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Characterization of *Desulfovibrio giganteus* sp. nov., a Sulfate-reducing Bacterium Isolated from a Brackish Coastal Lagoon

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Summary

In the course of ecological studies in Berre Lagoon, a mediterranean brackish coastal lagoon (Marseille, France), a new slightly halophilic sulfate-reducing bacterium was isolated from anoxic sediments enriched with lactate plus sulfate, and cysteine-HCl as reductant. Because of its morphology and the incomplete oxidation of organic substrates, the isolated strain 8601 was assigned to the genus *Desulfovibrio*, resembling *Desulfovibrio gigas*. However, it differed from this species in some morphological and physiological characteristics: only one polar flagellum the utilization of methanol, isopropanol, glycerol and cysteine as energy source. Therefore a new species *Desulfovibrio giganteus*, is described.

Key words: *Desulfovibrio giganteus* – Sulfate-reducing bacteria – Halophily

Introduction

In most ecological studies, sulfate-reducing bacteria have been isolated from anoxic sites by using lactate as energy and carbon source. Strains that grow on lactate are known in all genera of sulfate-reducing bacteria with the exception of the genus *Desulfobacter*. However, from brackish anoxic environments, *Desulfovibrio* is the most commonly isolated genus (*Caumette*, 1986), probably because of its greatest growth rate on lactate. In Berre Lagoon, *Le Gall* (1963) isolated a new species of sulfate-reducing bacteria that grow on lactate, *Desulfovibrio gigas*. Recent ecological investigations on sulfate-reducing bacteria of anoxic layers of Berre Lagoon, showed that most of the isolated strains belong to the species *Desulfovibrio desulfuricans* or *Desulfovibrio baculatus* (paper in prep.). However, several enrichments on lactate contained large vibrios resembling *D. gigas*. These vibrios were finally isolated when Na_2S was replaced by cysteine hydrochloride as reductant (*Le Gall*, 1967; *Le Gall*, pers. com.). In the present study, the isolated strain 8601 was compared to *Desulfovibrio gigas* strain 9332. On the basis of its cytological and physiological properties, the isolate was assigned to the genus *Desulfovibrio*. Furthermore the strain was characterized with respect to its morphology, its substrate utilization and ecophysiological properties in order to establish its taxonomic position. As a result, the isolated strain 8601 is described as a new species, *Desulfovibrio giganteus*.

Material and Methods

Source of strains. Strain 8601 was isolated from the upper layer of anoxic sediment of Berre Lagoon ("Etang de Berre") near Marseille. The sediment was rich in organic matter (65 g of organic matter per kg of sediment) and total volatile sulfide (20 mmol · l^{-1} of sediment), and was polluted by urban and industrial sewages. During sampling time, the temperature and salinity of the upper water were 17 °C and 18 g NaCl · l^{-1}, respectively. *D. gigas* strain 9332 was kindly provided by the Laboratoire de Chimie Bacterienne (CNRS, Marseille).

Medium, culture conditions and isolation. The anaerobic medium described by *Pfennig* et al. (1981) was supplemented with 20 g NaCl · l^{-1} and 3 g $MgCl_2$ · l^{-1} throughout the experiments. The isolation was performed at 30 °C and pH 7.5 with cysteine-HCl (5 mmol · l^{-1} as reductant in instead of Na_2S, using the agar shake dilution method described by *Pfennig* et al. (1981). The tubes were gassed with a mixture of 80% N_2 and 20% CO_2, and sealed anaerobically with buthyl rubber stoppers, without adding paraffin and dithionite. To check its purity, the isolate was inoculated into a medium containing 2.5% yeast ex-

tract, 2.5% peptone and 2.5% biotrypcase, without sulfate. If growth occurred, the culture was examined microscopically. After isolation, the strain was cultivated with Na_2S as reductant, because cysteine can be used as energy source. Temperature, pH and salinity requirements were determined by optical density measurements at 580 nm, after 2 days of growth. Substrate utilization was tested in basal medium supplemented with the different substrates. Both growth and sulfide production were measured to verify substrate utilization.

Morphology. Phase contrast photomicrographs were performed with an Optiphot Nikon microscope on agar slides according to the method of *Pfennig* and *Wagener* (1986). Electron photomicrographs were obtained with a transmission electron microscope Hitachi EM 600, using the negative staining method with 1% phosphotungstic acid.

