Desulfatiferula berrensis sp. nov., a n-alkene-degrading sulfate-reducing bacterium isolated from estuarine sediments

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A novel sulfate-reducing bacterium designated strain BE2801T was isolated from oil-polluted estuarine sediments (Berre Lagoon, France). Cells were Gram-stain-negative, motile, slightly curved or vibrioid rods. Optimal growth of strain BE2801T occurred at 30–32 °C, 0.5–1.5% NaCl (w/v) and pH 7.2–7.4. Strain BE2801T grew with C4 to C20 fatty acids or C12 to C20 n-alkenes as electron donors. Acetate and carbon dioxide were the oxidation products. The major cellular fatty acids were C16:0, C16:1ω7c and C18:1ω7. The DNA G+C content was 50.2 mol%. 16S rRNA and dsrAB gene sequence analysis indicated that strain BE2801T was a member of the family Desulfobacteraceae within the class Deltaproteobacteria. DNA–DNA hybridization with the most closely related taxon demonstrated 14.8 % relatedness. Based on phenotypic and phylogenetic evidence, strain BE2801T (=DSM 25524T=JCM 18157T) is proposed to be a representative of a novel species of the genus Desulfatiferula, for which the name Desulfatiferula berrensis sp. nov. is suggested.

The genus Desulfatiferula was first proposed by Cravo-Laureau et al. (2007), and members are mesophilic, Gram-negative sulfate-reducing bacteria that incompletely oxidize organic substrates. At the time of writing, only one species of this genus, Desulfatiferula olefinivorans, had been described and it is able to degrade unsaturated aliphatic hydrocarbons (n-alkenes) (Cravo-Laureau et al., 2007). Among sulfate-reducing bacteria known to oxidize aliphatic hydrocarbons (Grossi et al., 2008), two were reported exclusively to degrade n-alkenes: Desulfatibacillum alkenivorans strain PF2803T (Cravo-Laureau et al., 2004b) and D. olefinivorans strain LM2801T (Cravo-Laureau et al., 2007). Here, we describe a novel sulfate-reducing bacterium, strain BE2801T, which oxidizes fatty acids and long-chain n-alkenes and suggest that strain BE2801T should be classified as a representative of a novel species of the genus Desulfatiferula.

Strain BE2801T was isolated from brackish sediments found in a retention basin that recovers water from a wastewater treatment plant of a petrochemical factory located on the shore of the Berre Lagoon (Berre, France). Sediment samples were collected using a plastic core sampler capped with a butyl stopper and stored at 4 °C until use. Enrichment and cultivation methods have been described by Cravo-Laureau et al. (2004a). Enrichment cultures were supplemented with sterile 1-eicosene (C20H40, 1.0 mM) as substrate and sodium dithionite (0.12 mM) as an additional reductant (Widdel & Bak, 1992), and incubated at 30 °C in the dark. Strain BE2801T was purified from an enrichment culture free of sediments as previously described (Cravo-Laureau et al., 2004a). The purity of the strain was confirmed by its lack of growth in natural lagoon-water medium supplemented with glucose.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and dsrAB gene sequences of strain BE2801T are HE613444 and HE613445, respectively.
(3 mM) and yeast extract (0.5 g l⁻¹) under oxic and anoxic conditions and in AC Medium (Difco), and with microscopic observation. The maintenance and growth of pure cultures were carried out in a synthetic sulfate-reducing growth medium (Cravo-Laureau et al., 2004a) containing 1.2 % NaCl (w/v). Cultures were supplemented with 1-alkenes (1.0 mM) or 1-hexadecene (1.4 mM) and sodium dithionite (0.12 mM).

Cells of strain BE2801ᵀ were short, slightly curved or vibrioid rods, and were motile due to polar flagella. Using the Gram-staining reaction and KOH method (Buck, 1982) cells stained Gram-negative.

