Desulfatibacillum alkenivorans sp. nov., a novel n-alkene-degrading, sulfate-reducing bacterium, and emended description of the genus *Desulfatibacillum*

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An alkene-degrading, sulfate-reducing bacterium, strain PF2803^T, was isolated from oil-polluted sediments (Fos Harbour, France). The cells were found to be Gram-negative, non-sporulating, non-motile and to have a slightly curved rod shape. Optimum growth occurred at 1 % (w/v) NaCl, pH 6.8 and 28–30 °C. Strain PF2803^T oxidized alkenes (from C₈ to C₂₃). The G + C content of the genomic DNA was 57.8 mol% (HPLC). On the basis of 16S rRNA gene sequence analyses, strain PF2803^T belongs to the family 'Desulfobacteraceae' in the class 'Deltaproteobacteria', with *Desulfatibacillum aliphaticivorans* as its closest relative (99.6 % identity). Comparative sequence analyses of the dissimilatory sulfite reductase (*dsrAB*) gene supported the affiliation of strain PF2803^T to the genus *Desulfatibacillum*. DNA–DNA hybridization with its closest taxon demonstrated 48.4 % similarity. On the basis of the results of physiological and genetic analyses, strain PF2803^T is identified as a novel species of the genus *Desulfatibacillum*, for which the name *Desulfatibacillum alkenivorans* sp. nov. is proposed. The type strain is PF2803^T (=DSM 16219^T=ATCC BAA-924^T).

To date, 12 sulfate-reducing bacterial strains that degrade hydrocarbons have been isolated (Spormann & Widdel, 2000; Cravo-Laureau *et al.*, 2004). Of these strains, only five were able to oxidize aliphatic hydrocarbons (Aeckersberg *et al.*, 1991, 1998; Rueter *et al.*, 1994; So & Young, 1999; Cravo-Laureau *et al.*, 2004). One of them was recently described as a member of a novel genus with the species name *Desulfatibacillum aliphaticivorans* (Cravo-Laureau *et al.*, 2004). Here we report on the isolation and characterization of a novel species within this genus, for which we propose the name *Desulfatibacillum alkenivorans* sp. nov.

Several sulfate-reducing bacterial strains were isolated from oil-polluted sediments of a sewage plant of Fos Harbour

(France). Prior to isolation, sediments (3/4) recovered by water (1/4) under a small gas phase were stored at room temperature for about 5 years in a plastic bottle under natural light conditions until use. The bottle was hermetically sealed and oxygen diffusion was limited. Enrichment and culture methods were as previously described (Cravo-Laureau *et al.*, 2004). The sulfidogenic enrichment culture was obtained by incubating a homogeneous fraction (12 ml) of the plastic-bottle contents with natural seawater medium (48 ml) (Cravo-Laureau *et al.*, 2004) under a gas mixture (N₂/CO₂, 90:10) using anaerobic techniques (Pfennig *et al.*, 1981). Enrichment culture was supplemented with 1-tetradecene (1.4 mM final concentration) as the energy and carbon source. Sodium dithionite (0.12 mM) was added as an additional reducing agent (Widdel & Bak, 1992). The enrichment culture was incubated at 30 °C in the dark, without agitation. After several subcultures with 1-tetradecene, pure cultures were obtained by repeated passage through a deep-agar dilution series according to Pfennig & Trüper (1981), with sodium octanoate (2 mM) as substrate. The purity of the strains was confirmed by the absence of growth in natural seawater medium supplemented with glucose (3 mM) and yeast extract (0.5 g l⁻¹) under aerobic and anaerobic

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The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene and *dsrAB* gene sequences of strain PF2803^T are respectively AY493562 and AY504426.

A phase-contrast micrograph of cells of strain PF2803^T is available as supplementary material in IJSEM Online.

conditions, as well as in Ac medium (Difco), and by microscopic observations. Pure cultures were routinely maintained in a synthetic sulfate-reducing bacterial culture medium (Cravo-Laureau *et al.*, 2004) with 1.5% (w/v) NaCl. Cultures were supplemented with 1-tetradecene (1.4 mM) and sodium dithionite (0.12 mM).