Chemical determinations. H_2S was measured spectrophotometrically as colloïdal CuS (*Cord-Ruwisch,* 1985). Growth was quantified by measuring optical density at 580 nm. Alcohols and volatile fatty acids were analysed by gas chromatography with a gas chromatograph Delsi serie 30 (column, 1 m long, filled with porapak Q; temperature 190 °C; 20 psi N_2 as carrier gas; injector temperature 150 °C; detector temperature 250 °C). Acetone and isopropanol were separated with a 2.5 m column, filled with chromosorb WAW at 110 °C under the following conditions: injector temperature 180 °C; detector temperature 110 °C; 26 psi N_2 as carrier gas.

Determination of pigments. The cells (5 g wet weight) were suspended in 10 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 1 mg pancreatic deoxyribonuclease I and disrupted by passing them twice through a French press (Aminco) at 15 000 $lb.in^{-2}$.

The broken cell suspension was centrifuged at 25 000 g for 20 min to remove cell debris. The resulting cell-free extract was separated into a supernatant and a particulate fraction by centrifugation at 140 000 g for 2 h. The supernatant was considered as the soluble fraction. The dark gelatinous pellet was resuspended in the same buffer and represented the particulate fraction. Cell-free extracts were examined for cytochromes and bisulfite reductase using a Cary 219 recording spectrophotometer.

DNA base composition. Whole cell DNA was extracted after disruption of the cells and purification according to *Marmur* (1961), at the German Collection of Micro-organisms (DSM, Göttingen, Germany). The mol % of the DNA was determined by Dr. *Hippe* at the DSM.

Results

Morphology

Strain 8601 is a pleomorphic bacterium consisting of both straight and curved rods (Fig. 1). The cells were 5.0–10.0 μm long and 1.0 μm wide, motile by means of a single polar flagellum (Fig. 2). Both straight and curved cells could be aggregated in chains of 4 to 6 cells forming

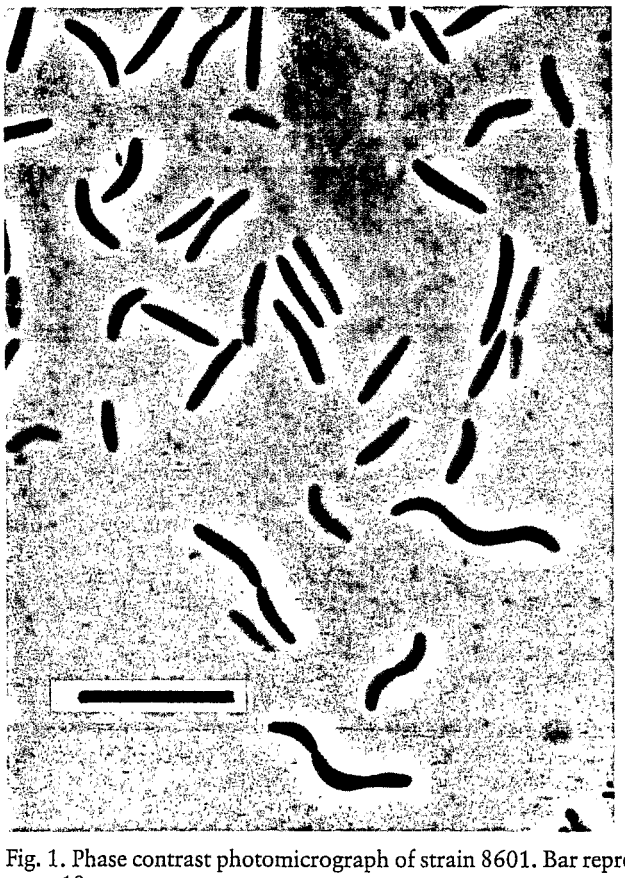

Fig. 1. Phase contrast photomicrograph of strain 8601. Bar represents 10 μm.

