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Studies on Dissimilatory Sulfate-Reducing Bacteria that Decompose Fatty Acids

III. Characterization of the Filamentous Gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov.

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Abstract. Gliding motility, ultrastructure and nutrition of two newly isolated filamentous sulfate-reducing bacteria, strains 5ac10 and 4be13, were investigated. The filaments were always attached to surfaces. Growth was supported by addition of insoluble aluminum phosphate or agar as substrata for gliding movement. Electron microscopy of ultrathin sections revealed cell walls characteristic of Gram-negative bacteria; the undulated structure of the outer membrane may pertain to the translocation mechanism. Intracytoplasmic membranes were present. Acetate, higher fatty acids, succinate or fumarate served as electron donors and carbon sources. Strain 5ac10 grew also with lactate, but not with benzoate that was used only by strain 4be13. Strain 5ac10 was able to grow slowly on H_2 plus CO_2 or formate in the presence of sulfate without additional organic carbon source. The capacity of complete oxidation was shown by stoichiometric measurements with acetate plus sulfate. Both strains contained b- and c-type cytochromes. Desulfoviridin was detected only in strain 5ac10. The two filamentous gliding sulfate reducers are described as new species of a new genus, *Desulfonema limicola* and *Desulfonema magnum*.

Key words: Filamentous anaerobes – Gliding motility – Cell wall structure – Anaerobic acetate oxidation – Fatty acids – Anaerobic benzoate oxidation – Sulfate reduction – Desulfoviridin – Cytochromes – Genus *Desulfonema*

The term "gliding bacteria" describes an assemblage of Gram-negative prokaryotes sharing merely the special feature of gliding motility. These bacteria do not necessarily have close genetic relationships. For a comprehensive presentation see Reichenbach (1981) and Reichenbach and Dworkin (1981). The group comprises both unicellular and multicellular, filamentous species. Gliding forms occur among the phototrophic and chemotrophic bacteria utilizing inorganic or organic electron donors and carbon sources. The majority of gliding bacteria so far studied under defined conditions is obligately aerobic. Some *Cytophaga* species are facultative anaerobes (Bachmann 1955; Anderson and Ordal 1961; Veldkamp 1961). *Sphaerocytophaga* and *Capnocytophaga* isolated from oral cavities were reported to have a purely fermentative metabolism (Gräf 1961; Newman et al. 1976; Leadbetter et al. 1979). For members of the filamentous Pelonemataceae (Skuja 1956, 1974) anaerobic growth was supposed because of their occurrence in anaerobic hypolimnia rich in H_2S (Hirsch 1981); however, pure cultures of these bacteria have never been studied and their type of metabolism is unknown.

Recently, two strains of strictly anaerobic filamentous gliding bacteria were isolated that conserve energy for growth by dissimilatory sulfate reduction (Widdel 1983). In this paper, the pure cultures are morphologically and physiologically characterized and described as two new species of a new genus, *Desulfonema limicola* and *Desulfonema magnum*. The latter is of special interest because it oxidizes benzoate to CO_2. The anaerobic degradation of benzoate has been studied several times in natural methanogenic associations (Tarvin and Buswell 1934; Nottingham and Hungate 1969; Ferry and Wolfe 1976; Keith et al. 1978). Mineralization of an aromatic compound by sulfate-reducing bacteria has not been observed so far (Evans 1977).

Materials and Methods

Sources of Organisms

The filamentous gliding-sulfate reducing bacteria, strains 5ac10 and 4be13, were enriched from marine sediments and isolated into pure cultures by anaerobic washing with sterile medium and by dilution series in soft agar tubes as described in a separated paper (Widdel 1983).

