Desulfatiferula olefinivorans gen. nov., sp. nov., a long-chain n-alkene-degrading, sulfate-reducing bacterium

Cristiana Cravo-Laureau,1 Cindy Labat,2 Catherine Joulian,2† Robert Matheron3 and Agnès Hirschler-Réa2

1Equipe Environnement et Microbiologie, IPREM UMR 5254, IBEAS Université de Pau et des Pays de l’Adour, BP1155, 64013 Pau cedex, France
2Laboratoire de Microbiologie et Biotechnologie des Environnements Chauds, IRD, UMR_D 180 IFR-BAIM, case 925, Universités de Provence et de la Méditerranée, 163 avenue de Luminy, 13288 Marseille cedex 9, France
3Laboratoire d’Ecologie Microbienne, IMEP UMR 6116, Université Paul Cézanne, Faculté des Sciences et Techniques de Saint-Jérôme, 13397 Marseille cedex 20, France

A novel anaerobic, long-chain alkene-degrading, sulfate-reducing bacterium, strain LM2801T, was isolated from brackish sediment of a wastewater decantation facility of an oil refinery (Berre lagoon, France). Cells of strain LM2801T were Gram-negative, motile, slightly curved or vibrioid rods. Its optimum growth conditions were 30–36 °C, 6–10 g NaCl l−1 and pH 7.5. Strain LM2801T incompletely oxidized long-chain alkenes (from C14 to C23) and fatty acids (C14 to C24). The DNA G+C content was 45.5 mol%. Sequence analyses of the 16S rRNA and dsrAB genes indicated that the strain was a member of the family Desulfobacteraceae within the Deltaproteobacteria. This novel isolate possesses phenotypic and phylogenetic traits that do not allow its classification as a member of any previously described genus. Therefore, strain LM2801T is described as a member of a new genus, Desulfatiferula gen. nov., of which Desulfatiferula olefinivorans sp. nov. is the type species. The type strain of Desulfatiferula olefinivorans is LM2801T (≡DSM 18843T =JCM 14469T).

Several strains of sulfate-reducing bacteria able to oxidize aliphatic hydrocarbons have been isolated (Widdel et al., 2007). Among these strains, five are known to oxidize alkenes (Aeckersberg et al., 1991, 1998; So & Young, 1999; Cravo-Laureau et al., 2004a, b), unsaturated hydrocarbons commonly produced in plants but also occasionally present in small quantities in crude oils (Curiale & Frolov, 1998). Among these five complete-oxidizing strains, only one degrades alkenes exclusively (Cravo-Laureau et al., 2004b). Here, we report on the isolation and characterization of a novel isolate that exclusively oxidizes long-chain alkenes and fatty acids incompletely to acetate, strain LM2801T.

Strain LM2801T was isolated from brackish sediment collected from a wastewater decantation facility that recovers wastewater from an oil refinery treatment plant (Berre, France). Sediment samples were collected using a plastic core sampler capped with a butyl stopper. Samples were stored at 4 °C until use. Enrichment and cultivation methods were described by Cravo-Laureau et al. (2004a). Enrichment cultures were supplemented with 1-eicosene (C20H40; 1.0 mM) as substrate and sodium dithionite (0.12 mM) as additional reductant (Widdel & Bak, 1992) and incubated at 30 °C in the dark. Eicosene was sterilized via hot filtration (cellulose membrane, 0.2 μm) and added hot to the culture medium. The strain was purified from an enrichment culture free of sediment as described previously (Cravo-Laureau et al., 2004a). The purity of the strain was confirmed by the absence of growth in natural lagoon-water medium supplemented with glucose (3 mM) and yeast extract (0.5 g l−1) under aerobic and anaerobic conditions and in AC medium (Difco) and by microscopic observations. Maintenance and growth of pure culture were achieved using a synthetic sulfate-reducing growth medium (Cravo-Laureau et al., 2004a) with 0.7% (w/v) NaCl. Cultures were supplemented with 1-eicosene (1.0 mM) and sodium dithionite (0.12 mM).