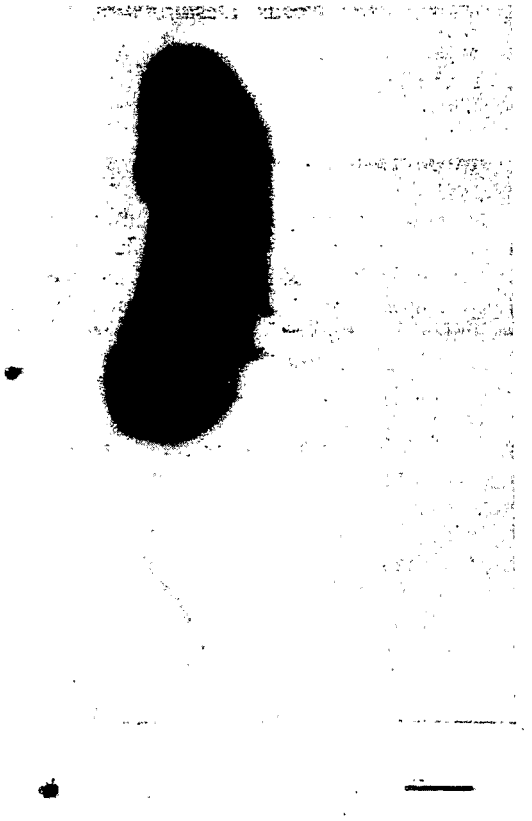

Fig. 2. Transmission electron micrograph of strain 8601. Note the single polar flagellum. Bar represents 1 μm.

"snakes" in old cultures. When the NaCl concentration was about 45 g · l^{-1}, only straight rods were visible in culture.

Physiological properties

Optimal temperature and pH were 35 °C and 7.5, respectively, for both strains 8601 and *D. gigas*. In contrast to *D. gigas*, strain 8601 required at least 2 g NaCl · l^{-1} for growth (Fig. 3). Strain 8601 can be described as a slightly but strictly halophilic bacterium whose optimal growth occurred between 2 and 25 g NaCl · l^{-1}. It grew up to 50 g NaCl · l^{-1}, after several subcultures (Fig. 3). Optimal growth required biotin as growth factor for both strains 8601 and *D. gigas*.

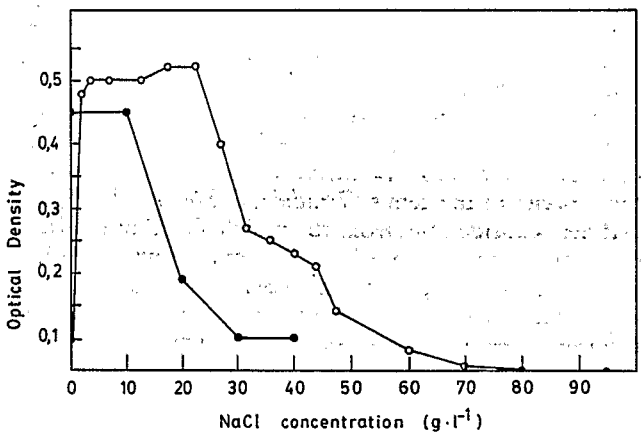

Fig. 3. Effect of NaCl concentration on the growth of strain 8601 (○) and *D. gigas* (●) cultivated on lactate. O. D. was measured at 580 nm.

Substrates as energy sources tested for both strains are listed in Table 1. Generally, when utilized, the substrates supported growth except for formate, methanol, isopropanol, isobutanol and pentanol with strain 8601, and for formate, ethanol, propanol, butanol, isobutanol and pentanol with *D. gigas*, even when 10 mmol · l^{-1} acetate were provided. Both strains did not oxidize the following substrates (mmol · l^{-1}) even with acetate as carbon source: malonate (10), citrate (10), tartronate (10), α-oxoglutarate (20), acetate (20), propionate (20), butyrate (10), palmitate (2), stearate (2), benzoate (10), acetone (20), glucose (20), fructose (20), lysine (10), serine (10), threonine (10), glutamate (10), alanine (10), choline (20), methylamine (20) and trimethylamine (20). Neither lactate nor glycerol were fermented by either strain. The electron acceptors for both strains are listed in Table 1. Nitrate but not dinitrogen was an alternative nitrogen source for strain 8601. H_2 serves as energy source with acetate as carbon source.

Pigments and mol % G+C

In the soluble and particulate fractions, the reduced spectra exhibited the characteristic absorption band of *c*-type cytochromes with maxima at 418, 522 and 551 nm.