Media and Conditions of Cultivation

The natural sea water medium used for enrichment and isolation of strains 5ac10 and 4be13 (Widdel 1983) was replaced in the following studies by defined, synthetic salt water media. Basal media containing sulfate, mineral salts, iron, trace elements, bicarbonate, sulfide and vitamins were prepared by the technique described for *Desulfobacter* (Widdel and Pfennig 1981). For strain 5ac10, the medium contained 13.5 g NaCl and 2.2 g $MgCl_2 \cdot 6\,H_2O$ per l; the pH was about 7.6. Strain 4be13 was grown with 20 g NaCl, 5 g $MgCl_2 \cdot 6\,H_2O$ and 1.4 g $CaCl_2 \cdot 2\,H_2O$ per l at pH 6.9–7.0. In the trace element stock solution, the amounts of H_3BO_3 and $CuCl_2 \cdot 2\,H_2O$ were lowered down to 6 and 2 mg per l, respectively (SL 10, application 1 ml per l medium).

In order to support gliding movement, an artificial light sediment of aluminum phosphate was precipitated in the medium before inoculation: per l of medium, 5 ml of a sterile solution of 48 g $AlCl_3 \cdot 6\,H_2O$/l was added; the pH was readjusted with 1.6 ml of a solution of 106 g Na_2CO_3/l. Alternatively, the gliding filaments were grown in a viscous medium prepared with 2 g washed agar per l.

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Organic substrates were added from sterile stock solutions before inoculation. The method for testing H_2 as electron donor and the application of dithionite as additional reductant has been described for *Desulfobacter* (Widdel and Pfennig 1981). For test of autotrophic growth, the bacteria were subcultured at least four times in mineral medium without organic compound except required vitamins (Table 1).

For stimulation of growth, a neutralized, autoclaved mixture of organic acids modified after Bryant (1973) was used. Per l, the stock solution contained 5 g isobutyric acid, 5 g *n*-valeric acid, 5 g isovaleric acid, 5 g 2-methylbutyric acid, 2 g *n*-caproic acid, 2 g *n*-heptanoic acid, 2 g *n*-octanoic acid, and 45 g succinic acid; application: 1 ml per l of medium.

Strains were grown at 28–30° C. The settled filaments were whirled up twice a day by shaking.

Preparation of Cell Filaments for Ultrathin Sections

For electronmicroscopical studies, the isolated strains were grown without sediment or agar in which case the filaments sticked together in dense clumps. The preparation and evaluation methods described by Walther-Mauruschat et al. (1977) were followed with a slight modification. For fixation, the cells were not embedded in agar. Instead, the cell clumps were washed twice in saline buffer, 0.05 mmol K-phosphate/l, pH 6.8, containing 24 g NaCl/l, and fixed at 20° C for 5 h in saline buffer with added glutaraldehyde. Afterwards, they were washed seven times and postfixed in saline buffer with OsO_4 for 5 h.

Chemical and Biochemical Determinations. Sulfide was determined photometrically by the methylene blue method of Cline (1969). Acetate was measured gaschromatographically as described for *Desulfobacter* (Widdel and Pfennig 1981). For identification of pigments, cells from 10 l cultures with artificial sediment of aluminum phosphate were harvested by slow centrifugation. To remove the bulk of the sediment, cells were washed twice in 0.5 l of a solution of 36 g *tri*-sodium citrate per l, pH 6.5. Cytochromes, desulfoviridin and carbon monoxide-reacting pigments were examined as described for *Desulfobacter*. The DNA base ratio was determined by Dr. H. Hippe, Göttingen, FRG, using the thermal denaturation method.

Growth Experiments. As turbidity measurements were not possible with the inhomogenously growing filaments, only approximate doubling times were calculated from the volume of the inoculum, V_i, the culture volume, V_c, and the growth time, t_g, during which the culture reached again the H_2S concentration and the estimated cell density of the inoculum. For mere exponential growth, the doubling time is $t_d = 0.3 \cdot t_g / \log(V_c/V_i)$. Assuming a sigmoid growth curve, the shortest doubling time would be even less.

Before measuring stoichiometry of acetate oxidation, strains 5ac10 and 4be13 were grown with acetate and benzoate, respectively, in sulfate media with inorganic sediment and growth-promoting supplements. After growth ceased, the settled filaments and sediment were washed anaerobically in sterile medium without organic compounds, and transferred into 50 ml bottles containing minimal medium with acetate plus sulfate; for controls, only either acetate or sulfate was added. Sulfide and acetate were measured immediately after inoculation and again after 2 weeks of incubation.