Physiological tests were performed as described by Cravo-Laureau et al. (2004a). Results of the physiological characterization (pH, salinity and temperature) with sodium octanoate (2 mM) as growth substrate are given in the species description. Strain BE2801ᵀ used butyrate (C₄:0, 5 mM), octanoate (C₈:0, 2 mM), myristate (C₁₄:0, 2 mM), palmitate (C₁₆:0, 2 mM), stearate (C₁₈:0, 2 mM) and arachidate (C₂₀:0, 1 mM) as electron donors and carbon sources. Growth on behenate (C₂₂:0, 1 mM) or lignocerate (C₂₄:0, 1 mM) was not observed. Growth was obtained with H₂/CO₂ (80 : 20, v/v, 1 bar) and slight growth was obtained with 10 mM pyruvate and 10 mM butanol. Absence of growth was observed with the following substrates (mM in parentheses, except where stated): formate (5), acetate (10), propionate (10), isobutyrate (5), valerate (5), lactate (10), citrate (5), glutarate (5), z-ketoglutarate (5), malate (10), succinate (10), fumarate (10), tartrate (2), glycolate (2), acetone (5), ethanol (10), glycerol (10), glucose (5), fructose (5), glucurionate (10), glycine (5), cysteine (5), methionine (10), aspartate (5), glutamate (5), betaine (5), phenol (0.5), benzoate (5), indol (0.25), thioacetamide (2), peptone (0.5 g l⁻¹), Casamino-acids (0.5 g l⁻¹) and yeast extract (0.5 g l⁻¹). Pyruvate (10 mM) was fermented. Malate, lactate and fumarate were not fermented. Sulfate (20 mM), sulfite (5 mM), elemental sulfur (0.8 g l⁻¹), DMSO (10 mM) and fumarate (10 mM) acted as electron acceptors. Slight growth was also observed with nitrate (10 mM) and thiosulfate (10 mM). Desulfovibrin was absent in this strain. N₂, NH₄Cl and yeast extract were used as nitrogen sources, but not nitrate or glutamate. Vitamins were not required for growth.

Cellular fatty acids were analysed as previously described (Grossi et al., 2011). The major cellular fatty acids of strain BE2801ᵀ cells grown with sodium octanoate were C₁₆:0, C₁₆:1₀₇c and C₁₈:₀_VO₇ (Table 1). C₁₆:0 has already been reported to be the predominant fatty acid of strain BE2801ᵀ (Grossi et al., 2011). Three fatty acids (C₁₂:0, C₁₄:₁₀₁₃, C₁₅:₀ 3-OH) observed after growth with octanoate were absent in cells grown with hexadecene (Table 1).

The fatty acid composition of the two novel strains grown on 1-alkenes has been previously reported (Grossi et al., 2011), and the average chain length of the cellular fatty acids of each strain was correlated with that of the alkene substrate. Linear saturated fatty acids and monounsaturated or hydroxylated homologues were the main fatty acids synthesized by both strains (Grossi et al., 2011).

Hydrocarbon utilization by strain BE2801ᵀ was tested using 250 mg aliphatic hydrocarbons l⁻¹ (n-alkanes: C₅–C₂₀, pristine, squalane, cyclohexane, or n-alkanes: C₁₂–C₂₀, or 100 mg aromatic hydrocarbons l⁻¹ (toluene, naphthalene, phenanthrene or fluoranthene). Strain BE2801ᵀ was able to oxidize and grow with all the n-alkenes tested (C₁₂ to C₂₀). Growth with alkanes or aromatic hydrocarbons was not observed. To our knowledge, strain BE2801ᵀ is the third strain of hydrocarbonoclastic sulfate-reducing bacteria reported to oxidize exclusively n-alkenes as a hydrocarbon substrate, the others being D. alkenivorans strain PF₂₈₀³ (Cravo-Laureau et al., 2004b) and D. olefinivorans strain LM₂₈₀¹ (Cravo-Laureau et al., 2007). Interestingly, these three strains are able to use only a very limited number of substrates in addition to alkenes as electron donors and carbon sources.