Table 1. Electron donors and acceptors utilized by strain 8601 in comparison with *Desulfovibrio gigas*

Characteristics	Strain 8601	D. gigas
Substrates utilisation (mmol · l^{-1})		
Lactate (20) + sulfate	+	+
Pyruvate (20) + sulfate	+	+[a]
Pyruvate (20), no sulfate	+	−[a]
Malate (20) + sulfate	−	−[a]
Malate (20), no sulfate	−	+[a]
Succinate (20) + sulfate	−	+
Fumarate (20) + sulfate	−	+
Fumarate (20), no sulfate	−	+
H2 (2 bar) + acetate (10) + sulfate	+	+
Formate (20) + sulfate	+[b]	+[b]
Methanol (20) + sulfate	+[b]	−
Ethanol (20) + sulfate	+	+[b]
Propanol (20) + sulfate	+	+[b]
Isopropanol (20) + sulfate	+[b]	−
Butanol (10) + sulfate	+	+[b]
Isobutanol (10) + sulfate	+[b]	+[b]
Pentanol (10) + sulfate	+[b]	+[b]
Glycerol (20) + sulfate	+	−
Cysteine (20) + sulfate	+	−
Electron acceptors[1] (mM)		
Sulfate (20)	+	+
Sulfite (3)	+	+
Thiosulfate (10)	+	+
Elemental sulfur	−	+[c]
Fumarate (20)	−	+
Nitrate (5)	−	−
Msp. hungatei	+[d]	+[d]

[1] with lactate as electron donor, in optimal growth conditions.
[a] from *Widdel* and *Pfennig* (1984).
[b] utilization without growth, even when 10 mM acetate was provided.
[c] from *Widdel* (1987).
[d] interspecies hydrogen transfer between SRB and methanogens.

In the soluble fraction, the reduced and oxidized spectra exhibited the characteristic absorption band of desulfoviridin at 624 nm. Furthermore the fluorescence test for desulfoviridin (*Postgate*, 1959) was positive. The mol % G+C of the DNA of strain 8601 was 55.5.

Discussion

Desulfovibrio strain 8601 was isolated from anoxic brackish sediments only when cysteine-HCl was used as reductant. With Na_2S as reductant, the large vibrioid cells were always dominated by small vibrios resembling *D. desulfuricans* or *D. baculatus* which were subsequently isolated. Probably, these small forms grew faster than the larger ones with lactate as energy and carbon source. When Na_2S was replaced by cysteine-HCl (*Le Gall*, pers. com.), the large vibrios were easily isolated most probably because of an increase in the redox potential of the medium (from −350 mV to −230 mV) which favoured their growth. They may also have been favoured because they oxidized cysteine as shown in Results, although they

preferably grew faster on lactate. Therefore strain 8601 was isolated.

Morphologically, under phase contrast microscopy, strain 8601 appeared similar to *Desulfovibrio gigas* strain 9332. However some important morphological differences were noted between both strains. *D. gigas* is known to be a curved rod, 5.0–10.0 µm long and 1.2–1.5 µm wide (*Widdel* and *Pfennig,* 1984), motile by means of lophotrichous flagella. In contrast, at low NaCl concentration, strain 8601 appeared as a pleomorphic sulfate-reducing bacterium with both straight and curved rods of the same size. The cells were motile by means of a single polar flagellum which is a common characteristic of the genus *Desulfovibrio,* but not of *D. gigas* (*Widdel* and *Pfennig,* 1984). At higher NaCl concentration (about 45 $g \cdot l^{-1}$), only straight rods were observed, suggesting a modification of the cell form with increasing salinity. *Desulfovibrio* strain 8601 needed NaCl for growth and tolerated up to 50 $g \cdot l^{-1}$ for a good growth, whereas *D. gigas* grew without salts and was even inhibited by NaCl concentrations higher than 20 $g \cdot l^{-1}$. So far, only two species of *Desulfovibrio* have been described as halophilic micro-organisms: *D. desulfuricans subsp. aestuarii* and *Desulfovibrio salexigens* (*Postgate* and *Campbell,* 1966). The latter has a salinity range similar to that of the isolated strain 8601. Thus, *Desulfovibrio* strain 8601 can be described as a slightly halophilic bacterium, whose optimum is near 20 g $NaCl \cdot l^{-1}$, which ist the common salinity of the brackish water in Berre Lagoon.

Strain 8601 and *D. gigas* differ also in their physiological properties. Unlike *D. gigas,* strain 8601 degraded methanol, isopropanol, glycerol and cysteine. However, only cysteine and glycerol allowed good growth. Like the isolate of *Stams* et al. (1985), strain 8601 oxidized glycerol and cysteine to acetate.

