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Studies on Dissimilatory Sulfate-Reducing Bacteria that Decompose Fatty Acids

I. Isolation of New Sulfate-Reducing Bacteria Enriched with Acetate from Saline Environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov.

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Abstract. Three strains (2ac9, 3ac10 and 4ac11) of oval to rod-shaped, Gram negative, nonsporing sulfate-reducing bacteria were isolated from brackish water and marine mud samples with acetate as sole electron donor. All three strains grew in simple defined media supplemented with biotin and 4-aminobenzoic acid as growth factors. Acetate was the only electron donor utilized by strain 2ac9, while the other two strains used in addition ethanol and/or lactate. Sulfate served as electron acceptor and was reduced to H_2S. Complete oxidation of acetate to CO_2 was shown by stoichiometric measurements with strain 2ac9 in batch cultures using sulfate, sulfite or thiosulfate as electron acceptors. With sulfate an average growth yield of 4.8 g cell dry weight was obtained per mol of acetate oxidized; with sulfite or thiosulfate the growth yield on acetate was about twice as high. None of the strains contained desulfoviridin. In strain 2ac9 cytochromes of the b- and c-type were detected. Strain 2ac9 is described as type strain of the new species and genus, *Desulfobacter postgatei*.

Key words: Anaerobic acetate oxidation – Saline environments – Sulfate reduction – Sulfite – Thiosulfate – Growth yields – Cytochromes – Species description – *Desulfobacter postgatei*

A new physiological type of the strict anaerobic, dissimilatory sulfate-reducing bacteria was previously described and named *Desulfotomaculum acetoxidans* (Widdel and Pfennig 1977, 1981; Widdel 1980). In contrast to all authenticated species known so far, the new species was able to utilize acetate and some other simple organic compounds as electron donors and to oxidize them completely to CO_2. *Desulfotomaculum acetoxidans*, morphologically characterized by long, rod-shaped cells with pointed ends and the formation of spores, was repeatedly enriched from piggery waste, cow manure, rumen content and dung-contaminated freshwater habitats. The present paper reports on the isolation and study of three pure cultures of acetate-oxidizing, sulfate-reducing bacteria from anaerobic brackish water and marine mud samples. These NaCl- and $MgCl_2$-dependent bacteria form nonsporing, oval to rod-shaped cells which differ greatly from *Desulfotomaculum acetoxidans*. This new type of sulfate reducing bacteria is described here as a new species and genus, *Desulfobacter postgatei*.

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Materials and Methods

Sources of Organisms. The following three strains were isolated in pure culture from enrichment cultures inoculated with samples from nature: 1. Strain 2ac9 from the anaerobic sediment of a brackish water ditch near the Jadebusen (North Sea). 2. Strain 3ac10 from anaerobic mud flat of the Jadebusen. 3. Strain 4ac11 from marine sludge with decaying algae from the coast of Helgoland (North Sea).

Media and Conditions of Cultivation. The basal medium had the following composition (values in g/l): Na_2SO_4, 3.0; KH_2PO_4, 0.2; NH_4Cl, 0.3; KCl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.15; from the amounts of NaCl and $MgCl_2 \cdot 6H_2O$ given per l three types of media with different salinities resulted: freshwater medium with 1.0 g NaCl and 0.4 g $MgCl_2 \cdot 6H_2O$, brackish water medium with 7.0 g NaCl and 1.2 g $MgCl_2 \cdot 6H_2O$, marine medium with 20.0 g NaCl and 3.0 g $MgCl_2 \cdot 6H_2O$. To the autoclaved, cooled medium the following components were added from sterile stock solutions: Trace element solution SL 7 (see below), 1 ml/l; bicarbonate solution with 84 g $NaHCO_3$/l, CO_2-saturated and autoclaved under CO_2 atmosphere, 30 ml/l; sulfide solution with 120 g $Na_2S \cdot 9H_2O$/l, autoclaved under N_2 atmosphere, 3.0 ml/l; vitamin solution of Pfennig (1978) containing biotin, 4-aminobenzoic acid, 10 mg pantothenate/l (changed), pyridoxamine, nicotinic acid and 20 mg thiamine/l (changed), 5 ml/l; vitamin B_{12} solution with 50 mg/l, 1 ml/l. The trace element solution SL 7 contained in 1 l (without complexing agents): HCl, 25%, 10 ml; $FeCl_2 \cdot 4H_2O$, 1.5 g; $CoCl_2 \cdot 6H_2O$, 190 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; $ZnCl_2$, 70 mg; H_3BO_3, 62 mg; $Na_2MoO_4 \cdot 2H_2O$, 36 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; $CuCl_2 \cdot 2H_2O$, 17 mg; at first the ferrous chloride was dissolved in the hydrochloric acid, while the other components were added after addition of distilled water. The pH of the medium (normally between 7.1 and 7.3) was adjusted with sterile diluted HCl or Na_2CO_3 solution. The medium was prepared in a special 5 l glas vessel under an atmosphere of 90% N_2 and 10% CO_2. Between the additions of stock solutions and for stirring and bottling, the vessel was closed, so that the weak excess pressure (5 kPa) of the gas mixture was maintained to avoid penetration of air. The medium was dispensed into sterile rubber sealed screw cap bottles, which were completely filled.

