

A Member of the Delta Subgroup of Proteobacteria from a Pyogenic Liver Abscess Is a Typical Sulfate Reducer of the Genus *Desulfovibrio*

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Received 10 July 2000/Returned for modification 3 September 2000/Accepted 11 November 2000

Strain FH26001/95 (ATCC 700045) was previously isolated from a pyogenic liver abscess from a human. Comparative 16S rRNA gene sequence analysis showed that this strain is related to members of the delta subgroup of the proteobacteria, within a cluster of sulfate-reducing bacteria (*Desulfovibrio* spp.) and non-sulfate-reducing bacteria (*Bilophila wadsworthia* and *Lawsonia* spp.). The phenotype of strain FH26001/95 was found to be typical of members of the genus *Desulfovibrio*. Growth and substrate transformations were possible at oxygen concentrations of 2 to 5% (vol/vol) but not at oxygen concentrations of 21% (vol/vol) in air. Its isolation from an infection in a human suggests that some members of the genus *Desulfovibrio* can be considered opportunistic pathogens.

Sulfate-reducing bacteria of the genus *Desulfovibrio* are typical inhabitants of anaerobic sediments and anaerobic digesters and have been reported to occur in the human digestive tract (3, 11). *Desulfovibrio* spp. have been isolated from clinical specimens associated with infections of humans (16, 19, 20, 22, 26, 29), but these have been assigned to the genus *Desulfovibrio* mainly on the basis of morphology and by comparative sequence analysis of their 16S rRNA genes. However, the genera *Desulfovibrio*, *Lawsonia*, and *Bilophila* are phylogenetically entwined (9, 18, 23, 27, 29) within the delta subgroup of the proteobacteria. *Lawsonia* spp. have been detected as intracellular pathogens of the ileum of a range of mammals and birds (6, 9, 15, 23, 24, 28). They are not known to be able to use sulfate as an electron acceptor, and their metabolism is unstudied. *Bilophila wadsworthia* has been isolated from specimens from patients with appendicitis, human feces and an anaerobic digester (2, 18, 27). This species is not able to reduce sulfate as an electron acceptor (2, 18). Assignment of strains to one of these three genera will require their phenotypic characteristics to be determined.

Strain FH26001/95 (ATCC 700045) was isolated from fluid from a liver abscess (29). Comparative 16S rRNA gene sequence analysis showed that this strain belongs to the delta subgroup of the proteobacteria, within the cluster of sulfate-reducing bacteria (*Desulfovibrio* spp.) and non-sulfate-reducing bacteria (*Bilophila wadsworthia* and *Lawsonia* spp.). We have characterized this isolate using methods commonly applied to *Desulfovibrio* spp. and found it to be a typical sulfate-

reducing bacterium, with the characteristics that allow an unequivocal assignment to the genus *Desulfovibrio*.

General characteristics. Strain FH26001/95 was able to grow in the sulfide-reduced, bicarbonate-buffered medium FM (13) at pH 7.2 with 10 mM sodium lactate, 20 mM Na₂SO₄, and 1 g of yeast extract per liter (incubated at 37°C). Completely filled 20-ml screw-cap tubes or 50-ml screw-cap bottles were normally used; the lids were lined with rubber seals. Yeast extract (filter sterilized [0.2- μ m pore size]), sulfur compounds and other electron acceptors, and carbon sources and electron donors were added from sterile stock solutions before inoculation.

Cells of strain FH26001/95 were curved rods, with a typical vibrioid or spiral form, mostly about 4 μ m long, but varying from 2 to 12 μ m in length and 0.7 to 0.8 μ m in diameter. The G+C content of the genomic DNA from cells of strain FH26001/95 was 62.8 mol% (standard deviation = 0.03 mol%; $n = 6$), as determined by a high-performance liquid chromatography method (12). Pale tan-colored lens-shaped colonies were formed in agar-deep cultures (25), made by using medium FM with lactate and sulfate. Other characteristics of strain FH26001/95 have been reported by Tee et al. (29).

Electron donors with sulfate as electron acceptor. Potential electron donors were added to sterile media from separately sterilized (autoclaved or filter sterilized in the case of heat-labile substrates) and neutralized (NaOH or HCl) stock solutions of D isomers of sugars (final concentration in the medium, 2 mM) or L isomers of organic and amino acids (final concentration, 10 mM). H₂ was added in a mixture with CO₂ (H₂-CO₂; 4:1 [vol/vol]) by the Hungate technique (30) into the gas phase of half-filled bottles sealed with black rubber stoppers (Aldrich Chemical Co., Castle Hill, New South Wales, Australia).