The ecological significance of methanol and isopropanol degradation by strain 8601 is not clear: although both substrates were well degraded, they never supported growth even if acetate was provided. However, under the conditions of anoxic brackish sediments, these substrates might be utilized in addition to other substrates, thus contributing to bacterial growth as it was shown for other alcohols (*Postgate,* 1984). However, *Widdel* (1986) reported that in estuarine mud, the addition of methanol led to methane production but never to sulfate reduction, indicating that this substrate does not allow high sulfate reduction in these environments. The ability of sulfate-reducing bacteria to oxidize methanol, isopropanol, glycerol and cysteine has rarely been reported. So far, the oxidation of these four substrates by one and the same species, has never been observed. In fact, *Braun* and *Stolp* (1985) isolated a sulfate-reducing bacterium resembling *Desulfovibrio vulgaris* and *D. desulfuricans* able to degrade methanol without growth, after cultivation on pyruvate, malate or fumarate, but not on lactate. In contrast strain 8601 degraded methanol without growth after transfer from lactate. *Cord-Ruwisch* et al. (1986) reported poor growth of *Desulfovibrio* strain JJ (DSM 3604) with methanol. *Nanninga* and *Gottschal* (1986) isolated a sulfate-reducing bacterium able to grow on methanol with 10 $mmol \cdot l^{-1}$ acetate plus 0.001% yeast extract and 0.001% casamino-acids. However, in its ability to grow on glycerol by sulfate reduction or by fermentation, producing 3-hydroxypropionate or 3-hydroxypropionate plus 1,3-propanediol respectively, and in its morphology, this isolate differs from strain 8601. Conversion of isopropanol to acetone as observed in our strain is also known to occur in cultures of *Desulfococcus multivorans* and enrichments of *Desulfovibrio*-like species as well as in methanogenic bacteria (*Widdel,* 1986).

Strain 8601 is also characterized by lacking a fumarate reductase system, since it did not reduce fumarate. This is correlated with the lack of *b*-type cytochrome in the cell-free extracts, which is one of the characteristic electron carriers involved in this system (*Gottschalk* and *Andreesen,* 1979). In contrast, *D. gigas* contains the fumarate reductase system (Table 1; *Gottschalk* and *Andreesen,* 1979) and cytochrome *b* (*Widdel,* 1986). *Desulfovibrio* strain 8601 and *D. gigas* differ also in their G+C base ratios. For the genus *Desulfovibrio,* the mol % G+C of the DNA is ranging between 46.1 and 61.2 (*Widdel* and *Pfennig,* 1984). The mol % G+C of the DNA of strain 8601 is 55.5, as in *D. desulfuricans* (55 ± 1) which is the type species of the genus (*Widdel* and *Pfennig,* 1984), but it differs considerably from the mol % G+C of the DNA of *D. gigas* which ist 60.2 (*Widdel* and *Pfennig,* 1984).

Thus, on the basis of morphological, physiological and DNA differences between strain 8601 and *D. gigas,* we propose a new species, *Desulfovibrio giganteus.*

Description of *Desulfovibrio giganteus:*

Desulfovibrio giganteus sp. nov. (*gi.gan'teus.* L. adj. *giganteus* giant, gigantic)
Morphology: straight to curved rods, 1.0 µm by 5.0 to 10.0 µm. Motile by means of single polar flagellum.
pH range: 6.5 to 8.5; optimum at 7.5.
Temperature range: 15 to 40 °C; optimum at 35 °C.
Slightly halophilic bacterium with optimum salinity between 2 and 25 g $NaCl \cdot l^{-1}$.
Salinity range: 2 to 50 g $NaCl \cdot l^{-1}$.
Strictly anaerobic, reduces sulfate, sulfite and thiosulfate with production of sulfide. Elemental sulfur, fumarate and nitrate do not serve as electron acceptors.
Substrates oxidized by sulfate reduction: lactate, pyruvate, H_2, formate, methanol, ethanol, propanol, isopropanol, butanol, isobutanol, pentanol, glycerol, cysteine.
Substrate fermented: pyruvate.
Formate, methanol, isopropanol, isobutanol and pentanol do not support growth even when acetate is provided.
Incomplete oxidation except for methanol and formate.
c-type cytochrome and desulfoviridin are present.
mol % G+C of DNA: 55.5
Habitat: stagnant anoxic mud and sediments of brackish coastal lagoons and coastal marine environment.
type strain: *Desulfovibrio giganteus* strain 8601, isolated from anoxic sediments of Berre Lagoon ("étang de Berre") near Marseille, deposited with the German Collection of Micro-organisms (DSM), under the number DSM 4123.

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