**Taxonomic Description**

**Desulfovibrio senegalensis** sp. nov., a mesophilic sulfate reducer isolated from marine sediment

Abdoulaye Thioye,¹,² Zouhaier Ben Ali Gam,² Malick Mbengue,¹ Jean-Luc Cayol,² Manon Joseph-Bartoli,² Coumba Touré-Kane³ and Marc Labat²*³

**Abstract**

Several strains of sulfate-reducing bacteria were isolated from marine sediments recovered from Hann Bay (Senegal). All were related to members of the genus *Desulfovibrio*. A strictly anaerobic, mesophilic and moderately halophilic strain designated BLaC¹ was further characterized. Cells of strain BLaC¹ stained Gram-negative and were 0.5 μm wide and 2–4 μm long, motile, rod-shaped and non-spor-forming. The four major fatty acids were anteiso-C₁₅:₀, iso-C₁₅:₀, iso-C₁₇:₀ and anteiso-C₁₇:₀. Growth was observed from 15 to 45 °C (optimum 60 °C) and at pH 5.5–8 (optimum pH 7.5). The salinity range for growth was 5–65 g NaCl l⁻¹ (optimum 30 g l⁻¹). Yeast extract was required for growth. Strain BLaC¹ was able to grow on lactate and acetate in the presence of sulfate as an electron acceptor. Sulfate, thiosulfate and sulfite could serve as terminal electron acceptors, but not fumarate, nitrate or elemental sulfur. The DNA G+C content was 55.8 mol%. 16S rRNA gene sequence analysis assigned strain BLaC¹ to the family *Desulfovibrionaceae*; its closest relative was *Desulfovibrio oxyclinae* DSM 19275T (93.7% similarity). On the basis of 16S rRNA gene sequence comparisons and physiological characteristics, strain BLaC¹ is proposed as representing a novel species of *Desulfovibrio*, with the name *Desulfovibrio senegalensis* sp. nov. The type strain is BLaC¹ (=DSM 101509T=JCM 31063T).

Sulfate-reducing bacteria (SRB) can be found in many engineered and natural environments where sulfate is present. In marine ecosystems, SRB contribute significantly to the mineralization of organic matter [1]. To date, more than 72 species of the genus *Desulfovibrio* have been described. *Desulfovibrio* species often occur in marine anaerobic systems. Many marine *Desulfovibrio* species have been isolated, including *Desulfovibrio gigas* [2, 3], *D. africanus* [4], *D. inopinatus* [5], *D. bizzertensis* [6], *D. frigidas* [7], *D. alkalitolerans* [8], *D. marinus* [9], *D. marinisediminis* [10], *D. portus* [11], *D. oceanic* [12] and ‘*D. hontreensis’* [13]. In this study, we undertook bacterial isolation of SRB sampled from marine sediment recovered from Hann Bay, Dakar, Senegal. Of the different members of the genus *Desulfovibrio* that were isolated, one, designated strain BLaC¹, is proposed to represent a novel species.

Enrichment was initiated on SRB medium from CFG services. Isolation was performed using an anaerobic enrichment medium containing (per litre of distilled water): 0.3 g KH₂PO₄, 0.2 g K₂HPO₄, 0.3 g NH₄Cl, 1 g NaCl, 0.5 g KCl, 0.15 g CaCl₂, 0.4 g MgCl₂, 0.5 g cysteine HCl, 0.5 g yeast extract (Difco) (all w/v), 1 ml mineral element solution [14] and 1 ml 0.1% (w/v) resazurin. The pH was adjusted to 7.2 with 10 M KOH. This enrichment medium was boiled under a stream of O₂-free N₂ gas, and cooled to room temperature; 5 ml aliquots were distributed in Hungate tubes under a stream of O₂-free N₂ gas. The N₂ gas phase was replaced with N₂/CO₂ (80:20), and the tubes were autoclaved. Before inoculation, 0.1 ml 2% Na₂S·9H₂O and 0.1 ml 10% NaHCO₃, lactate (20 mM) and sulfate (20 mM) were added. Enrichments were performed in Hungate tubes containing 5 ml of medium and inoculated with sample diluted to 10%. The tubes were incubated at 30 °C for 1 week. Cultures were purified by repeated use of the Hungate roll-tube method with medium solidified with 2% (w/v) agar (Difco). Several colonies that developed were harvested and cultured in the corresponding culture medium. The process of isolation was repeated several times until isolates were deemed axenic. Physiological optimal growth conditions were determined in duplicate experiments conducted in basal medium containing yeast extract

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**Keywords:** Desulfovibrio senegalensis; sulfate reducer; marine; sediment; Senegal.