Substrates were added to the bottles from sterile stock solutions before inoculation. When the only organic substrate was acetate, 10 ml from an autoclaved solution with 280 g $CH_3COONa \cdot 3H_2O$ per l were added per l of culture medium. After inoculation immediately before sealing, 0.2–0.6 ml of a freshly prepared, anaerobically filtersterilized dithionite solution with 50 g $Na_2S_2O_4$ per l were added per l of medium; alternatively, dithionite was also added as dry crystals (10–30 mg/l) with a sterile spatula. With addition of dithionite a much shorter lag period of freshly inoculated cultures was observed, especially when precultures were old. To test utilization of H_2, culture bottles were filled to one third with inoculated medium, gased with a mixture of 80% H_2 and 20% CO_2 and sealed with butyl rubber stoppers. Incubation of the hydrogen cultures occurred both stationary and on a shaker. All cultures were incubated at 29° C.

Isolation. Pure cultures were obtained by repeated application of the agar shake culture method in anaerobically sealed test tubes as described by

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Pfennig (1978); in addition to the paraffin layer the tubes were closed with Wright Burry seals. Before solidification of the agar medium dithionite solution was aseptically added to each tube from a 0.1 ml pipette which was at the same time used for gentle mixing starting at the highest dilution.

To check purity, the isolates were inoculated into media with 0.1% yeast extract plus H_2, formate, lactate, pyruvate or sugars as substrates. After incubation the media were examined microscopically.

Chemical Determinations. H_2S was determined photometrically after reaction with N,N-dimethylphenylenediamine to form methylene blue as described by Pachmayr (1960). Acetate was determined by gas chromatography on Porapak QS 100–120 mesh, column length 2.0 m, 180° C; carrier gas N_2 with 5% CO_2, 70 ml/min; detector FID. Before injection 0.98 ml of the culture supernatant was mixed with 0.02 ml of 25% HCl.

Characterization of Pigments. Cell material of a well grown 5 l culture was washed and sonicated in 8 ml K-phosphate buffer, pH 7.0, 50 mmol/l. Cell debris were removed by centrifugation at $4 \cdot 10^5\ m \cdot s^{-2}$ for 15 min. The cell-free extract was recentrifuged at $15 \cdot 10^5\ m \cdot s^{-2}$ for 90 min. The membrane pellet was washed once with 5 ml phosphate buffer and centrifuged again. Both supernatants were combined (cytoplasmic fraction). The membrane pellet was resuspended in 8 ml phosphate buffer (membrane fraction). For the detection of desulfoviridin the spectrum of the cytoplasmic fraction was measured against buffer as blank and checked for a peak at 630 nm; also, the fluorescence test of Postgate (1959) was applied. The carbon monoxide difference spectrum was obtained from the dithionite-reduced cytoplasmic fraction after gasing in the dark for 1 min with CO. Cytochromes of the *b*- and *c*-type were distinguished by extraction of lyophilized cytoplasmic or membrane fraction with acetone-HCl as described by Weston and Knowles (1973). Redox difference spectra were obtained from the pyridine hemochromes.

DNA Base Composition. The mol per cent guanine plus cytosine of the DNA were determined by Dr. H. Hippe, Göttingen, with the thermal denaturation method.