Quantitative growth experiments were performed with 1-hexadecene as substrate (5 µl hexadecene per tube corresponding to 17.4 µmol per 15 ml culture) (Cravo-Laureau et al., 2004a). Soluble sulfide was measured by colorimetry based on the methylene blue formation reaction (Cline, 1969). The exact sulfide content of standards was determined by iodometric titration (Vogel, 1961). Sulfate analyses were performed by ion chromatography (Metrohm) using a Metrosep A Supp 1 column. Acetate concentration was determined by HPLC (Thermo Scientific) using an Aminex HPX-87H-300 × 7 column. CO₂ in the gas phase was determined by GC (2100, Perichrom) after acidification of the culture (H₂PO₄, 10 mM). 1-Hexadecene concentrations were determined

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Growth substrate</th>
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<tbody>
<tr>
<td></td>
<td>Octanoate</td>
</tr>
<tr>
<td>C₁₀:₀</td>
<td>0.7</td>
</tr>
<tr>
<td>C₁₂:₀</td>
<td>3.0</td>
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<tr>
<td>C₁₄:₀</td>
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<tr>
<td>C₁₅:₀</td>
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<tr>
<td>C₁₆:₀</td>
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<tr>
<td>C₁₇:₀</td>
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<tr>
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<td>1.7</td>
</tr>
<tr>
<td>C₁₆:₁₀⁹</td>
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<tr>
<td>C₁₆:₁₀⁷c</td>
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</tr>
<tr>
<td>C₁₆:₁₀⁷t</td>
<td>0.9</td>
</tr>
<tr>
<td>C₁₈:₁₀⁹</td>
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<tr>
<td>C₁₈:₁₀⁷</td>
<td>9.0</td>
</tr>
<tr>
<td>C₁₄:₄ 3-OH</td>
<td>3.7</td>
</tr>
<tr>
<td>C₁₅:₅ 3-OH</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 1. Fatty acid composition (relative %) of strain BE2801ᵀ grown on octanoate and 1-hexadecene (data from Grossi et al., 2011)
Strain BE2801\textsuperscript{T} oxidizes substrates incompletely to acetate. After 37 days of incubation, 14.1 ± 1.1 \textmu mol hexadecene and 73.5 ± 14.4 \textmu mol sulfate were consumed, while 63.5 ± 12.6 \textmu mol soluble sulfide, 75.1 ± 14.5 \textmu mol acetate, 59.7 ± 14.8 \textmu mol CO\textsubscript{2} and 196.4 ± 32 \textmu g biomass were produced. Thus, experimentally, 1 mol hexadecene required 5.2 ± 1 mol sulfate. These values approximate to the theoretical equation for the incomplete oxidation of hexadecene, forming acetate and CO\textsubscript{2}:

\[
\text{C}_{16}\text{H}_{32} + 20/3 \text{SO}_4^2- + 4/3 \text{H}^+ \rightarrow 20/3 \text{HS}^- + 16/3 \text{CH}_3\text{COO}^- + 16/3 \text{CO}_2 + 16/3 \text{H}_2\text{O}
\]

Strain BE2801\textsuperscript{T} oxidizes substrates incompletely to acetate and CO\textsubscript{2}, as do several other sulfate-reducing bacteria (Rabus et al., 2006). This is not the case for the two other alkene oxidizers, \textit{D. olefinivorans} strain LM2801\textsuperscript{T} and \textit{D. alkenivorans} strain PF2803\textsuperscript{T}. The former oxidizes alkenes to acetate as the sole end product, while the latter mineralizes alkenes entirely to CO\textsubscript{2}.

The DNA G+C content of strain BE2801\textsuperscript{T}, determined at the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (DSMZ) according to a standard protocol (Mesbah et al., 1989), was 50.2 mol\%. The methods for genomic DNA purification, PCR amplification, sequence alignment and comparative analyses of 16S rRNA genes and \(\gamma\)- and \(\beta\)-subunits of the dissimilatory sulfite reductase (\textit{dsrAB}) genes have been described previously (Cravo-Laureau et al., 2004a). Sequencing was performed by GATC Biotech (Konstanz, Germany). Analysis of the almost complete sequence (1417 bp) of the 16S rRNA gene of strain BE2801\textsuperscript{T} (Fig. 1) revealed that this novel isolate belongs to the family \textit{Desulfobacteraceae} within the class \textit{Deltaproteobacteria} of the phylum \textit{Proteobacteria}. The closest relative of strain BE2801\textsuperscript{T} is \textit{D. olefinivorans} strain LM2801\textsuperscript{T} (97\% similarity), an alkene-degrading strain isolated from brackish sediments of a wastewater decantation system of an oil refinery. Strain BE2801\textsuperscript{T} was not closely phylogenetically (maximum 91\% similarity) to other known alkene-degrading strains (CV2803\textsuperscript{T}, PF2803\textsuperscript{T}, AK-01, Pnd3 and Hxd3; Grossi et al., 2008) in the family \textit{Desulfobacteraceae} (Fig. 1). The affiliation of strain BE2801\textsuperscript{T} to the family \textit{Desulfobacteraceae} was confirmed by DsrAB amino acid sequence analysis (349 amino acids; Fig. 2). DNA–DNA hybridizations with strain BE2801\textsuperscript{T} and \textit{D. olefinivorans} (DSM 18843\textsuperscript{T}) were performed at the DSMZ by the method of De Ley et al. (1970) modified by Escara & Hutton (1980) and Huß et al. (1983). The relatedness between the two strains is 14.8\%. This value is well below the threshold value of 70\% accepted for the delineation of a novel bacterial species (Wayne et al., 1987). Based on phylogenetic, biochemical and bioenergetic properties, we propose the affiliation of strain BE2801\textsuperscript{T} to the genus \textit{Desulfatiferula}.”