**Abbreviation:** SRB, sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of BLaC¹ is KT767981.
(0.5 g l\(^{-1}\)) and lactate (20 mM) with sulfate as the electron acceptor. For pH growth experiments, the culture medium was adjusted to the desired pH using anaerobically prepared stock solutions of NaHCO\(_3\) (10 %) for pH between 7 and 8, Na\(_2\)CO\(_3\) (8 %) for pH above 8 and HCl (100 mM) for pH below 7. The temperature range for growth was determined using the same medium adjusted to the optimum growth pH. For studies of NaCl requirements, NaCl was weighed directly into the tubes at concentrations ranging from 0 to 80 g NaCl l\(^{-1}\), before dispensing basal medium without NaCl. The tubes were incubated at 37 °C. Growth was measured by inserting tubes directly into a model Cary 50 Scan spectrophotometer (Varian) and measuring OD\(_{580}\).

Genomic DNA was extracted according to the protocol described for the Wizard Genomic DNA purification kit (Promega). 16S rRNA genes were amplified using primers Fd1 (5’-AGAGTTTGATCCTGGCTCAG-3’) and Rd1 (5’-AAGGAGGTGATCCAGCC-3’). The nucleotide sequence (1420 bases) was manually aligned using the sequence alignment editor BioEdit [15]. Reference sequences were obtained from the Ribosomal Database Project II [16] and GenBank databases [17]. Pairwise evolutionary distances based on 1298 unambiguous nucleotides were computed by the Jukes and Cantor [18] method. The phylogenetic tree obtained by the neighbour-joining method [19] is shown in Fig. 1.

The fatty acid composition of strain BLaC\(^{T}\) was determined at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) using 2×10 ml of liquid culture [20]. The cellular fatty acid composition of strain BLaC\(^{T}\) was determined on the same basal medium to which yeast extract was added (0.5 %) and cultures of strain BLaC\(^{T}\) were stopped at the end of the exponential phase of growth and sent to DSMZ for fatty acid analysis. Fatty acids were extracted using the method of Miller [21], with the modifications of Kuykendall et al. [22], and the profile of cellular fatty acids was analysed by GC with the Microbial Identification System (MIDI) using GC analysis according to the MIDI Microbial Identification system (e.g. Method: TSBA40).

Enrichment cultures were positive after 1 week of incubation at 30 °C, and microscopic examination revealed the presence of motile rod-shaped bacteria. Two strains of similar morphology were isolated (BLaC\(^{T}\) and BlaC2); the strain designated BLaC\(^{T}\) was taken for further characterization.

Cells of strain BLaC\(^{T}\) were strictly anaerobic, rod-shaped, 0.5 µm wide and 2–4 µm long, and occurred singly or in pairs. They were motile and non-spore-forming. Electron microscopy showed a Gram-stain-negative cell-wall structure (Fig. 2). Strain BLaC\(^{T}\) was a strictly anaerobic bacterium unable to grow under aerobic conditions, and

![Phylogenetic dendrogram based on 1298 unambiguous base pairs of 16S rRNA gene sequence data, showing the position of strain BLaC\(^{T}\) (DSM 101509\(^{T}\)). Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Numbers in parentheses are GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences. Bar, 5 % estimated sequence divergence.](Downloaded from www.microbiologyresearch.org by IP: 53163.10.11 On: Fri, 07 Dec 2018 16:19:00)
mesophilic, growing optimally at 40 °C (range 15–45 °C). The optimum pH for growth was around 7.5. The isolate grew optimally with 3 % added NaCl, and no growth occurred above 6.5 % (w/v) NaCl.

Strain BLaC1T utilized sulfate (20 mM), thiosulfate (20 mM) and sulfite (2 mM) as electron acceptors, but not fumarate (20 mM) or elemental sulfur (0.1 %). Nitrate (20 mM) was not reduced. The sulfur production test was carried out photometrically with colloidal CuS [23], and nitrate or nitrite reduction was assayed using specific sticks (Quanto-fix; Macherey Nagel).

