

File ID        uvapub:1651  
Filename      17154y.pdf  
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SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type            article  
Title            Cleavage of dimethylsulfoniopropionate and reduction of acrylate by  
                  Desulfovibrio acrylicus sp. nov  
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Faculty        FNWI: Institute for Biodiversity and Ecosystem Dynamics (IBED), FNWI  
Year            1996

FULL BIBLIOGRAPHIC DETAILS:

<http://hdl.handle.net/11245/1.123288>

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## ORIGINAL PAPER

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## Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus* sp. nov.

Received: 22 March 1996 / Accepted: 8 May 1996

**Abstract** From anoxic intertidal sediment, a dimethylsulfoniopropionate (DMSP)-cleaving anaerobe (strain W218) was isolated that reduced the acrylate formed to propionate. The bacterium was vibrio- to rod-shaped and motile by means of multiple polar flagella. It reduced sulfate, thio-sulfate, and acrylate, and used lactate, fumarate, succinate, malate, pyruvate, ethanol, propanol, glycerol, glycine, serine, alanine, cysteine, hydrogen, and formate as electron donors. Sulfate and acrylate were reduced simultaneously; growth with sulfate was faster than with acrylate. Extracts of cells grown in the presence of DMSP contained high DMSP lyase activities (9.8 U/mg protein). The DNA mol% G+C was 45.1. On the basis of its characteristics and the 16S rRNA gene sequence, strain W218 was assigned to a new *Desulfovibrio* species for which the name *Desulfovibrio acrylicus* is proposed. A variety of other sulfate-reducing bacteria (eight of them originating from a marine or saline environment and five from other environments) did not reduce acrylate.

**Key words** Dimethylsulfoniopropionate · Dimethylsulfide · Acrylate · Anaerobic respiration · Sulfate-reducing bacterium · *Desulfovibrio acrylicus* sp. nov.

**Abbreviations** DMSP Dimethylsulfoniopropionate · DMS Dimethylsulfide

### Introduction

Dimethylsulfoniopropionate (DMSP), an important osmolyte of many marine algae and some plants, is the major precursor of dimethylsulfide (DMS) in the marine environment. DMS is thought to play a role in climate regulation through its atmospheric oxidation products methanesulfonic acid and sulfuric acid (Charlson et al. 1987) and to act as a carrier of sulfur from the marine to the terrestrial environment (Lovelock et al. 1972). Microbial breakdown of DMS and DMSP has received considerable attention in recent years. Several aerobic marine bacteria have been shown to degrade DMSP either by cleavage to DMS and acrylate or via an initial demethylation (Kiene 1990; Taylor and Gilchrist 1991; Diaz et al. 1992; Ledyard et al. 1993; Visscher and Taylor 1994; De Souza and Yoch 1995). Relatively high concentrations of DMSP are found in marine sediments (Kiene 1988; Visscher et al. 1994). In anoxic sediments, DMSP has been found to be either demethylated to 3-mercaptopropionate, with 3-methiolpropionate as a possible intermediate, or cleaved to DMS and acrylate (Kiene and Visscher 1987; Kiene and Taylor 1988). The combined activities of marine DMSP-demethylating *Desulfobacterium* strains (Van der Maarel et al. 1993) and certain 3-methiolpropionate-demethylating *Methanosarcina* strains (Van der Maarel et al. 1995) might be responsible for the observed conversion of DMSP to 3-mercaptopropionate. Anaerobic marine DMSP-cleaving bacteria have not been described. The only known DMSP-cleaving anaerobe, a strain of *Clostridium propionicum*, was isolated from freshwater (Wagner and Stadtman 1962) and fermented acrylate to propionate and acetate. This report describes an anaerobic marine DMSP-cleaving bacterium that does not ferment acrylate, but uses it only as an electron acceptor.

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## Materials and methods

### Organisms, media, and growth conditions

Strain W218 was isolated from anoxic intertidal sediment from the Wadden Sea near Westmiedland, The Netherlands. The sediment sampling and preparation of diluted sediment slurry have been described previously (Van der Maarel et al. 1995). A bicarbonate-buffered, sulfide-reduced basal medium with a N<sub>2</sub>/CO<sub>2</sub> (80/20%) headspace according to Heijthuisen and Hansen (1989) and supplemented with 0.05–1 g/l yeast extract (Difco, Detroit, Mich., USA) was used. From an enrichment culture, a pure culture was obtained by repeated plating on a sulfide-reduced agar medium with 10 mM DMSP as substrate in the presence of 1 g/l yeast extract; plating was carried out in an anaerobic chamber. Sulfate, acrylate, and substrates were added from neutralized stock solutions (1 M). Incubations were in 120-ml bottles with 50 ml medium at 28°C (for the enrichment cultures and isolation) or 30°C.