Results

Observations on Isolated Filaments by Light Microscopy

Morphological characteristics of the filamentous sulfate-reducing bacteria strains 5ac10 and 4be13 are summarized in Table 1.

The cells contained granules of poly-β-hydroxybutyric acid (Figs. 1–4) which in the pellet of disrupted cells formed a white layer soluble in chloroform.

Strain 5ac10 did not glide continuously along its longitudinal axis; the highest velocity observed was about 1 $\mu m \cdot s^{-1}$. The most conspicious motility character was a twitching or jerkey swinging when the filaments came out of the sediment particles. Strain 4be13 moved more regularly with a speed of 4 $\mu m \cdot s^{-1}$. Simultaneously, the filaments rotated. When stumbling against an obstacle, the cells creeped backwards after some seconds. An inversion of the rotation could not be observed so that both right- and left-handed screw motility seemed to occur. In artificial sediment of aluminum phosphate, both gliders left slimy tubes behind that were visible by the precipitate (for strain 4be13 see Fig. 3). Similar tracks formed by gliding filaments have been shown by Castenholz (1973) and Strohl and Larkin (1978). Gliding movement of filaments sticking together sometimes resulted in regular double twists (Fig. 4).

Ultrastructure

Electron microscopy of ultrathin sections exhibited a dense cytoplasm with light areas (Figs. 6 and 8) probably resulting from the removal of poly-β-hydroxybutyric acid granules.

In both strains, intracytoplasmic membranes were detected (Figs. 5 and 7). They appeared mainly irregularly arranged, and only some formed radial parallel layers (Figs. 5 and 8). Between the cytoplasmic membrane and the outer membrane, a dark layer could be distinguished from the less electron-dense interspace. The outer membrane was undulated. In strain 4be13, the waves were more regular and of higher contrast than in strain 5ac10. At the inner side of the cytoplasmic membrane, a row of dense particles was situated (Fig. 7 and 10). Adjacent to the cell envelope, parallel layers of low electron density (Figs. 5, 9 and 10) and a few radial fibers (Fig. 11) were observed.

Judging from the cell wall structure, the filamentous gliding sulfate reducers have to be classified with the Gram-negative bacteria although the two strains stained Gram-variable to Gram-positive.

Growth Conditions and Nutrition

In the defined salt water medium, strain 5ac10 grew as well as in natural sea water medium. When the concentrations of NaCl and $MgCl_2$ were half as high, growth was already retarded, and in freshwater medium the filaments were damaged. Strain 4be13 continued to grow only with more than 4 mmol $CaCl_2$/l when transferred from natural sea water medium to defined salt water medium; at lower $CaCl_2$ concentrations, these filaments became immotile, swelled and died off. Thus, nearly the Ca^{2+} concentration of natural sea water (Turekian 1969) was applied for cultivation. NaCl and $MgCl_2$ were also important for strain 4be13, and optimum growth occurred only when the concentrations were not significantly lower than indicated.

Table 1. Morphological and physiological characteristics of the filamentous gliding sulfate-reducing bacteria *Desulfonema limicola* strain 5ac10 and *Desulfonema magnum* strain 4be13. Substrate concentrations (in parentheses) are given in mmol/l. Symbols: ++ good growth; + slow growth; − tested but not utilized; · not tested