Stoichiometric Measurements and Cell Material Determination. The stoichiometry of substrate oxidation was measured in batch cultures in 1 l bottles that were sealed anaerobically with rubber stoppers; the gas phase above the medium was kept very small. Starting with sulfate-free medium and the same acetate concentration of 20.0 mmol per l in each case, the electron acceptors sulfate, sulfite or thiosulfate were added in different exactly known growth limiting amounts from stock solutions. In blanks the electron acceptor was omitted. Considering its toxicity, sulfite was added stepwise in smaller amounts during growth. To avoid major changes of pH with sulfate or sulfite as electron acceptor, small calculated volumes of sterile HCl were added during growth, in the case of sulfite always before addition of the electron acceptor. To thiosulfate cultures sterile Na_2CO_3 solution was added in the same way. After growth ceased, H_2S, acetate and the exact culture volume were determined. Cells were harvested by centrifugation, washed once with 10 mmol per l Na-phosphate buffer, pH 4.5, transferred with distilled water to light glass beakers and dried to constant weight at 80° C.

Complete reduction of added sulfate was shown by addition of $BaCl_2$ solution after acidification of part of the medium. No $BaSO_4$ turbidity occurs with sulfate-free media or when sulfate is completely reduced.

Results

Enrichments and Isolation

Enrichment studies with anaerobic freshwater medium containing acetate as sole organic substrate and sulfate as electron acceptor showed, that the sulfate-reducing bacteria which could be isolated under these conditions were always of the *Desulfotomaculum acetoxidans* type (Widdel 1980; Widdel and Pfennig 1981). The fact, that *Desulfotomaculum acetoxidans* was regularly obtained from dung-contaminated habitats or rumen fluid and animal manure itself as well as the temperature optimum of 36° C indicated that this bacterium occurs primarily intestinal.

Further enrichment cultures with acetate were inoculated with anaerobic mud samples from brackish water and marine habitats. To account for their higher salinity, basal media with higher concentrations of NaCl and $MgCl_2$ were used (brackish water and marine medium). In all these enrichments intense formation of H_2S was observed, although no bacterial turbidity could be detected in the supernatant of the first passages. Transfers were made after intense mixing of the sediment. After some transfers oval to rod-shaped bacteria with rotund ends were observed in the medium; a part of the cells was motile, but after several transfers most bacteria became immotile. They grew well, but marine bacteria often showed tendency to form clumps which settled to the bottom. In none of these saline enrichments *Desulfotomaculum acetoxidans* was observed. No further growth took place when the enrichments were transferred to freshwater medium. One brackish water strain (2ac9) and two marine strains (3ac10 and 4ac11) were isolated in pure culture by repeated application of the agar shake culture method.

Salt-requiring acetate-oxidizing sulfate reducers which morphologically resembled the brackish water and marine forms could also be enriched from village ditches far away from the sea coast (region of Hannover and Konstanz, Bodensee), when sterile brackish water medium was used. With freshwater medium such sulfate reducers could not be enriched; but when grown once well in brackish water medium, they developed further in freshwater medium, although to lower densities.

Morphology

The three strains isolated (2ac9, 3ac10, 4ac11) formed oval to rod-shaped cells with rounded ends (Fig. 1); the cells of strain 3ac10 were more slender. Dimensions of cells are given in Table 1. Strain 4ac11 was motile by means of a single polar flagellum. The other two strains were immotile, although electron microscopy exhibited some cells of strain 3ac10 with single polar flagella. All three sulfate reducers were Gram negative.

Growth Conditions and Nutrition

Strain 2ac9 grew in brackish water or marine medium, but not in freshwater medium. Strains 3ac10 and 4ac11 grew only in marine medium; at lower concentrations of NaCl and $MgCl_2$ growth was significantly retarded.

Tests for the growth factor requirements revealed that of the seven vitamins added originally, strains 2ac9 and 4ac11 required biotin and 4-aminobenzoic acid for growth while strain 3ac10 required only biotin. With these vitamins added all three strains could be grown in defined minimal media.