The following strains were grown in the media as described in the Deutsche Sammlung von Mikroorganismen und Zellkulturen catalogue of strains (1993): *Desulfococcus multivorans* DSM 2059 (5 mM benzoate), *Desulfosarcina variabilis* DSM 2060 (5 mM benzoate), *Desulfobacter postgatei* DSM 2034 (10 mM acetate), *Desulfovibrio salexigens* DSM 2638, *Desulfovibrio vulgaris* DSM 1744, *Desulfovibrio halophilus* DSM 5663, *Desulfovibrio* sp. strain HDv DSM 6830, *Desulfotomaculum kuznetsovii* DSM 6115 (60°C), *Desulfobulbus propionicus* DSM 2032, *Desulfovibrio desulfuricans* strain BH (laboratory collection) and *Desulfovibrio gigas* NCIMB 9332 were grown in the freshwater medium of Kremer and Hansen (1987) and *Desulfovibrio* sp. strain 20020 (DSM 3099) and *Desulfovibrio* sp. strain 20028 (DSM 3100) were grown in their marine medium. *Desulfuromonas acetoxidans* DSM 864 and *Desulfuromonas* sp. strain PM1 (laboratory collection) were cultivated on fumarate as described by Heijthuisen and Hansen (1989). The incubation temperature was 30°C and the growth substrate was 20 mM lactate unless otherwise indicated.

### Growth measurements

Growth rates were determined by following the increase in optical density at 660 nm in screw-capped tubes (17 ml) with 10 ml medium using a Biotron BTR 101 colorimeter. Molar growth yields of two cultures grown in 250 ml mineral medium with 0.1 g/l yeast extract plus 20 mM lactate and 20 mM sulfate or 10 mM acrylate were determined. Cultures were harvested at the end of the exponential growth phase by centrifugation (10 min at 16,000 × g), and the pellet was washed twice with 10 mM potassium phosphate (pH 7.1) containing NaCl (25 g/l) and MgCl<sub>2</sub>·6H<sub>2</sub>O (3 g/l).

### Preparation of cell-free extracts and DMSP lyase assay

Cells were grown in the basal medium with 20 mM sulfate, 40 mM lactate, 5 mM DMSP, and 1 g/l yeast extract. Twelve hours before harvesting of the cells, an additional 3 mM DMSP was added. The following steps were performed under air: After harvesting the cells by centrifugation (10 min at 16,000 × g) and washing in 25 mM potassium phosphate (pH 7.1), a cell-free extract was prepared by three additional passages of the cells through a French pressure cell operated at 106 MPa, followed by 30 min centrifugation at 39,000 × g. DMSP lyase was assayed by measuring the absorbance increase at 220 nm due to the formation of acrylate ( $\epsilon_{220}$  1.14 mM<sup>-1</sup> cm<sup>-1</sup>) with a Hitachi U-1100 spectrophotometer. The assay mixture (1.0 ml) contained 25 μmol potassium phosphate (pH 7.1) and 5 μmol DMSP; the reaction was started by adding an appropriate volume of cell extract. Protein in cell-free extracts was measured according to Bradford (1976).

### DNA analysis

The G+C content of the DNA was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) by HPLC based on the method of Mesbah et al. (1989). For 16S rRNA gene sequence analysis, cells were suspended in 2 ml 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA and incubated for 30 min at 37°C with lysozyme (2 mg/ml), followed by lysis of the cells with SDS (0.5% w/v), *N*-lauroylsarcosine (1% w/v), and RNase (50 μg/ml). Subsequently, the lysed cell suspension was incubated for 30 min at 55°C with proteinase K (100 μg/ml). DNA was extracted and precipitated according to Sambrook et al. (1989). The 16S rRNA gene was amplified via the polymerase chain reaction (PCR) using a set of oligonucleotide primers corresponding to positions 50–68 (5'-AACACATGCAA-GTCGAACG-3') and 1378–1392 (5'-ACGGCGGTGTGTAC-3') of *Escherichia coli* numbering. The PCR reaction mixture (100 μl) contained 20 ng DNA, 10 pmol of each primer, 10 μl Vent<sub>R</sub> polymerase buffer (New England Biolabs, Beverly, Mass., USA), 20 pmol of each nucleoside triphosphate and 2 units of Vent<sub>R</sub> DNA Polymerase (New England Biolabs). The reaction mixture was preincubated at 94°C for 3 min, and subsequently subjected to 26 cycles of 94°C for 60 s, 55°C for 120 s, and 72°C for 120 s. In the final cycle of the PCR reaction the incubation at 72°C was extended to 300 s. The amplified 16S rRNA gene was ligated into *Sma*I-digested pBlueScribe (Stratagene, La Jolla, Calif., USA). The nucleotide sequence of both DNA strands of the 16S rRNA gene was determined via dideoxy sequencing with T7 DNA polymerase (Pharmacia, Uppsala, Sweden) and [<sup>35</sup>S]dATP using six oligonucleotide primers complementary to conserved sequences in the 16S rRNA gene corresponding to positions 342–356 (5'-TACGGGAGGCAGCAG-3'), 500–517 (5'-CAGCAGCCGCG-GTAATAC-3'), 900–919 (5'-AAACTCAAATGAATTGACGG-3'), and 1115–1130 (5'-CAACGAGCGCAACCCT-3') of *E. coli* numbering. To rule out possible artifacts introduced in the 16S rRNA gene by PCR, the nucleotide sequences of two independently amplified 16S rRNA genes were determined. The nucleotide sequence was aligned manually with other 16S rRNA gene sequences derived from the NCBI collection. The sequence phylogeny was analyzed using PHYLIP 3.5c (Felsenstein 1985) and PAUP (Swofford 1993). A distance matrix was calculated with DNADIST using the two parameter model of Kimura (1980). A phylogenetic tree was constructed via the neighbour-joining method of Saitou and Nei (1987) as implemented in the NEIGHBOR program.