The G+C content determined by the Identification Service of the DSMZ was 55.8 mol% based on the method of Mesbah et al. [24]. This G+C content value fell within the range described for species of the genus Desulfovibrio [25].

Analysis of the almost-complete sequence (1420 bp) of the 16S rRNA gene of strain BLaC1T grouped it among members of the family Desulfovibrionaceae. The phylogenetic tree shown in Fig. 1 indicates that the isolate was closely related to Desulfovibrio oxyclinae DSM 11498T (93.7 % 16S rRNA gene sequence similarity), Desulfovibrio tunisiensis DSM 19275T (93.5 %) and Desulfovibrio halophilus DSM.

Fig. 2. Electron microscopy of strain BLaC1T showing a typical Gram-stain-negative cell-wall structure. Bar, 100 nm.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Vibrio</td>
<td>Vibrio</td>
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<td>Cell size (µm)</td>
<td>0.5×2–4</td>
<td>0.5×1.23</td>
<td>0.5×1–2.5</td>
<td>0.6×2.5–5</td>
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<td>Source</td>
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<td>Microbial mat</td>
<td>Exhaust water</td>
<td>Solar lake</td>
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<td>Temperature range (optimum) (°C)</td>
<td>15–45 (40)</td>
<td>NR (35)</td>
<td>15–45 (37)</td>
<td>15–40 (35)</td>
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<td>pH range (optimum)</td>
<td>6.3–8.5 (7.5)</td>
<td>NR (7.0–7.5)</td>
<td>4.0–9.5 (7.0)</td>
<td>5.5–8.5 (7.0)</td>
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<td>NaCl range (optimum) (% w/v)</td>
<td>0.5–6.5 (3)</td>
<td>2.5–22.5 (5–10)</td>
<td>0.3–7.0 (4)</td>
<td>3.0–18.0 (6–7)</td>
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<tr>
<td>DNA G+C content (mol %)</td>
<td>55.8</td>
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<td>H₂CO₃</td>
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<td>Lactate</td>
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<td>Nitrate</td>
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Table 1. Comparison of the morphological and physiological properties of strain BLaC1T and its closest relatives

Strains: 1, BLaC1T (data from this study); 2, D. oxyclinae DSM 11498T [28]; 3, D. tunisiensis DSM 19275T [26]; 4, D. halophilus DSM 5663T [27]. +, Positive; −, negative; NR, not reported.
Strain BLaC1\textsuperscript{T} is a strictly anaerobic, heterotrophic, mesophilic and halotolerant micro-organism of the domain \textit{Bacteria}. Analysis of the 16S rRNA gene sequence of strain BLaC1\textsuperscript{T} indicates that it belongs to the genus \textit{Desulfovibrio} with \textit{D. oxyclinae} as the phylogenetically nearest recognized species. Strain BLaC1\textsuperscript{T} is a non-spore-forming bacterium like \textit{D. oxyclinae} (strain P1B\textsuperscript{T}), \textit{D. tunisiensis} (strain RB22\textsuperscript{T}) and \textit{D. halophilus} (strain SL8903\textsuperscript{T}), but differs in some morphological and physiological characters (Table 1). First, cells of strain BLaC1\textsuperscript{T} are straight rod-shaped unlike those of the three closest strains P1B\textsuperscript{T}, RB22\textsuperscript{T} and SL8903\textsuperscript{T}, which present a typical vibrio shape when grown on medium containing lactate as the electron donor and sulfate as the terminal electron acceptor. All four strains (BLaC1\textsuperscript{T}, P1B\textsuperscript{T}, RB22\textsuperscript{T} and SL8903\textsuperscript{T}) were mesophilic, and none grew above 45 °C, but strain BLaC1\textsuperscript{T} had a higher optimum (40 °C) than strain RB22\textsuperscript{T} (37 °C), strain P1B\textsuperscript{T} (35 °C) or strain SL8903\textsuperscript{T} (35 °C). Strain BLaC1\textsuperscript{T} differed from its closest relatives in its salt tolerance. Strain BLaC1\textsuperscript{T} was halotolerant and required only 0.5% (w/v) NaCl, whereas strains P1B\textsuperscript{T} and SL8903\textsuperscript{T} were moderately halophilic bacteria, requiring 2.5 and 3.0% NaCl, respectively. Strain BLaC1\textsuperscript{T} had the lowest optimum of growth with NaCl (3%) compared with its closest relative, RB22\textsuperscript{T} (5–10%). Strain BLaC1\textsuperscript{T} could grow at up to 6.5% NaCl, whereas strains RB22\textsuperscript{T}, SL8903\textsuperscript{T} and its closest relative P1B\textsuperscript{T} could grow at up to 7.0, 18.0 and 22.0% NaCl, respectively.