Cells of strain LM2801 were short, slightly curved or vibrioid rods (see Supplementary Fig. S1, available in

†Present address: BRGM, Service Environnement et Procédés, Unité Biotechnologies, 3 av. Claude Guillemin, 45060 Orléans, France.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and dsrAB gene sequences of strain LM2801T are respectively DQ826724 and DQ826725.

A phase-contrast photomicrograph of cells of strain LM2801T is available as supplementary material with the online version of this paper.
Physiological tests were performed as described by Cravo-Laureau et al. (2004a). Growth was monitored indirectly by measuring sulfide production according to Cline (1969). Results of the physiological characterization (pH, salinity and temperature) with sodium palmitate (2 mM) as growth substrate are given in the species description. Strain LM2801$^T$ used myristate ($C_{14:0}$, 2 mM), palmitate ($C_{16:0}$, 2 mM), stearate ($C_{18:0}$, 2 mM), arachidate ($C_{20:0}$, 1 mM), behenate ($C_{22:0}$, 1 mM) and lignocerate ($C_{24:0}$, 1 mM) as electron donors and carbon sources. Growth on alkenes was particularly slow; moreover, strain LM2801$^T$ is able to use only a very few substrates besides alkenes as electron donors and carbon sources.

Quantitative growth experiments with 1-eicosene were carried out according to Cravo-Laureau et al. (2004a). Sulfide was determined colorimetrically by 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 a turbidimetric method (Kolmert et al., 2000) after removing sulfide with zinc carbonate. Acetate concentration was determined by HPLC (Waters 600E equipped with a Phenomenex Rexex organic acids column) after removing sulfide with zinc carbonate. 1-Eicosene concentrations were determined by gas chromatography after extraction with pentane (Cravo-Laureau et al., 2004a). After 50 days of incubation, 0.94 ± 0.04 mM eicosene and 6.03 ± 0.74 mM sulfate were consumed, while 6.48 ± 0.19 mM sulfide and 9.3 ± 2.79 mM acetate were produced. These values are close to the following theoretical reaction:

$$C_{20}H_{40} + 5SO_2^- \rightarrow 5H_2S + 10CH_3COO^-$$

The 1-eicosene degradation balance shows that strain LM2801$^T$ oxidized the hydrocarbon incompletely to acetate as end product.

The G+C content of the DNA of strain LM2801$^T$, determined at the DSMZ using HPLC according to a standard protocol described by Mesbah et al. (1989), was 45.5 mol%. The methods for genomic DNA purification, PCR amplification, sequence alignment and comparative analyses of genes encoding the 16S rRNA and the $\gamma$- and $\beta$-subunits of the dissimilatory sulfite reductase ($dsrAB$), dendrogram construction and bootstrap analysis have been described previously (Cravo-Laureau et al., 2004a). Sequencing was done by Genome Express (Grenoble, France). Analysis of the almost-complete sequence (1353 bp) of the 16S rRNA gene of strain LM2801$^T$ revealed that this novel isolate belongs to the family Desulfoviridaceae within the class Deltaproteobacteria (Fig. 1). This analysis also shows that strain LM2801$^T$ is distantly related to other alkene-oxidizing sulfate-reducing strains, including Hxd3 (Aeckersberg et al., 1991), PnD3 (Aeckersberg et al., 1998), AK-01 (So & Young, 1999), Desulfatibacillum aliphaticivorans CV2803$^T$ (Cravo-Laureau et al., 2004a) and Desulfatibacillum alkenivorans PF2803$^T$ (Cravo-Laureau et al., 2004b) (Fig. 1), with less than 92 % 16S rRNA gene sequence identity. Strain LM2801$^T$ grouped in a new cluster with two sulfate-reducing strains, R-ButA1 and R-CaprA1, which were isolated from rice fields (Wind et al., 1999). However, the 16S rRNA gene sequences of strain LM2801$^T$ and these two strains are only distantly related (96.2 % identity). Other known species of the Desulfoviridaceae are phylogenetically distant (less than 90 % identity) from strain LM2801$^T$ (Fig. 1). The phylogenetic position of strain LM2801$^T$ within the family Desulfoviridaceae was supported by deduced DsrAB sequence analyses (322 amino acids) (Fig. 2). Based on phylogenetic, biochemical and physiological differences (Table 1) between strain LM2801$^T$ and strains R-ButA1