Restriction fragment analysis was done by incubating the 16S rRNA gene cloned in the pBlueScribe vector or PCR-amplified 16S rDNA (positions 50–1392) from a culture with one of the following restriction enzymes (1 U for 60 min): *Taq*I, *Sau*3a, *Rsa*I, and *Alu*I, followed by separation of the fragments on a 2% agarose gel.

### Chemicals and analytical procedures

DMSP was synthesized from DMS and acrylic acid according to Chambers et al. (1987). The purity was checked by analyzing the amount of DMS or acrylate before and after alkaline treatment and by determining the amount of organic carbon present.

Acetate, propionate, and acrylate were determined after removal of the cells by centrifugation and analyzed on a Packard 437 gas chromatograph (Packard, Delft, The Netherlands) equipped with a flame ionization detector. A 1.4-m glass column (inner diameter, 2 mm) was used with Chromosorb WAW 100/120 mesh coated with 10% SP-1000 + 1% H<sub>3</sub>PO<sub>4</sub> (Chrompack Nederland, Bergen op Zoom, The Netherlands). Injector and detector temperature were 220 and 240°C, respectively; a temperature program (115–140°C, with an increase of 1.3°C/min) was used. Volatile fatty acids were extracted in 0.3 ml diethyl ether after the addition of 0.2 g NaCl, 0.1 ml internal standard (20 mM valerate), and 10 μl formic acid to 0.2 ml sample; 1 μl of the ether phase was injected directly on the column. DMS was analyzed as described by Van der Maarel et al. (1993). DMSP was determined as acrylate after overnight treatment

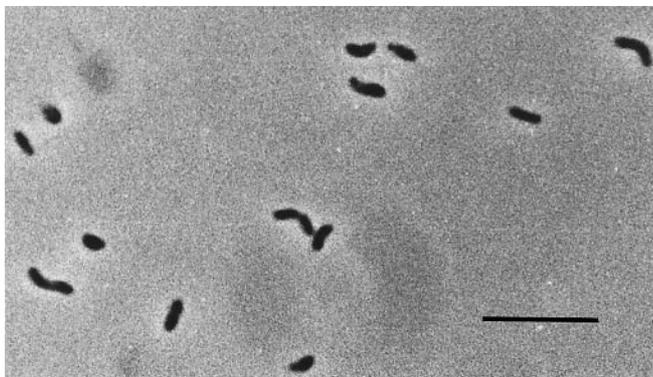
with 1 M NaOH. Hydrogen was determined by the method described for measuring methane (Heijthuijsen and Hansen 1989). Sulfide was measured colorimetrically (Trüper and Schlegel 1964). The presence of desulfovibrin in cell extract was determined according to Postgate (1959). Cytochromes were detected by recording an air-oxidized/dithionite-reduced difference spectrum of cell-free extract. Protein was determined according to Lowry et al. (1951) after treatment of the cell pellet with 1 M NaOH at 100°C for 10 min; before protein analysis, the pellet obtained after centrifugation and washing with 10 mM phosphate buffer (pH 7.1) containing 25 g/l NaCl and 3 g/l  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  was treated with 5 ml methanol to remove interfering sulfur compounds.

## Results

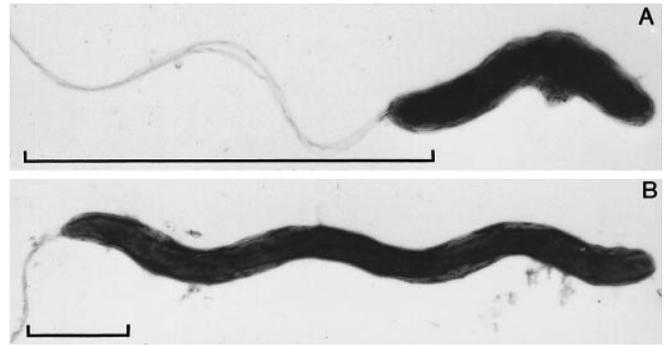
### Enrichment, isolation, and general characteristics of strain W218