Strain FH26001/95 was able to grow with hydrogen, lactate, pyruvate, malate, fumarate, formate, ethanol, propanol, butanol, and alanine in the presence of 20 mM Na₂SO₄ and 1 g of

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TABLE 1. Characteristics of strain FH26001/95 and members of the genera *Desulfovibrio*, *Bilophila*, and *Lawsonia*

Character	Strain FH26001/95	<i>Desulfovibrio</i> spp. ^a	<i>Bilophila wadsworthia</i> ^a	<i>Lawsonia intracellularis</i> ^a
G+C content (mol%)	63	61–67	39–40	36 ^b
Sulfate reduction	+ ^c	+	–	–
Sulfite reduction	+	+	+	NR
Desulfovibrin	+ ^a	+	+	NR
Obligate intracellular growth	–	–	–	+

^a Data from Baron et al. (2), Laue et al. (18), McOrist et al. (23), Tee et al. (29), and Widdel and Bak (30).

^b R. T. Good and R. A. Strugnell (personal communication).

^c Symbols and abbreviations: +, character present; –, character absent; NR, not reported.

yeast extract per liter. Sulfide (assayed qualitatively by a copper precipitation test [7]) was produced with these electron donors. Acetate, propionate, butyrate, succinate, citrate, glycerol, fructose, galactose, glucose, ribose, aspartate, glutamate, glycine, isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine did not support growth or sulfide production. The production of organic acids and alcohols was analyzed by high-pressure liquid chromatography with a refractive index detector (8). Acetate was produced from lactate, pyruvate, ethanol, and alanine, but when growing with propanol or butanol, the corresponding fatty acid (propionate or butyrate) was produced. Acetate and smaller amounts of succinate (<10% of end-product carbon) were produced when the strain was grown with fumarate or malate. When it was grown with lactate plus Na₂SO₄, the balances of substrates and products showed that 2 mol of lactate was oxidized to 2 mol of acetate coupled to the reduction of 1 mol of sulfate to 1 mol of sulfide (determined colorimetrically [5]). The range of electron donors and the stoichiometry of substrate catabolism are typical of those of members of the genus *Desulfovibrio* (30).

Electron acceptors and fermentation. Strain FH26001/95 was able to utilize nitrate, sulfate, thiosulfate, elemental sulfur (prepared as described elsewhere [13]), and taurine as electron acceptors with lactate as the electron donor. Nitrite (determined with sulfanilic acid and *N,N*-dimethyl-1-naphthylamine [17]) and ammonia (4) were detected as end products of nitrate reduction, but N₂ gas (assayed by including a Durham tube in the medium) was not produced. Sulfide was the end product of reduction of sulfur-containing electron acceptors. Fumarate was not used as an electron acceptor. The use of sulfate as a terminal electron acceptor is a diagnostic characteristic of *Desulfovibrio* spp. (30).

Strain FH26001/95 was able to ferment pyruvate to acetate and presumably H₂ and CO₂ (assays for these gases were not conducted). Lactate, malate, fumarate, ethanol, propanol, butanol, glycerol, alanine, aspartate, glutamate, fructose, galactose, formate, and hydrogen did not support growth in the absence of a suitable electron donor.

Other requirements for growth. Strain FH26001/95 was able to grow with pyruvate or with lactate plus nitrate in anaerobically prepared medium FM reduced with 1.5 mM Na₂S, with 3 mM cysteine, or without a reducing agent (when 0.5 mM cysteine was added as a sulfur source). Cysteine-reduced medium FM was prepared by adding 5 ml of a freshly prepared, filter-sterilized 0.4 M L-cysteine solution instead of Na₂S.

Strain FH26001/95 was able to utilize sulfate, sulfide, thiosulfate, taurine, cysteine, and cystine (all tested at 1 mM with

respect to sulfur atoms) as sulfur sources when the strain was grown under anoxic conditions with lactate plus nitrate in unreduced medium. Yeast extract (at 1 g per liter) and methionine (1 mM) did not serve as sulfur sources for growth. Strain FH26001/95 required yeast extract for growth, and the yeast extract could not be replaced by a mixture of 26 growth factors.

Strain FH26001/95 is a *Desulfovibrio* sp. The phenotypic characteristics of strain FH26001/95 are consistent with its assignment to the genus *Desulfovibrio* (Table 1). Species of the genus *Desulfovibrio* are differentiated on the basis of only a few phenotypic characters (30). A detailed comparative study encompassing strain FH26001/95 and its apparent close relatives (16, 19, 22) will be required to elucidate the taxonomic status of this group of opportunistic pathogens.