Of the two sulfate-reducing isolates, strain PF2803^T was chosen for taxonomic description. The cell walls of strain PF2803^T produced a Gram-negative reaction in classical Gram staining and with the KOH method (Buck, 1982). The cells were non-motile, polymorphic rod-shaped and sometimes slightly curved (see supplementary figure in IJSEM Online).

Physiological characteristics (pH, salinity and temperature ranges) were determined as previously described (Cravo-Laureau *et al.*, 2004). The results are presented in the species description. Under optimal growth conditions, the doubling time, with sodium octanoate as substrate, was about 22 h. The doubling time of strain PF2803^T growing on hydrocarbon could not be determined because of the aggregation of the cells to hydrocarbon droplets. Therefore measurements of optical density, as well as centrifugation of cells, were not possible. The results of electron-donor and carbon-source utilization are presented in Table 1. Malate and pyruvate were not fermented. Strain PF2803^T used sulfate (20 mM), thiosulfate (10 mM) and sulfite (5 mM) but not nitrate (10 mM), fumarate (10 mM) or elemental sulfur (0.8 g l⁻¹) as electron acceptors. Cells of the new isolate did not contain desulfovibrin. Disproportionation

of thiosulfate and sulfite was not observed. N₂, NH₄Cl and yeast extract were each used as a nitrogen source, but neither nitrate nor glutamate was used. Vitamins were not essential for growth.

Aliphatic hydrocarbons (n-alkanes, C₅ to C₂₀; n-alkenes, C₈ to C₂₃) were added at a final concentration of 250 p.p.m., whereas aromatic hydrocarbons (benzene, toluene, xylene, *p*-cymene, naphthalene and phenanthrene) were added at a final concentration of 100 p.p.m. Strain PF2803^T was able to oxidize n-alkenes from C₈ to C₂₃. Growth on n-alkanes or aromatic hydrocarbons was not observed. This is the first report of a sulfate-reducing bacterium that, of all the hydrocarbons, exclusively degrades alkenes. Quantitative growth experiments on 1-tetradecene were carried out according to Cravo-Laureau *et al.* (2004). Sulfide was determined colorimetrically (Cline, 1969). The exact sulfide content of standards was determined by iodometric titration (Vogel, 1961). Measurements of sulfate were performed using the turbidimetric method (Tabatabai, 1974) after removal of sulfide with zinc carbonate. The concentrations of 1-tetradecene were determined by GC after extraction with pentane (Cravo-Laureau *et al.*, 2004). After 50 days incubation, strain PF2803^T oxidized 0.92 ± 0.13 mM tetradecene, consumed 12.01 ± 1.41 mM sulfate and produced 11.5 ± 1.15 mM sulfide. The complete oxidation of 1-tetradecene corresponds to the following theoretical reaction: C₁₄H₂₈ + 10.5 SO₄²⁻ → 10.5 S²⁻ + 14 H₂O + 14 CO₂.

Our experimental results give values that are close to this

Table 1. DNA G+C contents and physiological characteristics of strains PF2803^T and *D. aliphaticivorans* DSM 15576^T

+, Growth; -, no growth; (+), slight growth. All strains were positive for utilization of the following (mM, except where stated otherwise): H₂/CO₂ (1 bar), pyruvate (10), crotonate (2), isobutyrate (5), butyrate (5), valerate (5), octanoate (2), nonanoate (2), palmitate (5), stearate (2) and butanol (10). All strains were negative for utilization of the following (mM, except where stated otherwise): formate + acetate (5/10), lactate (10), citrate (5), 2-oxoglutarate (5), tartrate (2), glycolate (2), thioglycolate (2), acetone (5), ethanol (10), methanol (10), 2-propanol (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).