An enrichment culture with anoxic intertidal sediment as inoculum in mineral medium with 1.5 mM DMSP and 500 mg/l yeast extract resulted within a few days in the rapid formation of DMS and the transient accumulation of acrylate. After transfer into fresh medium with 3.5 mM DMSP, an enrichment culture was obtained that converted DMSP to DMS and acrylate, which was slowly degraded further. Microscopically, mainly vibrio- and rod-shaped cells were present. A pure culture of a DMSP-cleaving bacterium, strain W218, was obtained by using anoxic agar plates with 10 mM DMSP and 1 g/l yeast extract; colonies of strain W218 were beige. The pure culture, which grew poorly on DMSP and 1 g/l yeast extract, grew much better in basal media with lactate and sulfate and was shown to be a facultatively acrylate-reducing strain of *Desulfovibrio* (see below).

Cells ranged from short rods to vibrios (Fig. 1) and were motile. Electron microscopic examination revealed the presence of multiple flagella at one of the poles (Fig. 2). Motility was lost in some cases when colonies were repeatedly transferred to anoxic agar plates containing 20 mM acrylate and 2 g/l yeast extract. Spores were never observed. Cells of strain W218 stained gram-negative, behaved negative in the KOH lysis test (Gregersen 1978),



**Fig. 1** Phase contrast micrograph of strain W218 grown on lactate and sulfate, bar 10  $\mu\text{m}$

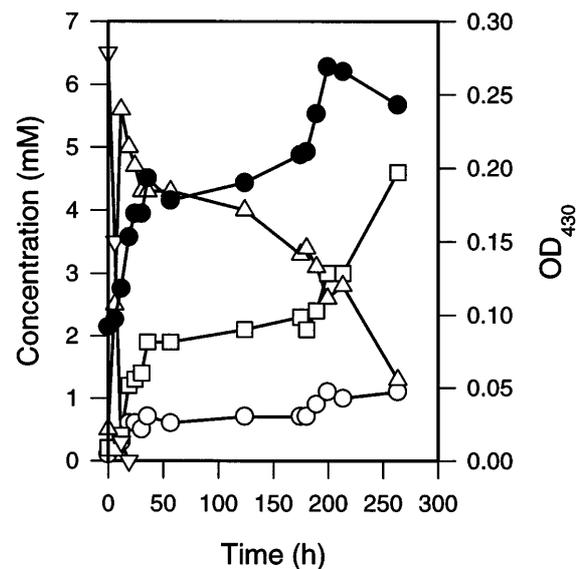


**Fig. 2** Electron micrograph of negatively stained (with 1% uranyl acetate) strain W218 grown on lactate and sulfate (A) or lactate and acrylate (B), bars 5  $\mu\text{m}$

and showed a gram-negative cell wall structure in electron micrographs (data not shown). The strain was catalase-negative. Desulfovibrin was detected in cell extracts. Absorption spectra of dithionite-reduced *minus* air-oxidized cell extract showed peaks at 422, 524, and 553 nm, which are indicative for *c*-type cytochromes. The G+C content of the DNA of strain W218 was 45.1 mol%.

### Growth characteristics

When grown in medium containing 0.5 g/l yeast extract and DMSP, strain W218 rapidly converted DMSP to DMS. After an increase in the optical density and the concentration of propionate and a decrease in the concentration of acrylate during the first 30 h, hardly any acrylate was further metabolized (Fig. 3). However, when extra yeast extract was added after 123 h, acrylate was metabolized fur-

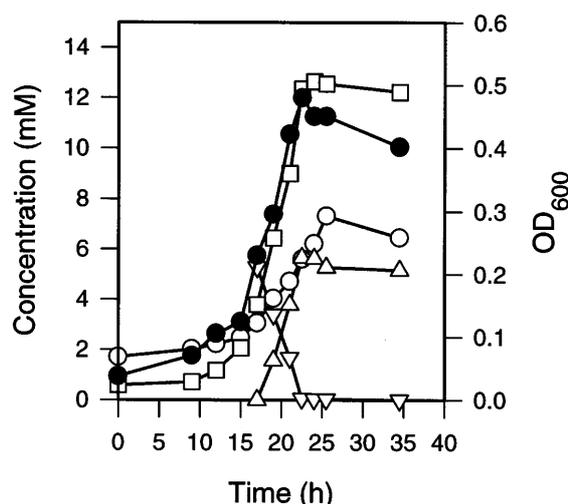


**Fig. 3** Growth of strain W218 on 6 mM DMSP and 0.05% yeast extract. At 123 h, extra 0.05% yeast extract was added; ● optical density, ▽ DMSP, △ acrylate, ○ acetate, □ propionate

ther, and the optical density and the concentration of propionate increased. At the end of the incubation (262 h), 5.2 mM acrylate had been converted, and 4.6 mM propionate and only 1.1 mM acetate had been formed. These results indicated that strain W218 did not ferment acrylate, but that it might have utilized acrylate as an electron acceptor. Indeed, strain W218 was able to grow on H<sub>2</sub> and acrylate; under these conditions, equimolar concentrations of propionate were formed. In a medium with 1 mM acetate without yeast extract under an atmosphere of H<sub>2</sub>/CO<sub>2</sub> (80/20%), the strain grew poorly, but still reduced acrylate to propionate.