Initiation of growth of freshly inoculated cultures, especially when precultures were old, was favoured markedly by the addition of dithionite as a further, strong reductant. When well growing, young cultures were transferred (about 5% inoculum) to fresh media, growth started immediately without addition of dithionite. With strain 2ac9 a doubling time of 20 h was measured in batch cultures. In a continuously gased anaerobic fermenter at constant pH and low H_2S concentration the doubling time was about 15 h (W. Badziong, pers. comm.). Strains 3ac10 and 4ac11 grew nearly just as fast

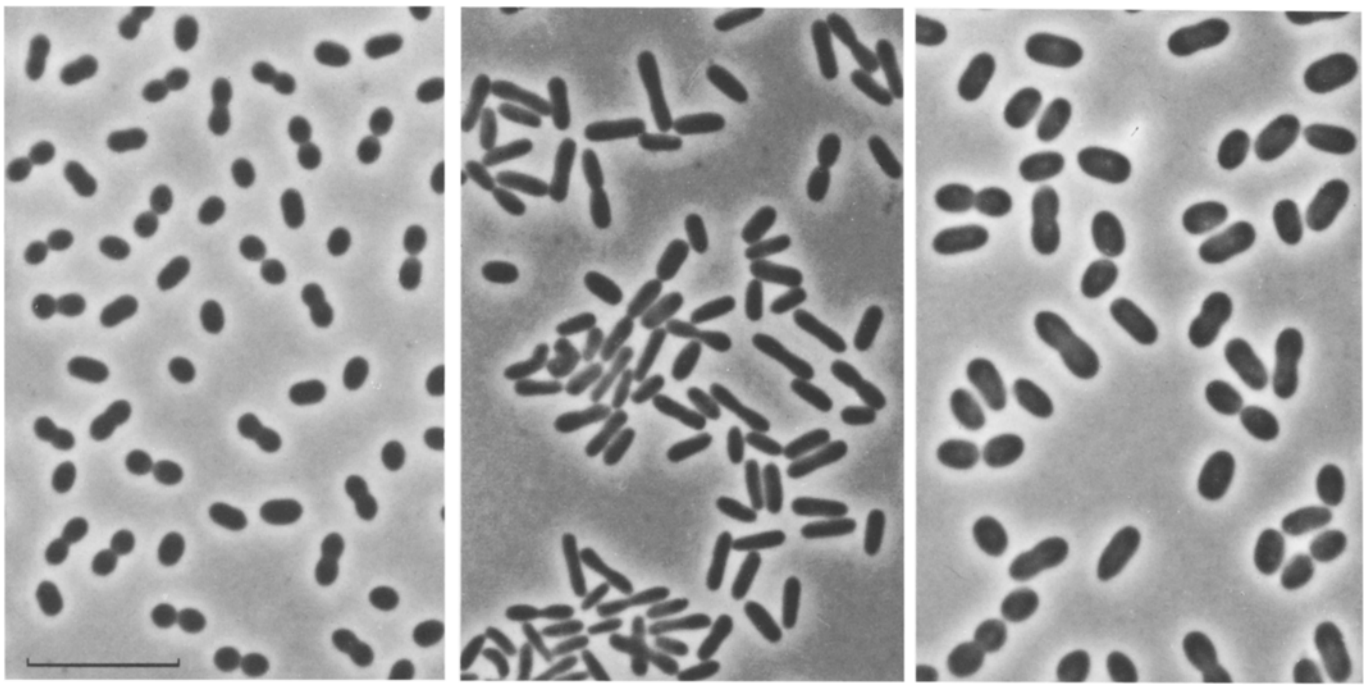

Fig. 1. Phase contrast photomicrographs of the acetate-oxidizing sulfate reducers isolated from saline environments. Bar equals 10 µm for all prints. *Left: Desulfobacter postgatei* strain 2ac9 from brackish water mud. *Middle:* Strain 3ac10 from marine mud. *Right:* Strain 4ac11 from marine mud

Table 1. Morphological and physiological characteristics of the three pure cultures of acetate-oxidizing sulfate reducers isolated from brackish water (*Desulfobacter postgatei* strain 2ac9) and marine mud samples (strains 3ac10 and 4ac11)

Characteristics	strain 2ac9 *Desulfobacter postgatei*	strain 3ac10	strain 4ac11
Width × length (µm)	1 – 1.5 × 1.7 – 2.5	1 – 1.2 × 2.5 – 3.5	1.5 – 2 × 2.5 – 3.5
Flagellation	no flagellum	sometimes single polar; but immotile	single polar; motile
Gram stain	negative	negative	negative
Growth factor requirements	biotin, 4-aminobenzoic acid	biotin	biotin, 4-aminobenzoic acid
pH-range of growth	6.2 – 8.5	5.8 – 8.5	6.1 – 8.3
pH-optimum	7.3	7.2	7.1
Temperature range of growth (°C)	10 – 37	10 – 37	10 – 37
Temperature optimum (°C)	32	32	31
Compounds tested as electron donors and carbon sources:			
Acetate	+	+	+
Lactate	–	(+)	(+)
Pyruvate	–	–	–
Ethanol	–	+	–