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and R-CaprA1, strain LM2801T is proposed as representing a novel species in a new genus, *Desulfatiferula olefinivorans* gen. nov., sp. nov.

**Description of Desulfatiferula gen. nov.**

*Desulfatiferula* (De.sul.fa.ti.fe’ ru.la. L. pref. de from; N.L. n. sulfas -atis sulfate; L. fem. n. *ferula* a staff, a small rod; N.L. fem. n. *Desulfatiferula* a rod-shaped sulfate-reducer).

Mesophilic sulfate-reducing bacteria. Gram-negative cells, motile by polar flagella. Rod-shaped, non-sporulating cells. Desulfoviridin is not detected. Organic substrates are oxidized incompletely. The type species is *Desulfatiferula olefinivorans*.

**Description of Desulfatiferula olefinivorans sp. nov.**


Cells are slightly curved or vibrioid rods (0.45 × 0.8–5.0 μm). Growth occurs at 16–38 °C (optimum 30–36 °C) and pH 6.6–8.3 (optimum pH 7.5). Growth occurs at NaCl concentrations of 0–50 g l⁻¹ (optimum 6–10 g NaCl l⁻¹), and at least 0.5 g MgCl₂·6 H₂O is required for growth. Only sulfate is used as an electron acceptor. C₁₄ to C₂₄ fatty acids serve as electron donors as well as alkenes (C₁₄–C₂₃). Vitamins are required. The DNA G+C content of the type strain is 45.5 mol% (HPLC).

The type strain, LM2801T (=DSM 18843T =JCM 14469T), was isolated from oil-polluted sediment (Berre lagoon, France).

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**Table 1. Main characteristics of sulfate-reducing bacteria phylogenetically close to strain LM2801T**

Desulfoviridin was not detected in the three strains. All strains were unable to use H₂CO₃, formate, acetate, propionate, lactate and ethanol as electron donors and carbon sources. Data for reference strains were taken from Wind *et al.* (1999). +, Growth; −, no growth; (+), slight growth; NR, not reported.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LM2801T</th>
<th>R-CaprA1</th>
<th>R-ButA1</th>
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<tr>
<td><strong>Morphology</strong></td>
<td>Curved rod</td>
<td>Curved rod</td>
<td>Curved rod</td>
</tr>
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<td><strong>Cell size (μm)</strong></td>
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<td>0.6–0.7</td>
<td>0.6–0.8</td>
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<td><strong>DNA G+C content (mol%)</strong></td>
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<td>52.44</td>
<td>52.19</td>
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<td><strong>Electron donors and carbon sources</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>+ (C₁₄–C₂₄)</td>
<td>NR</td>
<td>NR</td>
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<td>Pyruvate</td>
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<td>Alkenes</td>
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</table>

*Fermented.*

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**Fig. 1.** Phylogenetic tree based on comparative analyses of 16S rRNA gene sequences of strain LM2801T and its relatives. Bar, 2% sequence difference. Solid circles, bootstrap >70%; open circles, 50% <bootstrap <70%. Sequence accession numbers are given in parentheses.

**Fig. 2.** Phylogenetic tree based on comparative analyses of deduced DsrAB amino acid sequences of strain LM2801T and its relatives. Bar, 2% sequence difference. Solid circles, bootstrap >70%; open circles, 50% <bootstrap <70%. Sequence accession numbers are given in parentheses.
References