In the presence of acrylate as an electron acceptor and 1 g/l yeast extract, strain W218 was able to grow on several amino acids (glycine, L-serine, L-alanine, and L-cysteine), organic acids (L-lactate, fumarate, succinate, malate, pyruvate, and formate), alcohols (ethanol, propanol, and glycerol), and H<sub>2</sub>; organic substrates were tested at 10 mM. Lactate was oxidized to acetate. Growth was also observed with yeast extract, peptone, or casamino acids (all 1 g/l) as substrates. No growth was observed with the following compounds (all at 10 mM unless otherwise mentioned): L-threonine, L-asparagine, L-methionine, L-glutamate, L-histidine, L-arginine, L-lysine, L-aspartate, adenine, thymine, acetate, propionate, butyrate, 3-methylpropionate, 3-mercaptopropionate, betaine, choline, sarcosine, nicotinate, glycolate, methanol, 2,3-butanediol, glucose, galactose, fructose, lactose, maltose, mannose, xylose, sorbitol, cellobiose, melibiose, cellulose (1 g/l), pectin (1 g/l), xylan (1 g/l), and inulin (1 g/l). Growth on lactate plus acrylate was fastest between 30 and 37°C, at a pH of 7.4, and with an NaCl concentration of 18 g/l (range: 6–40 g/l). Other electron acceptors were tested with H<sub>2</sub> as an electron donor in the presence of 0.5 g/l yeast extract; both sulfate and thiosulfate gave good growth and were reduced to sulfide. No growth was observed with nitrate, elemental sulfur, sulfite (5 mM), crotonate, vinylacetate, acrylamide, cinnamate, caffeate, 3-methylpropionate, and 3-mercaptopropionate as potential electron acceptors; unless otherwise indicated the soluble compounds were added to 10 mM. No growth (with 1 g/l yeast extract as a substrate) was observed in stationary cultures under air.

Growth with sulfate as electron acceptor resulted in rod- to vibrio-shaped cells (usually 2–4 µm long and 0.8 µm wide). However, cells grown with acrylate became longer and tended to become spiral-shaped, as is shown for an extreme example in Fig. 2. Acrylate was reduced simultaneously with sulfate when 5 mM acrylate was added to a culture growing on 25 mM lactate and 20 mM sulfate (Fig. 4). The maximum specific growth rate on lactate and sulfate was considerably higher than on lactate and acrylate; the molar growth yield on lactate plus acrylate was slightly higher than on lactate and sulfate (Table 1). A concentration of 40 mM acrylate as electron acceptor resulted in a longer lag phase and lower growth rate and molar growth yield. Since addition of propionate (up to 40 mM) did not affect the growth characteristics, the results indicate that higher concentrations of acrylate are inhibitory.



**Fig. 4** Simultaneous reduction of acrylate (5 mM; added at  $t = 16$  h) and sulfate (10 mM) by strain W218 with lactate (25 mM) as substrate. Cells were pregrown on sulfate and lactate; ● optical density, ○ sulfide, ▽ acrylate, △ propionate, □ acetate

**Table 1** Lactate oxidation with sulfate or acrylate as electron acceptor by strain W218 in media with 0.1 g/l yeast extract. Values of the molar growth yields ( $Y_{mol}$ ) and on the substrate conversions are the means of two cultures; values of the specific growth rate ( $\mu_{max}$ ) are the means of three cultures. Carbon and electron recoveries were calculated assuming  $C_6H_{10.84}N_{1.4}O_{3.07}$  as empirical formula for cell material (Stouthamer 1979)