Oxygen relations. Strain FH26001/95 was able to grow in opposing gradients of O₂ and lactate in the absence of other electron acceptors. Gradient systems were set up in foil-capped, 250-ml, "tall-form" glass beakers with a height of 115 mm and an internal diameter of 58 mm. The gradient systems contained two layers, the lower with the growth substrate and the upper with the preparation being inoculated. The lower layer consisted of 25 ml made up of equal volumes of 2MOPS-FM and a 3% (wt/vol) molten washed-agar preparation (30), mixed just prior to addition to the sterile beaker. 2MOPS-FM contained the components of medium FM at double the normal concentrations, except that sodium sulfide and sodium bicarbonate were omitted, and 50 ml of 1 M 3-(*N*-morpholino)propanesulfonic acid (MOPS; adjusted to pH 7.0 with NaOH and autoclaved), 10 ml of filter-sterilized 10% (wt/vol) yeast extract, and 25 ml of filter-sterilized 20 mM L-cystine (adjusted to pH 10 with NaOH) were added per liter. When a growth substrate was required, this layer was supplemented with sodium lactate to give a final concentration of 10 mM. Once this layer had solidified, the upper layer of 75 ml was added. This upper layer consisted of equal volumes of 2MOPS-FM and a 1% (wt/vol) molten washed-agar preparation. The two components were mixed, cooled to 42°C, and inoculated with 0.75 ml of a stationary-phase culture of strain FH26001/95 before being poured into the beaker to form a layer 28 mm deep. After 3 to 6 days of incubation at 37°C in air, a plate of cells with a thickness of about 200 μm formed 6 mm below the agar surface. No growth or plate formation was observed if lactate was omitted from the system or if the cultures were incubated anaerobically in anaerobic jars with GasPak Plus anaerobic system envelopes (Becton Dickinson and Co., Cockeysville, Md.) or in uninoculated controls. When incubated anaerobically with lactate in the upper agar layer and with 20

mM Na₂SO₄ in both agar layers, up to five plates of cells formed at depths of 10 to 20 mm below the surface (8 to 18 mm above the lactate-containing layer).

Oxygen concentrations in the aerobically incubated agar-stabilized gradient cultures were measured polarographically with a 760 needle oxygen electrode (Diamond General, Ann Arbor, Mich.). The surface of the gradient system (0 mm reference position) was determined by first inserting the E207 reference electrode (SDR Clinical Technology, Middle Cove, New South Wales, Australia) into the agar near the wall of the beaker and then lowering the needle electrode on a vernier-scaled manipulator into the agar until a signal was obtained. Oxygen concentrations were then read at increments of 0.1 mm. The needle electrode-reference electrode-polarographic amplifier system was calibrated with air- and N₂-saturated distilled water before the measurements were made (and was checked again after the measurements were made). The oxygen concentration at the position of the plate of cells was 19 to 24 μM, equivalent to a headspace partial pressure of about 2% (vol/vol) O₂. The cells in the plate were collected with a Pasteur pipette and were immediately viewed by phase-contrast microscopy with a video monitor calibrated with a stage micrometer. These cells were motile, with a rapid darting motility at a rate of 50 to 60 μm s⁻¹. The rapid darting motility of the cells presumably played a role in maintaining the highly defined plates.

Cultures under different O₂ partial pressures were grown in anaerobically prepared medium FM without the addition of sodium sulfide. Aliquots of 30 ml were dispensed into 200-ml glass bottles which were closed with black rubber stoppers, and the headspace was immediately flushed with O₂-free N₂ plus CO₂ (N₂-CO₂; 4:1 [vol/vol]). These cultures were supplemented with 10 mM sodium lactate, 1 g of yeast extract per liter, and 0.5 mM L-cystine. Oxygen was then added with a syringe to give the desired final partial pressure. Growth was observed with lactate in the presence of 5% (vol/vol) O₂ in the headspace, at which the dissolved O₂ concentration would be 54 μM at 37°C. A total of 4.1 mM lactate was utilized and 3.5 mM acetate formed. No growth or lactate degradation was observed with 0, 10, or 20% (vol/vol) O₂ in the headspace. Growth was observed in parallel cultures without O₂ but with 20 mM Na₂SO₄, and all the added lactate was oxidized to acetate.

Desulfovibrio vulgaris has been shown to prefer and grow at O₂ concentrations of 0.24 to 0.48 μM (14). Marschall et al. (21) found that *Desulfovibrio desulfuricans* strain CSN formed plates in oxygen-sulfide gradients at positions where the O₂ concentration was up to 9 μM. *D. desulfuricans* strain Holland SH-1 grew optimally in continuous culture at a dissolved O₂ concentration of 16 μM and was able to tolerate concentrations of 45 μM and greater (1). We observed growth of strain FH26001/95 in oxygen-lactate gradients at about 20 μM dissolved O₂ and in liquid cultures at initial dissolved O₂ concentrations of about 50 μM. The free dissolved O₂ concentration in arterial blood is about 110 μM, while that in venous blood is about 45 μM (10). The dissolved O₂ concentration in tissues will be lower. The oxygen tolerance of strains like FH26001/95 may be an essential factor in the ability of opportunistically pathogenic strains of *Desulfovibrio* spp. to survive in blood and infected tissues.

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