Symbols: + good growth; (+) slow growth; – tested but not utilized
Further electron donors tested but not utilized: Strain 2ac9: $H_2 + CO_2$, formate, propionate, butyrate, higher fatty acids, propanol, butanol, succinate, fumarate, malate, glucose, fructose, maltose, cellobiose. Strains 3ac10 and 4ac11: $H_2 + CO_2$, formate, propionate, butyrate, higher fatty acids
Electron acceptors utilized by strain 2ac9 for acetate oxidation: sulfate, sulfite, thiosulfate; tested but not utilized by strain 2ac9: elemental sulfur, fumarate, nitrate

as strain 2ac9. With excess acetate and sulfate, strain 2ac9 reached H_2S concentrations of more than 20 mmol per l.

With different electron donors instead of acetate growth and formation of H_2S in the presence of sulfate were compared to blanks without electron donor. Results are summarized in Table 1. Strain 2ac9 was highly spezialized in nutrition; acetate was the only electron donor utilized out of 20 substrates tested. Instead of sulfate, strain 2ac9 used sulfite or thiosulfate as electron acceptors, but not elemental sulfur (sulfur flower), fumarate or nitrate. Sulfur was even inhibitory: When it was added to a culture of strain 2ac9 with acetate and sulfate, no growth took place.

Table 2. Results of stoichiometric measurements with *Desulfobacter postgatei* strain 2ac9 on acetate as sole electron donor and organic carbon source and sulfate, sulfite or thiosulfate as electron acceptors. In 1 l batch cultures equal initial concentrations of acetate (20 mmol/l) and different growth limiting concentrations of sulfate, sulfite or thiosulfate had been given. The data represent the differences between the amounts obtained from cultures with electron acceptors and controls with only acetate and no added electron acceptor

Electron donor given	(mmol/l)	Hydrogen sulfide formed (mmol/l)	Cell dry weight formed (mg/l)	Acetate consumed totally (mmol/l)	Acetate consumed for cell material[a] (mmol/l)	Acetate dissimilated (mmol/l)	mol H_2S formed per mol acetate dissimilated	Growth yield: dry weight per mol acetate dissimilated
Sulfate	4.0	4.05	19.7	4.56	0.41	4.15	0.98/1	4.75
	8.0	7.84	39.5	9.11	0.81	8.30	0.94/1	4.76
	12.0	11.51	58.4	13.44	1.20	12.24	0.94/1	4.77
	16.0	15.98	78.8	17.98	1.62	16.36	0.98/1	4.82
Sulfite	3.0	3.07	25.7	2.82	0.53	2.29	4.0/3	11.22
	6.0	6.12	49.2	5.56	1.01	4.55	4.0/3	10.82
	9.0	9.35	75.2	8.63	1.55	7.08	4.0/3	10.62
	12.0	12.36	102.8	11.7	2.12	9.58	3.9/3	10.73
Thio-sulfate	3.0	6.16	29.6	3.81	0.61	3.20	1.9/1	9.25
	6.0	11.79	63.2	7.37	1.30	6.07	1.9/1	10.41
	9.0	17.09	82.9	10.93	1.71	9.22	1.9/1	8.99
	12.0	22.96	103.2	13.88	2.13	11.75	2.0/1	8.78

[a] Acetate consumed for cell material was calculated by the following equation: $17\,CH_3COO^- + 11\,H_2O \rightarrow 8\,(C_4H_7O_3) + 2\,HCO_3^- + 15\,OH^-$; thus, 0.0206 mmol acetate are required for 1.0 mg of cell dry weight

Pigments, G + C Content

In none of the three newly isolated strains desulfoviridin was detected. Strain 2ac9 was also checked for further pigments. The carbon monoxide difference spectrum of the cytoplasmic fraction of strain 2ac9 exhibited peaks at 412, 540 and 593 nm and troughs at 425, 554 and 617 nm. There were some similarities to the CO-difference spectrum of the sulfite reductase P582 in *Desulfotomaculum nigrificans* observed by Trudinger (1970). On the other hand, the distinctly marked trough at 554 nm, indicated also a CO-complex of a *c*-type cytochrome as reported by Weston and Knowles (1973). Thus, the CO-difference spectrum obtained from strain 2ac9 may be a composed one, and the presence of P 582 has to be confirmed by purification. In both the cytoplasmic and the membrane fraction *b*- and *c*-type cytochromes were identified. The redox-difference spectrum of pyridine hemochromes from acetone-HCl extraction exhibited maxima at 420, 525 and 556 nm, indicating protoheme of *b*-type cytochromes. *c*-Type cytochromes were identified as pyridine hemochromes in the residue of the acetone-HCl extraction by their absorption maxima at 415, 521 and 550 nm.