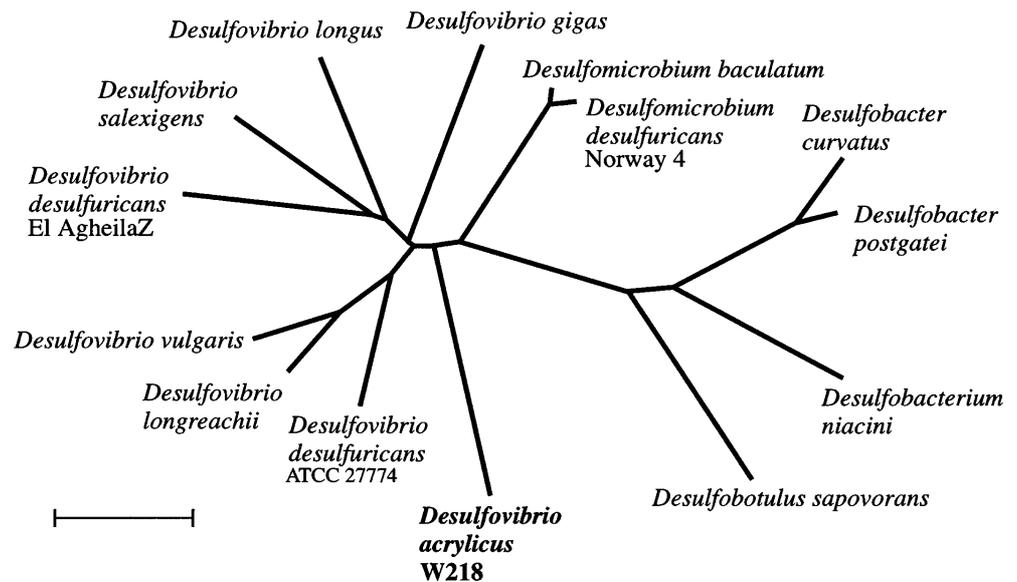
	Electron acceptor	
	Sulfate	Acrylate
Lactate used (mM)	17.2	5.2
Acetate formed (mM)	15.6	6.1
Electron acceptor used (mM)	7.0	11.4
$\mu_{max}$ ( $h^{-1}$ )	0.15	0.06
$Y_{mol}$ (g protein/mol substrate)	2.2	2.7
Carbon recovery (%)	97	107
Electron recovery (%)	95	107

Extracts prepared from cells grown in a medium with 20 mM sulfate and 40 mM lactate and DMSP (8 mM total, see Materials and methods) contained high activities of DMSP lyase [ $9.8 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$ ]. The apparent  $K_m$  for DMSP in cell extracts was approximately 1 mM. From the DMS production rate of a cell suspension, the apparent  $K_m$  for DMSP of whole cells was determined to be approximately 0.4 mM.

#### Phylogenetic position of strain W218

The morphological and physiological characteristics and the presence of desulfovirdin indicated that strain W218 is a member of the genus *Desulfovibrio*. This was confirmed by a phylogenetic analysis of the 16S rRNA gene of strain W218 (positions 48–1392). Strain W218 clearly groups within the genus *Desulfovibrio* (Fig. 5), but shows only approximately 85% similarity with other species of

**Fig. 5** Phylogenetic relationship of *Desulfovibrio acrylicus* W218 with other sulfate-reducing bacteria. The tree is based on a distance analysis of the 16S rRNA gene sequence (positions 48–1392) and was constructed using the neighbour-joining method as implemented in PHYLIP 3.5c. Bar 0.05 substitutions per sequence position



this genus. A maximum parsimony analysis using PAUP resulted in one most parsimonious tree with the same topology as the distance tree shown in Fig. 5. The sequence has been deposited with GenBank (U32578).

In view of the pleomorphic character of the culture, the purity of the culture was checked by isolation of 46 strains from individual colonies from a  $10^8$  dilution in lactate/sulfate agar tubes. All strains consisted of rod- to vibrio-shaped cells and reduced sulfate to sulfide upon transfer to fresh medium containing 20 mM lactate and 20 mM sulfate. When transferred to medium containing 20 mM lactate and 20 mM acrylate, all strains consisted of rod-shaped to small spiral-shaped cells and reduced the acrylate to propionate. The restriction fragment pattern of PCR amplified 16S rDNA from a culture on lactate/sulfate was identical to the pattern of the cloned 16S rRNA gene; both patterns matched the expected pattern based on the sequence information completely.

#### Acrylate reduction by other sulfate-reducing bacteria?

A wide variety of sulfate-reducing bacteria from different habitats and two sulfur-reducing strains were tested for the ability to reduce acrylate (see Materials and methods for a list of strains). None of the 15 strains was able to grow with the combination of their original growth substrate and acrylate as an electron acceptor.

## Discussion

Strain W218 is the first example of an anaerobic marine bacterium that is able to cleave DMSP. It has a higher DMSP lyase level than the aerobic *Alcaligenes* sp. strain M3A and *Pseudomonas doudoroffii* (de Souza and Yoch 1995). Unlike the DMSP-cleaving strain of *Clostridium propionicum* (Wagner and Stadtman 1962) and the acrylate-

utilizing *Clostridium propionicum* strain 19acry3 (Janssen 1991), strain W218 does not ferment acrylate, but reduces it to propionate with a suitable electron donor. Kiene and Taylor (1988) found that after the addition of DMSP to low micromolar concentrations, DMS and acrylate did not accumulate. They suggested that fermenting organisms like *C. propionicum* could be responsible for the cleavage of DMSP and the degradation of acrylate. Another indication for the involvement of fermenting organisms in the degradation of acrylate came from the observation of Kiene and Taylor (1989) that addition of micromolar levels of acrylate to sediment suspensions in the presence of molybdate as inhibitor of sulfate reduction resulted in the formation of acetate and propionate in a 1:2 ratio. The nature and the quantitative importance of fermenting bacteria in the degradation of DMSP remains to be examined.