	Desulfonema limicola strain 5ac10	*Desulfonema magnum* strain 4be13
Length × width of one cell (μm)	2.5−3.5 × 2.5−3	9−13 × 6−8
Length of filaments (μm)	50−1000	100−2000
Growth factor requirement	biotin	biotin, 4-aminobenzoic acid, B_{12}
pH Range of growth	6.5−8.8	6.6−7.5
pH Optimum	7.6	7.0
Temperature range of growth	15−36	15−37
Temperature optimum	30	32
mol% G + C of DNA	34.5	41.6
Compounds tested as electron donors and carbon sources:		
$H_2 + CO_2$ autotroph	+	−
$H_2 + CO_2$ + acetate (1)	++	−
Formate (10) autotroph	+	−
Formate + acetate (1)	++	++
Acetate (15)	+	+
Propionate (10)	++	++
Butyrate (5)	++	++
Isobutyrate (5)	++	++
Valerate (5)	++	++
2-Methylbutyrate (5)	++	+
Isovalerate (5)	++	++
Caproate (3)	++	++
Heptanoate (1.5)	++	++
Octanoate (1)	++	++
Pelargonate (0.5)	++	++
Decanoate (0.5)	++	++
Laurate (0.5)	++	−
Myristate (1)	+	−
Palmitate (1)	−	−
Lactate (10)	++	−
Pyruvate (10)	++	−
Succinate (10)	++	++
Fumarate (10)	++	++
Malate (10)	−	+
Benzoate (4)	−	++
4-Hydroxybenzoate (1)	·	++
Hippurate (2)	·	+
Phenylacetate (2)	·	++
3-Phenylpropionate (2)	·	++

Tested but not utilized by strains 5ac10 and 4be13: ethanol (10), propanol (5), butanol (5), stearate (1), cyclohexane-carboxylate (2), adipate (3), pimelate (2), glucose (5), fructose (5), maltose (2.5), cellobiose (2.5)
Compounds tested only with strain 4be13 but not utilized: 2-hydroxybenzoate (1), 3-hydroxybenzoate (1), nicotinate (1.5), uric acid (5)

The filamentous sulfate reducers were always attached to surfaces and did never grow suspended in the supernatant medium. Growth was favoured by addition of a light sediment of aluminum phosphate. Without this substratum, the filaments sticked together in dense clumps and developed only poorly. Growth and gliding movement were favoured also in viscous media containing 0.2% agar.

To test different organic compounds as electron donors, growth and formation of H_2S were compared to blanks without added substrates. Results are summarized in Table 1. Strains 5ac10 and 4be13 were also incubated with different electron acceptors in the presence of acetate or benzoate, respectively. Instead of sulfate, strain 5ac10 used sulfite (initial concentration given: 2 mmol/l) and thiosulfate (10 mmol/l); no growth occurred with fumarate, malate or nitrate as electron acceptors. Strain 4be13 reduced only sulfate. Elemental sulfur was inhibitory for both strains: when it was added in the presence of sulfate, no growth and no formation of H_2S took place. Growth was never observed in the absence of an electron acceptor; neither pyruvate or lactate nor fumarate or malate nor sugars were fermented. Thus, the gliding filaments obligately reduced oxidized sulfur compounds.

On acetate alone in a defined medium containing sulfate and vitamins, the strains grew very slowly with doubling times of about 100 h or even more. For isolation, a stimulatory supernatant from anaerobically fermented yeast extract and manure had been used additionally (Widdel 1983). With this complex growth-promoting addition, strains 5ac10 and 4be13 reached doubling times of about 30 h. The stimulant was necessary for cultivation of strain 5ac10 on acetate to obtain acceptable incubation times and cell yields; strain 4be13 grew already satisfactorily on benzoate without the complex additions. With the amounts of the growth stimulants alone

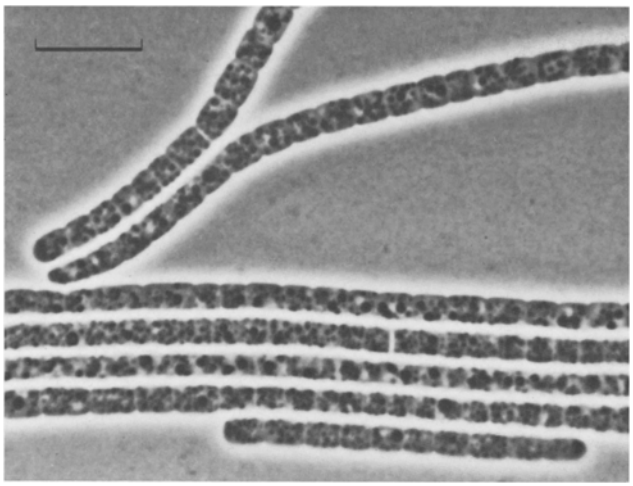


Fig. 1. Phase contrast photomicrograph of *Desulfonema limicola* strain 5ac10 grown with acetate plus sulfate; cells contain granules of poly-β-hydroxybutyric acid. Bar equals 10 µm