Compared with its three closest relatives, strain BLaC1\textsuperscript{T} showed differences in its capacity to utilize different substrates in the presence of sulfate as an electron acceptor. Strain BLaC1\textsuperscript{T} could not use fumarate, methanol or glyceral, unlike strain RB22\textsuperscript{T}, which displayed positive growth with all three substrates when sulfate was used as an electron acceptor.

Strain BLaC1\textsuperscript{T} could not use malate or butanol, unlike its closest relative strain P1B\textsuperscript{T}, which displayed positive growth with both substrates when sulfate was used as an electron acceptor. Strain BLaC1\textsuperscript{T} also differed from its closest relatives in the utilization of acetate. Only BLaC1\textsuperscript{T} used acetate as an electron donor in the presence of sulfate, unlike its three closest relatives (strains RB22\textsuperscript{T}, P1B\textsuperscript{T} and SL8903\textsuperscript{T}).

Both strain BLaC1\textsuperscript{T} and strain RB22\textsuperscript{T} were capable of autotrophic growth on H\textsubscript{2}/CO\textsubscript{2} in the presence of sulfate, but not strains P1B\textsuperscript{T} or SL8903\textsuperscript{T}. Strain BLaC1\textsuperscript{T} also differed from its three closest relatives in the range of electron acceptors utilized. All the strains could use sulfate, thiosulfate and sulfite as electron acceptors. However, unlike the other strains (RB22\textsuperscript{T}, P1B\textsuperscript{T} and SL8903\textsuperscript{T}) strain BLaC1\textsuperscript{T} did not reduce elemental sulfur.

Finally, strain BLaC1\textsuperscript{T} was markedly distinct from \textit{D. tunisiensis} [26] and \textit{D. halophilus} [27] by its DNA G+C content. Strain BLaC1\textsuperscript{T} had a G+C content of 55.8 mol%, whereas values for strains RB22\textsuperscript{T} and SL8903\textsuperscript{T} were 59.6 and 60.7 mol%, respectively.

Taking into account its phenotypic and phylogenetic characteristics, we propose that strain BLaC1\textsuperscript{T} be classified as representing a novel species of the genus \textit{Desulfovibrio} of the family \textit{Desulfovibrionaceae}, for which the name \textit{Desulfovibrio senegalensis} sp. nov. is proposed.

### DESCRIPTION OF \textit{DESULFOVIBRIO SENEGALENSIS} SP. NOV.

\textit{Desulfovibrio senegalensis} (se.ne.gal.en’sis. N.L. masc. adj. \textit{senegalensis} named after Senegal from where the sample of marine sediment used for isolation originated).

Cells are rod-shaped, anaerobic, mesophilic and moderately halophilic; spores are never observed. Growth is observed from 15 to 45 °C (optimum 40 °C) and at pH 5.5–8.0 (optimum 7.5). The salinity range for growth is 5–65 g NaCl l\textsuperscript{-1} (optimum 30 g l\textsuperscript{-1}). Sulfate, thiosulfate and sulfate are utilized as electron acceptors, but not elemental sulfur and fumarate. Nitrate is not reduced. Substrates utilized in the presence of sulfate as electron acceptors include lactate, acetate, formate and H\textsubscript{2}CO\textsubscript{2}. Malate, fumarate, ethanol, methanol, butanol, glycerol and propionate are not utilized with sulfate as an electron acceptor. The four major fatty acids are anteiso-C\textsubscript{15:0}, iso-C\textsubscript{15:0}, iso-C\textsubscript{17:0} and anteiso-C\textsubscript{17:0}.

The type strain, BLaC1\textsuperscript{T} (=DSM 101509\textsuperscript{T}=JCM 31063\textsuperscript{T}), was isolated from marine sediments recovered from Hann Bay, Senegal. The DNA G+C content of the type strain is 55.8 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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