Determination of the DNA base ratio of strain 2ac9 by thermal denaturation yielded a content of 45.9 mol % guanine + cytosine.

Stoichiometry of Acetate Oxidation

Complete anaerobic oxidation of acetate was shown by growth experiments with strain 2ac9 in the presence of equal initial concentrations of acetate (20.0 mmol/l) and different growth-limiting concentrations of sulfate, sulfite or thiosulfate. Results of growth experiments are listed in Table 2. Complete reduction of the added sulfate was shown by the $BaCl_2$ method. The molar ratios between acetate dissimilated and H_2S formed from either sulfate, sulfite or thiosulfate are in good agreement with the following equations [Eq. (2a) is Eq. (2) expressed for 1 mol acetate]:

$$CH_3COO^- + SO_4^{2-} \rightarrow 2\,HCO_3^- + HS^- \quad (1)$$
$$\Delta G^{\circ\prime} = -71.7\ \text{kJ}$$

$$3\,CH_3COO^- + 4\,SO_3^{2-} + H^+ \rightarrow 6\,HCO_3^- + 4\,HS^- \quad (2)$$

$$CH_3COO^- + \frac{4}{3}\,SO_3^{2-} + \frac{1}{3}\,H^+ \rightarrow 2\,HCO_3^- + \frac{4}{3}\,HS^- \quad (2a)$$
$$\Delta G^{\circ\prime} = -158.3\ \text{kJ}$$

$$CH_3COO^- + S_2O_3^{2-} + H_2O \rightarrow 2\,HCO_3^- + 2\,HS^- + H^+ \quad (3)$$
$$\Delta G^{\circ\prime} = -117.7\ \text{kJ}$$

The $\Delta G^{\circ\prime}$ values (for pH 7.0) were calculated from data of Thauer et al. (1977).

With sulfate the average growth yield was 4.8 g cell dry weight per mol of acetate dissimilated (obtained from a linear plot), while the average growth yield with sulfite was 10.8 g cell dry weight per mol acetate. With thiosulfate as electron acceptor the highest growth yield obtained was 10.4 g cell dry weight per mol of acetate oxidized; growth yields slightly decreased with increasing thiosulfate concentrations.

Discussion

Ecological Aspects

Attempts to enrich for acetate-oxidizing sulfate-reducing bacteria from anaerobic mud samples of freshwater habitats were succesful only in few cases. When H_2S was produced from acetate in freshwater enrichments, *Desulfotomaculum acetoxidans* developed. The fact that this bacterium could regularly be obtained from manure or rumen content in-

dicated, that *Desulfotomaculum acetoxidans* is primarily an intestinal bacterium which is introduced by pollution into freshwater ponds and ditches. Consistent with these results is the optimum growth temperature of *Desulfotomaculum acetoxidans* of about 36° C (Widdel and Pfennig 1977). In contrast to freshwater enrichments samples from anaerobic marine or brackish water habitats yielded sulfate-reducing bacteria in every case. Apparently, salt-requiring acetate-oxidizing sulfate reducers are widespread in marine sediments and belong to the regular anaerobic marine microflora (Laanbroek and Pfennig 1981). This conclusion is in agreement with the turnover measurements of other authors who showed that the way of terminal anaerobic decomposition of organic matter, particularly of acetate, depended on the availability of sulfate as external electron acceptor. In freshwater sediments, where sulfate occurs in limited amounts, methane and CO_2 are the predominant anaerobic end products (Winfrey and Zeikus 1977; Fallon et al. 1980). In marine sediments, however, complete anaerobic oxidation of organic matter via dissimilatory sulfate reduction is possible (Cahet 1975; Jørgensen 1977; Abram and Nedwell 1978; Oremland and Taylor 1978; Mountfort et al. 1980).