Characteristic	Strain PF2803 ^T	<i>D. aliphaticivorans</i> DSM 15576 ^T
DNA G+C content (mol%)	57.8	41.4
Electron donors and carbon sources (mM, except where otherwise stated):		
H ₂ /acetate (1 bar/10)	-	+
Formate (5)	-	+
Acetate (10)	-	(+)
Propionate (10)	-	(+)
Malate (10)	+	(+)
Succinate (10)	-	(+)
Fumarate (10)	-	+
Glycerol (10)	+	-
Alkanes (250 p.p.m.)	-	C ₁₃ to C ₁₈
Alkenes (250 p.p.m.)	C ₈ to C ₂₃	C ₇ to C ₂₃

Fig. 1. Phylogenetic tree based on comparative analyses of 16S rRNA gene sequences of strain PF2803^T and its relatives. Bar, 2 nucleotide substitutions per 100 nucleotides.

reaction, showing that strain PF2803^T completely oxidized hydrocarbon.

The G+C content of the DNA of strain PF2803^T, determined at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) using HPLC according to a standard protocol described by Mesbah *et al.* (1989), was 57.8 mol%. The methods used for extraction and purification of genomic DNA, for PCR amplification, for sequence alignment and for comparative analyses of 16S rRNA genes and α - and β -subunits of the dissimilatory-sulfite-reductase (*dsrAB*) genes have been described previously (Cravo-Laureau *et al.*, 2004). Sequencing was carried out by Genome Express (Grenoble, France). 16S rRNA gene sequence analysis (1295 bp) revealed that the novel isolate belonged to the class 'Deltaproteobacteria' and that its closest relative is *D. aliphaticivorans* (99.6 % identity) (Fig. 1). The phylogenetic position of the novel isolate within the genus *Desulfatibacillum* was supported by *dsrAB* sequence analyses (329 aa) (Fig. 2). DNA-DNA hybridization studies with strain PF2803^T and *D. aliphaticivorans* (DSM 15576^T) were performed at the DSMZ by the method of De Ley *et al.* (1970), as modified by Escara & Hutton (1980) and Huß *et al.* (1983). The relatedness value between the two strains is 48.4 %. Although this value is relatively high with regard to the fact that the G+C (% mol) contents of the DNAs are very different (Table 1), it is significantly below the value of 70 % recommended by Wayne *et al.* (1987) for strains of the same species. On the basis of physiological differences (Table 1) between strain PF2803^T and *D. aliphaticivorans*, DNA-DNA hybridization results and DNA G+C

contents, it is justified to consider strain PF2803^T as a novel species of the genus *Desulfatibacillum*, for which the name *Desulfatibacillum alkenivorans* is proposed.

Emended description of the genus *Desulfatibacillum* Cravo-Laureau *et al.* 2004

Alkanes and/or alkenes are used as substrates. The type species is *Desulfatibacillum aliphaticivorans*.

Description of *Desulfatibacillum alkenivorans* sp. nov.

Desulfatibacillum alkenivorans (al.ke.ni'vo.rans. N.L. n. *alkenum* alkene, a specific hydrocarbon molecule; L. v. *voro* to devour; N.L. part. adj. *alkenivorans* devouring alkenes).

Cells are slightly curved rods (0.68 × 1.2–4.5 µm). Growth occurs at 22–38 °C (optimum, 28–30 °C) and pH 6.2–8.0 (optimum, pH 6.8). Growth occurs at NaCl concentrations of 5–50 g l⁻¹ (optimum, 10 g NaCl l⁻¹). Sulfate, sulfite and thiosulfate are used as electron acceptors. H₂, pyruvate, malate, crotonate, isobutyrate, fatty acids (C₄ to C₁₈), butanol and glycerol serve as electron donors. Able to grow autotrophically. Alkenes (C₈ to C₂₃) are oxidized. No vitamins are required. The G+C content of the DNA is 57.8 mol% (HPLC).

The type strain is PF2803^T (=DSM 16219^T=ATCC BAA-924^T). Isolated from oil-polluted sediments of a sewage plant at Fos Harbour (France).

Fig. 2. Phylogenetic tree based on comparative analyses of amino acid sequences deduced from *dsrAB* genes of strain PF2803^T and its relatives. Bar, 5 substitutions per 100 amino acids.

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