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**Fig. 1.** Phylogenetic tree based on comparative analyses of 16S rRNA gene sequences of strain BE2801\textsuperscript{T} and its relatives. Accession numbers are shown in parentheses. Bar, 1 substitution per 100 nt. Percentages of 1000 bootstrap resampling are shown near the relevant nodes. Bootstrap values are shown for branches with more than 50\% bootstrap support.
physiological differences (Table 2) between strain BE2801<sup>T</sup> and \textit{D. olefinivorans} strain LM2801<sup>T</sup>, strain BE2801<sup>T</sup> is proposed to represent a novel species of the genus \textit{Desulfatiferula}, for which the name \textit{Desulfatiferula berrensis} sp. nov. is suggested.

\textbf{Description of \textit{Desulfatiferula berrensis} sp. nov.}

\textit{Desulfatiferula berrensis} (ber.ren'sis. N.L. fem. adj. berrensis of or belonging to Berre, France, pertaining to where the type strain was first isolated).

Cells are slightly curved or vibrioid rods (0.6–1.0–4.0 μm). The optimum temperature for growth is 30–32 °C (range 20–35 °C). The optimum pH is 7.2–7.4 (range 6.5–8.2). Growth occurs at concentrations of 0–4.4% NaCl (w/v) (optimum 0.5–1.5 % NaCl). Sulfate, sulfite, elemental sulfur, DMSO and fumarate are used as electron acceptors. Fatty acids (C4 to C20) and \textit{n}-alkenes (C12 to C20) serve as electron donors. Slight growth is possible with pyruvate or butanol as substrates. The strain is lithoautotrophic (H₂) and ferments pyruvate. Vitamins are not required.

The type strain, BE2801<sup>T</sup> (=DSM 25524<sup>T</sup> = JCM 18157<sup>T</sup>) was isolated from oil polluted sediments (Berre lagoon, France). The DNA G+C content of the type strain is 50.2 mol%.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Characteristics} & \textbf{1} & \textbf{2} \\
\hline
Morphology & Curved or vibrioid rods & Curved or vibrioid rods \\
Cell size (μm) & 0.6 × 1.0–4.0 & 0.45 × 0.8–5.0 \\
DNA G+C content (mol%) & 50.2 & 45.5 \\
Electron donors and carbon sources & & \\
H₂/CO₂ & + & – \\
Fatty acids & + (C<sub>4</sub>–C<sub>20</sub>) & + (C<sub>4</sub>–C<sub>24</sub>) \\
Pyruvate & (+)* & – \\
Malate & – & – \\
Succinate & – & – \\
Fumarate & (+) & – \\
Butanol & (+) & – \\
Alkanes & – & – \\
Alkenes & + (C<sub>12</sub>–C<sub>20</sub>) & + (C<sub>14</sub>–C<sub>23</sub>) \\
Oxidation products & Acetate and CO₂ & Acetate \\
\hline
\end{tabular}
\caption{Main characteristics of strains BE2801<sup>T</sup> and \textit{Desulfatiferula olefinivorans} DSM 18843<sup>T</sup>}
\end{table}

1, BE2801<sup>T</sup>; 2, \textit{Desulfatiferula olefinivorans} (DSM 18843<sup>T</sup>). +, Growth; –, no growth; (+), slight growth.

*Fermented.
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References