A result which cannot yet be understood is that salt-requiring acetate oxidizers, which resemble brackish water or marine forms, occur also far away from the sea coast. However, after adaptation to lower salt concentrations, growth was also possible in freshwater medium showing that these sulfate reducers were not as strictly dependent on higher NaCl and $MgCl_2$ concentrations as the strains isolated from brackish water or marine habitats.

Bioenergetical Aspects of Sulfate Reduction with Acetate

Growth yields of sulfate-reducing bacteria in the presence of different electron acceptors were determined by Senez (1962) and Vosjahn (1970) with pyruvate as electron donor; this substrate allows ATP formation by substrate phosphorylation in addition to electron transport. The measurements showed that growth yields with pyruvate plus sulfate and pyruvate plus sulfite were identical. For detailed growth yield determinations with *Desulfovibrio vulgaris* Badziong and Thauer (1978) used molecular hydrogen as sole electron donor and acetate plus CO_2 as carbon sources so that ATP formation by substrate phosphorylation was excluded. Growth yields with thiosulfate were about 2 to 3 times higher than with sulfate as electron acceptor. From maximal growth yields, which were obtained by extrapolation to infinite growth rates, it was concluded that the reduction of 1 mol sulfate with 4 mol H_2 is associated with a net synthesis of 1 mol ATP. A synthesis of 3 mol ATP was calculated for the reduction of 1 mol thiosulfate; from a bioenergetical point of view, the same amount of ATP should be synthesized during reduction of 1 mol sulfite with 3 mol H_2.

In our experiments acetate was the electron donor which – on the basis of present biochemical knowledge – does not allow net synthesis of ATP by substrate phosphorylation (an energy-rich phosphate bond formed in a hypothetical tricarboxylic acid cycle would be required for the activation of free acetate). Thus, all ATP must be obtained by electron transport. From our batch culture growth experiments no maximum growth yields at infinite growth rates can be extrapolated. Nevertheless, the growth yields on acetate allow some considerations on the energetics of the reduction of different electron acceptors.

The grams cell dry weight of *Desulfobacter postgatei* strain 2ac9 formed per kilojoule free energy [see Eqs. (1), (2a) and (3)] were 0.067 g/kJ with acetate and sulfate, 0.068 g/kJ with acetate and sulfite and 0.088 g/kJ (highest value) with acetate and thiosulfate. Thus, ATP formation for synthesis of cell material appears to occur with similar efficiency during growth on both electron acceptors sulfate or sulfite; with thiosulfate the efficiency was slightly higher. Sulfate activation via APS is sometimes understood as an energy-consuming process in the sense that a considerable part of free energy is wasted as entropy to allow sulfate activation. On the other hand, sulfate reduction with acetate is a reaction the free energy of which is only low [Eq. (1)]. To allow net synthesis of ATP, highly irreversible reaction steps during acetate oxidation and sulfate reduction should be avoided or kept minimal by the cells. If sulfate activation would result in a waste of a larger part of the total free energy, this should become apparent in a marked decrease in efficiency of the relatively low exergonic oxidation of acetate with sulfate as compared to sulfite as electron acceptor. The results presented here show, however, that growth on acetate plus sulfate is not less efficient than growth on acetate plus sulfite.

These results urge upon the question whether the hydrolysis of pyrophosphate liberated during sulfate activation via APS is obligate, or whether pyrophosphate may not be removed by immediate use as an ATP equivalent for special phosphorylations. The latter possibility was already mentioned by Thauer et al. (1977) and Badziong and Thauer (1978). Moreover, with various metabolic electron donors APS reduction to sulfite and AMP as well as further reduction of sulfite to sulfide are energetically very favourable processes (Thauer et al. 1977; Badziong and Thauer 1978), which should promote APS formation.

In the experiments of Badziong and Thauer (1978) the electron donor for the reduction of sulfate was H_2 and not acetate. The highest growth yield obtained with H_2 was 8.3 g cell dry weight per mol of sulfate, which is more than the growth yield of 4.8 g per mol of sulfate and acetate measured by us. If *Desulfovibrio vulgaris* and *Desulfobacter postgatei* are comparable, these results can be related to the $\Delta G^{\circ\prime}$ value, which is -152.2 kJ per mol sulfate when four mol H_2 are the electron donor (Badziong and Thauer 1978) but only -71.7 kJ per mol sulfate when acetate is the electron donor. One may conclude, that net ATP formed during electron transport by a given number of electrons does not only depend on the kind of the sulfur compound being reduced but also on the energy level of the electron donor: Electrons derived from H_2 of 1 atm appear to allow higher phosphorylation yields than electrons derived from acetate. A similar distinction was already expressed by Vosjahn (1970), who showed that the electron pair derived from the cleavage of pyruvate enters the electron transport system at a more negative level than the electron pair derived from lactate.

