gram-negative, facultatively anaerobic, polarly flagellated. 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_2. Exhibits cytochrome oxidase, catalase and gelatinase activity, and produces hydrogen sulfide from thiosulfate. Haemolyses 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^T was isolated from intertidal sediments. The G+C content of the DNA is 51·7 mol%. The type strain, SB2B^T, has been deposited with the American Type Culture Collection as ATCC 700329^T.

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