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

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A novel anaerobic, long-chain alkene-degrading, sulfate-reducing bacterium, strain LM2801^T, was isolated from brackish sediment of a wastewater decantation facility of an oil refinery (Berre lagoon, France). Cells of strain LM2801^T 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 LM2801^T 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 LM2801^T 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 LM2801^T (=DSM 18843^T =JCM 14469^T).

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 LM2801^T.

Strain LM2801^T was isolated from brackish sediment collected from a wastewater decantation facility that recovers wastewater from an oil refinery treatment plant

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *dsrAB* gene sequences of strain LM2801^T are respectively DQ826724 and DQ826725.

A phase-contrast photomicrograph of cells of strain LM2801^T is available as supplementary material with the online version of this paper.

(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^T were short, slightly curved or vibrioid rods (see Supplementary Fig. S1, available in

IJSEM Online) that were motile by means of polar flagella. Cells stained Gram-negative after the Gram staining reaction and the KOH method (Buck, 1982).

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 the last two was particularly slow. Slight growth was obtained with 5 mM butyrate. No growth was observed on the following substrates (mM, except where stated): H₂/CO₂ (1 bar), H₂ plus acetate (1 bar/10 mM), formate (5), acetate (10), formate plus acetate (5/10), propionate (10), isobutyrate (5), valerate (5), octanoate (2), nonanoate (2), decanoate (2), dodecanoate (2), lactate (10), pyruvate (10), citrate (5), α -ketoglutarate (5), malate (10), succinate (10), fumarate (10), crotonate (2), tartrate (2), glycolate (2), thioglycolate (2), acetone (5), ethanol (10), methanol (10), 2-propanol (10), butanol (10), glycerol (10), glucose (5), fructose (5), gluconate (10), glycine (5), alanine (5), serine (5), threonine (10), lysine (10), cysteine (5), methionine (10), aspartate (5), glutamate (5), betaine (5), phenol (0.5), benzoate (5), gallate (5), catechol (0.5), indole (0.25), nicotinate (2), thioacetamide (2), peptone (0.5 g l⁻¹), Casamino acids (0.5 g l⁻¹) and yeast extract (0.5 g l⁻¹). Malate, lactate, fumarate and pyruvate were not fermented. Sulfate (20 mM) served as an electron acceptor, whereas nitrate (10 mM), fumarate (10 mM), thiosulfate (10 mM), sulfite (5 mM), elemental sulfur (0.8 g l⁻¹) and DMSO (10 mM) were not used. Desulfovibrin was absent. N₂, NH₄Cl and yeast extract were used as nitrogen sources, but not nitrate or glutamate. Vitamins were required for growth.

Hydrocarbon utilization was tested with 250 mg aliphatic hydrocarbons l⁻¹ (n-alkanes, C₅–C₂₀; n-alkenes, C₈–C₂₃) and 100 mg aromatic hydrocarbons l⁻¹ (benzene, toluene, xylene, *p*-cymene, naphthalene and phenanthrene). Strain LM2801^T was able to oxidize and grow on n-alkenes from C₁₄ to C₂₃. Growth on alkenes was particularly slow; indeed, 6.5 mM sulfide was produced after 50 days of growth on eicosene whereas, in the presence of sodium palmitate, it reached 9 mM in 10 days. Growth on n-alkanes or aromatic hydrocarbons was not observed. Among sulfate-reducing bacteria able to oxidize aliphatic hydrocarbons (Widdel *et al.*, 2007), only *Desulfatibacillum alkenivorans* PF2803^T (Cravo-Laureau *et al.*, 2004b) oxidizes alkenes exclusively. Nevertheless, strain LM2801^T metabolizes only long-chain alkenes, whereas *Desulfatibacillum alkenivorans* oxidizes alkenes smaller than C₁₄. 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 Rezex 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:

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 γ - and β -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 *Desulfobacteraceae* 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 *Desulfobacteraceae* are phylogenetically distant (less than 90 % identity) from strain LM2801^T (Fig. 1). The phylogenetic position of strain LM2801^T within the family *Desulfobacteraceae* 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

Fig. 1. Phylogenetic tree based on comparative analyses of 16S rRNA gene sequences of strain LM2801^T and its relatives. Bar, 2% sequence difference. Solid circles, bootstrap >70%; open circles, 50% <bootstrap <70%. Sequence accession numbers are given in parentheses.

and R-CaprA1, strain LM2801^T 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. Desulfovibrin is not detected. Organic substrates are

Table 1. Main characteristics of sulfate-reducing bacteria phylogenetically close to strain LM2801^T

Desulfovibrin was not detected in the three strains. All strains were unable to use H₂/CO₂, 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.

Characteristic	LM2801 ^T	R-CaprA1	R-ButA1
Morphology	Curved or vibrioid rod	Curved rod	Curved rod
Cell size (μm)	0.45 × 0.8–5.0	0.6–0.7 × 2.1–2.6	0.6–0.8 × 2.1–2.6
DNA G + C content (mol%)	45.5	52.44	52.19
Electron donors and carbon sources			
Butyrate	(+)	+	+
Fatty acids	+ (C ₁₄ –C ₂₄)	NR	NR
Pyruvate	–	+*	–
Malate	–	–	+
Succinate	–	+	–
Fumarate	–	(+)	–
Alkanes	–	NR	NR
Alkenes	+ (C ₁₄ –C ₂₃)	NR	NR

*Fermented.

oxidized incompletely. The type species is *Desulfatiferula olefinivorans*.

Description of *Desulfatiferula olefinivorans* sp. nov.

Desulfatiferula olefinivorans [o.le.fi.ni.vo'rans. N.L. n. *olefinum* olefin; L. part. adj. *vorans* devouring; N.L. part. adj. *olefinivorans* olefin (alkene) devouring].

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 MgCl₂·6H₂O l⁻¹ 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, LM2801^T (=DSM 18843^T =JCM 14469^T), was isolated from oil-polluted sediment (Berre lagoon, France).

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Fig. 2. Phylogenetic tree based on comparative analyses of deduced DsrAB amino acid sequences of strain LM2801^T and its relatives. Bar, 5% sequence difference. Solid circles, bootstrap >70%; open circles, 50% <bootstrap <70%. Sequence accession numbers are given in parentheses.

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