Taxonomy

The three strains of salt-requiring, acetate-oxidizing sulfate-reducing bacteria, 2ac9, 3ac10 and 4ac11, are morphologically and nutritionally similar. While strain 2ac9 utilizes acetate exclusively, strain 4ac11 and 3ac10 oxidize in addition lactate or lactate and ethanol, respectively. Since all three strains did never show spore formation under any conditions, they cannot be classified together with the existing acetate-oxidizing, sporing bacterium of the emended genus

Desulfotomaculum (37.5 mol% GC). Clear differences also exist to the rod-shaped, nonmotile, desulfoviridin-containing intestinal sulfate reducers of the genus *Desulfomonas* (Moore et al. 1976) which carry out an incomplete oxidation of lactate or pyruvate and have a base ratio of 67 mol% GC.

It appears necessary, therefore, to establish a new genus and species for the oval to rod-shaped sulfate reducing bacteria that selectively enrich in media with acetate as the sole organic substrate. Strain 2ac9 was most intensively studied and is proposed as type strain of the new genus and species *Desulfobacter postgatei.*

Strains 3ac10 and 4ac11 are morphologically and nutritionally similar to strain 2ac9. Whether they can also be classified with the species *Desulfobacter postgatei* can be decided after determination of their GC content.

Genus *Desulfobacter* gen. nov.

De.sul.fo.bac'ter. L. pref. *de* from; L. n. *sulfur* sulfur; M. L. n. *bacter* masc. equivalent of Gr. fem. n. *bacteria* rod or staff; M. L. masc. n. *Desulfobacter* a rod-shaped sulfate reducer.

Rod-shaped to ellipsoidal cells, 1–2 by 1.7–3.5 µm, with rounded ends, single or in pairs. Motile forms with single polar flagella have been observed. Sometimes tendency to stick together in clumps forming a sediment. Gram negative.

Chemoorganotroph, metabolism respiratory. Strict anaerobes. Acetate is used as electron donor and carbon source and completely oxidized to CO_2. Other simple organic substrates may also be oxidized completely. Sulfate and other oxidized sulfur compounds serve as electron acceptors and are reduced to H_2S. Media containing a reductant and vitamins are necessary for growth; often higher concentrations of NaCl and $MgCl_2$ are required.

Temperature optimum: 28–32°C; growth may occur at 10°C.

Cells contain b- and *c-*Type cytochromes.

Desulfobacter postgatei sp. nov.

Post.ga'te.i. M. L. gen. n. *postgatei* of J. R. Postgate, an English microbiologist who has made the sulfate-reducing bacteria well-known by many studies.

Rod-shaped to ellipsoidal cells, 1–1.5 by 1.7–2.5 µm, with rounded ends, single or in pairs. Immotile or motile by single polar flagella. No spore formation. Gram negative.

Strict anaerobic chemoorganotroph. Acetate is completely oxidized to CO_2. Sulfate, sulfite, or thiosulfate serve as electron acceptors and are reduced to H_2S; not utilized: elemental sulfur, fumarate, nitrate, oxygen. Growth requires mineral media with sulfide as reductant and not less than about 5 g NaCl and 1 g $MgCl_2 \cdot 6\,H_2O$ per l. 4-Aminobenzoate and biotin are required as growth factors.

Selective enrichment with acetate plus sulfate at higher NaCl and $MgCl_2$ concentrations.

pH-range: 6.2–8.5, optimum at 7.3. Temperature range: 10–37°C, optimum at 32°C.

Cell membrane and cytoplasmic fraction contain *b-* and *c-*type cytochromes; protoheme of the former is extractable with acetone + HCl. Desulfoviridin not present.

DNA base ratio: 45.9 mol% G + C (thermal denaturation).

Habitats: anaerobic mud of brackish water or marine environments; occurrence has also been shown in freshwater mud by enrichment with salt water media.

Type strain: "Dangast", 2ac9, DSM 2034, deposited in: Deutsche Sammlung von Mikroorganismen, Göttingen.

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