Strain W218 is a sulfate-reducing bacterium that can also carry out the reduction of acrylate to propionate. This reduction can be considered as a novel type of anaerobic respiration. The ability to grow by the reduction of an unsaturated organic compound has been found for a variety of organisms. *Acetobacterium woodii* is able to reduce the acrylic side chain of caffeate (3,4-dihydroxyphenylacrylate) with  $H_2$  and to couple this process to the synthesis of ATP (Tschech and Pfennig 1984; Hansen et al. 1988). The most common and best understood type of anaerobic respiration with an unsaturated organic compound is the reduction of fumarate (Kröger et al. 1992). Organisms such as *Wolinella succinogenes*, *Escherichia coli*, and *Desulfovibrio gigas* are able to grow by the reduction of fumarate. The fumarate reductase is localized at the cytoplasmic side of the cell membrane and converts fumarate to succinate without energy-rich intermediates (Kröger et al. 1992). It would be interesting to know whether the mechanism of acrylate reduction in strain W218 is similar to the mechanism of fumarate reduction or to the propionate-forming pathway in bacteria with a non-randomiz-

ing propionic acid fermentation, where acrylyl-CoA is reduced (Brockman and Wood 1975).

Acrylate reduction is energetically more favorable than sulfate reduction: per mol of hydrogen consumed the  $\Delta G^\circ$  values are  $-75$  kJ/mol and  $-38$  kJ/mol, respectively (calculated from Thauer et al. 1977). At limiting electron donor concentrations, this thermodynamic advantage may be translated into a competitive advantage for the acrylate-reducing strain. This may explain why a sulfate-reducing bacterium is able to reduce acrylate in an environment where sulfate is present in high concentrations (up to 28 mM). The molar growth yield with acrylate as an electron acceptor was slightly higher than with sulfate. This was expected because in the activation of 1 mol of sulfate, 1 mol of ATP is eventually converted to 1 mol of AMP.

On the basis of its morphology, physiology, and 16S rRNA gene sequence, strain W218 clearly is a member of the genus *Desulfovibrio*. Devereux et al. (1990) concluded that *Desulfovibrio* comprises a coherent, but phylogenetically diverse group of species that is distinct from other groups of sulfate-reducing bacteria and proposed to create the family Desulfovibrionaceae, which includes at least five different lineages. Strain W218 does not group closely within one of these lineages and could represent a new lineage within the family. We propose to place strain W218 in the genus *Desulfovibrio* as the type strain of the new species *Desulfovibrio acrylicus*.

#### Description of *Desulfovibrio acrylicus* sp. nov.

*Desulfovibrio acrylicus* (acryl'icus L. adj. derived from *acidum acrylicum* acrylic acid). Small rods to vibrios; 2–4  $\mu\text{m}$  long and 0.8  $\mu\text{m}$  wide when grown with sulfate, but longer when grown with acrylate. Motile by multiple polar flagella; no spores formed. Gram-negative and catalase-negative. Strictly anaerobic.  $\text{H}_2$ , formate, lactate, fumarate, succinate, malate, pyruvate, ethanol, propanol, glycerol, glycine, serine, alanine, and cysteine serve as electron donor; sulfate, thiosulfate, or acrylate used as electron acceptor. Growth optimal at pH 7.4, a temperature of 30–37°C, and 18 g/l NaCl. Contains desulfovibrin and cytochromes of the *c*-type. Forms dimethylsulfide from dimethylsulfoniopropionate. G+C content of the DNA is 45.1 mol%. Habitat anoxic marine sediments. Type strain W218 deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ 10141). The 16S rRNA gene sequence has been deposited with GenBank (U32578).

**Acknowledgements** Thanks are due to Irma van der Veen for excellent technical assistance, Lubbert Dijkhuizen for valuable discussions, and Klaas Sjollemma for performing electron microscopy. This work was supported by the National Research Programme of the Netherlands Ministry of Housing, Physical Planning and the Environment (project NOLK/026/90) and by the European Union (project EV 5 V – CT 93–036).

#### References

- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Brockman HL, Wood WA (1975) Electron-transferring flavoprotein of *Peptostreptococcus elsdenii* that functions in the reduction of acrylyl-coenzyme A. *J Bacteriol* 124:1447–1453
- Chambers ST, Kunin CM, Miller D, Hamada A (1987) Dimethylthetin can substitute for glycine betaine as an osmoprotectant molecule for *Escherichia coli*. *J Bacteriol* 169:4845–4847
- Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* 326:655–661
- De Souza MP, Yoch DC (1995) Comparative physiology of dimethylsulfide production by dimethylsulfoniopropionate lyase in *Pseudomonas doudoroffii* and *Alcaligenes* sp. strain M3A. *Appl Environ Microbiol* 61:3986–3991
- Deutsche Sammlung von Mikroorganismen und Zellkulturen (1993) Catalogue of strains 1993. Braunschweig, Germany
- Devereux R, He S-H, Doyle CL, Orkland S, Stahl DA, LeGall J, Whitman B (1990) Diversity and origin of *Desulfovibrio* species: phylogenetic definition of a family. *J Bacteriol* 172:3609–3617
- Diaz MR, Visscher PT, Taylor BF (1992) Metabolism of dimethylsulfoniopropionate and glycine betaine by a marine bacterium. *FEMS Microbiol Lett* 96:61–66
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Gregersen T (1978) Rapid method for distinction of gram-negative from gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* 5:123–127
- Hansen B, Bokranz M, Schönheit, Kröger A (1988) ATP formation coupled to caffeate reduction by  $\text{H}_2$  in *Acetobacterium woodii* Nzva16. *Arch Microbiol* 150:447–451
- Heijthuijzen JHFG, Hansen TA (1989) Betaine fermentation and oxidation by marine *Desulfovibrio* strains. *Appl Environ Microbiol* 55:965–969
- Janssen PH (1991) Isolation of *Clostridium propionicum* strain 19acry3 and further characteristics of the species. *Arch Microbiol* 155:566–571
- Kiene RP (1988) Dimethyl sulfide metabolism in anoxic salt marsh sediment. *FEMS Microbiol Ecol* 53:71–78
- Kiene RP (1990) Dimethyl sulfide production from dimethylsulfoniopropionate in coastal seawater samples and bacterial cultures. *Appl Environ Microbiol* 56:3292–3297
- Kiene RP, Taylor BF (1988) Demethylation of dimethylsulfoniopropionate and production of thiols in anoxic marine sediments. *Appl Environ Microbiol* 54:2208–2212
- Kiene RP, Taylor BF (1989) Metabolism of acrylate and 3-mercaptopropionate. In: Saltzman ES, Cooper WJ (eds) Biogenic sulfur in the environment. American Chemical Society, Washington DC, pp 222–230
- Kiene RP, Visscher PT (1987) Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. *Appl Environ Microbiol* 53:2426–2434
- Kimura M (1980) A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Kremer DR, Hansen TA (1987) Glycerol and dihydroxyacetone dissimilation in *Desulfovibrio* strains. *Arch Microbiol* 147:249–256
- Kröger A, Geisler V, Lemma E, Theis F, Lenger R (1992) Bacterial fumarate respiration. *Arch Microbiol* 158:311–314
- Ledyard KM, DeLong EF, Dacey JWH (1993) Characterization of a DMSP-degrading bacterial isolate from the Sargasso Sea. *Arch Microbiol* 160:312–318
- Lovelock JE, Maggs RJ, Rasmussen RA (1972) Atmospheric dimethyl sulphide and the natural sulfur cycle. *Nature* 237:452–453

- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Mesbah M, Premachandran U, Whitman W (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* 39:159–167
- Postgate JR (1959) A diagnostic reaction of *Desulphovibrio desulphuricans*. *Nature* 183:481–482
- Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Stouthamer AH (1979) The search for correlation between theoretical and experimental growth yields. In: Quayle JR (ed) *International review of biochemistry*, vol 21. Microbial biochemistry. University Park Press, Baltimore, pp 1–47
- Taylor BF, Gilchrist DC (1991) New routes for aerobic biodegradation of dimethylsulfoniopropionate. *Appl Environ Microbiol* 57:3581–3584
- Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* 41:100–180
- Trüper HG, Schlegel HG (1964) Sulphur metabolism in Thiorhodaceae. 1. Quantitative measurements of growing cells of *Chromatium okenii*. *Antonie Van Leeuwenhoek* 30:225–238
- Tschech A, Pfennig N (1984) Growth yield increase linked to caffeine reduction in *Acetobacterium woodii*. *Arch Microbiol* 137:163–167
- Van der Maarel MJEC, Quist P, Dijkhuizen L, Hansen TA (1993) Anaerobic degradation of dimethylsulfoniopropionate to 3-S-methylmercaptopropionate by a marine *Desulfobacterium* strain. *Arch Microbiol* 160:411–412
- Van der Maarel MJEC, Jansen M, Hansen TA (1995) Methanogenic conversion of 3-S-methylmercaptopropionate to 3-mercaptopropionate. *Appl Environ Microbiol* 61:48–51
- Visscher PT, Taylor BF (1994) Demethylation of dimethylsulfoniopropionate to 3-mercaptopropionate by an aerobic marine bacterium. *Appl Environ Microbiol* 60:4617–4619
- Visscher PT, Kiene RP, Taylor BF (1994) Demethylation and cleavage of dimethylsulfoniopropionate in marine intertidal sediments. *FEMS Microbiol Ecol* 14:179–190
- Wagner C, Stadtman ER (1962) Bacterial fermentation of dimethyl- $\beta$ -propiothetin. *Arch Biochem Biophys* 98:331–336