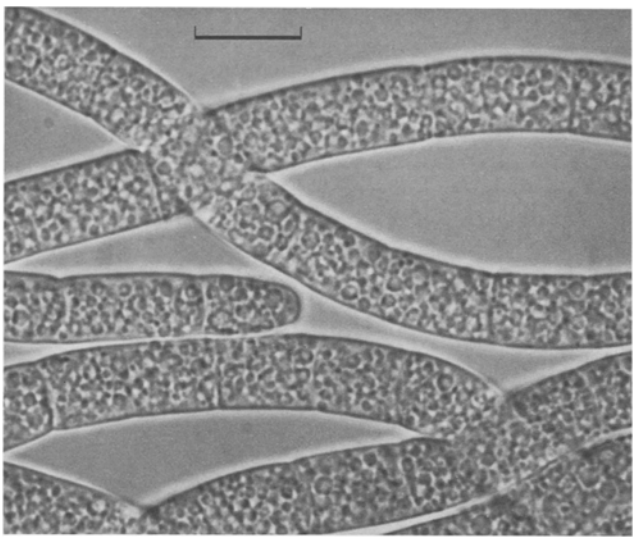


Fig. 2. Light field photomicrograph of *Desulfonema magnum* strain 4be13 grown with benzoate plus sulfate; cells contain granules of poly-β-hydroxybutyric acid. Bar equals 10 µm

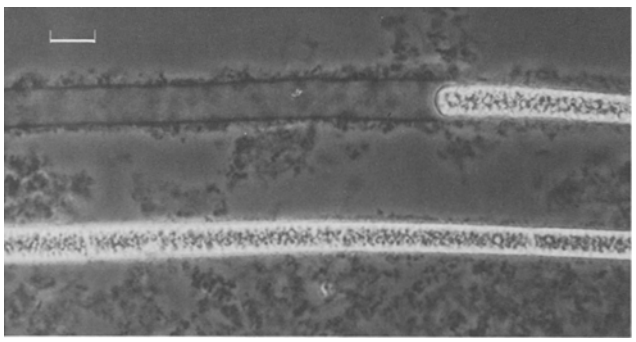


Fig. 3. Phase contrast photomicrograph of *Desulfonema magnum* strain 4be13 leaving a trail behind in the added artificial sediment of aluminum phosphate. Bar equals 10 µm

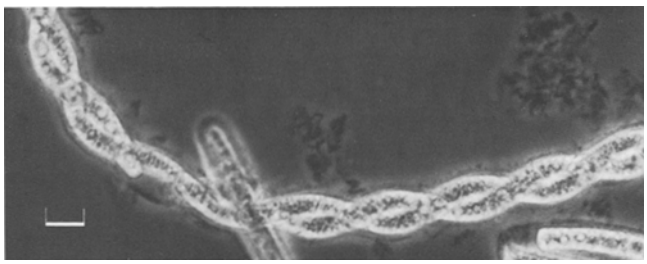


Fig. 4. Phase contrast photomicrograph of *Desulfonema magnum* filaments winding around themselves. Bar equals 10 µm

in the absence of acetate or benzoate, only low cell densities and H_2S concentrations were reached. A certain stimulatory effect on growth with acetate was also obtained by using small amounts (about 50 mg/l) of additional electron donors as formate, propionate, butyrate, valerate or succinate. For strain 5ac10 cultivated with acetate, the mixture of fatty acids plus succinate as indicated turned out to be as effective as the natural supplements, and was used to replace these undefined additions.

The seven vitamins added originally could be substituted by biotin in case of strain 5ac10 and by biotin plus 4-aminobenzoic acid and vitamin B_{12} in case of strain 4be13. The optimum temperatures and pH values for growth are given in Table 1. With inocula smaller than 2% of the total volume, or after transfer of old stock cultures, initiation of growth was favoured by addition of 10–20 mg sodium dithionite per l as a strong reductant.

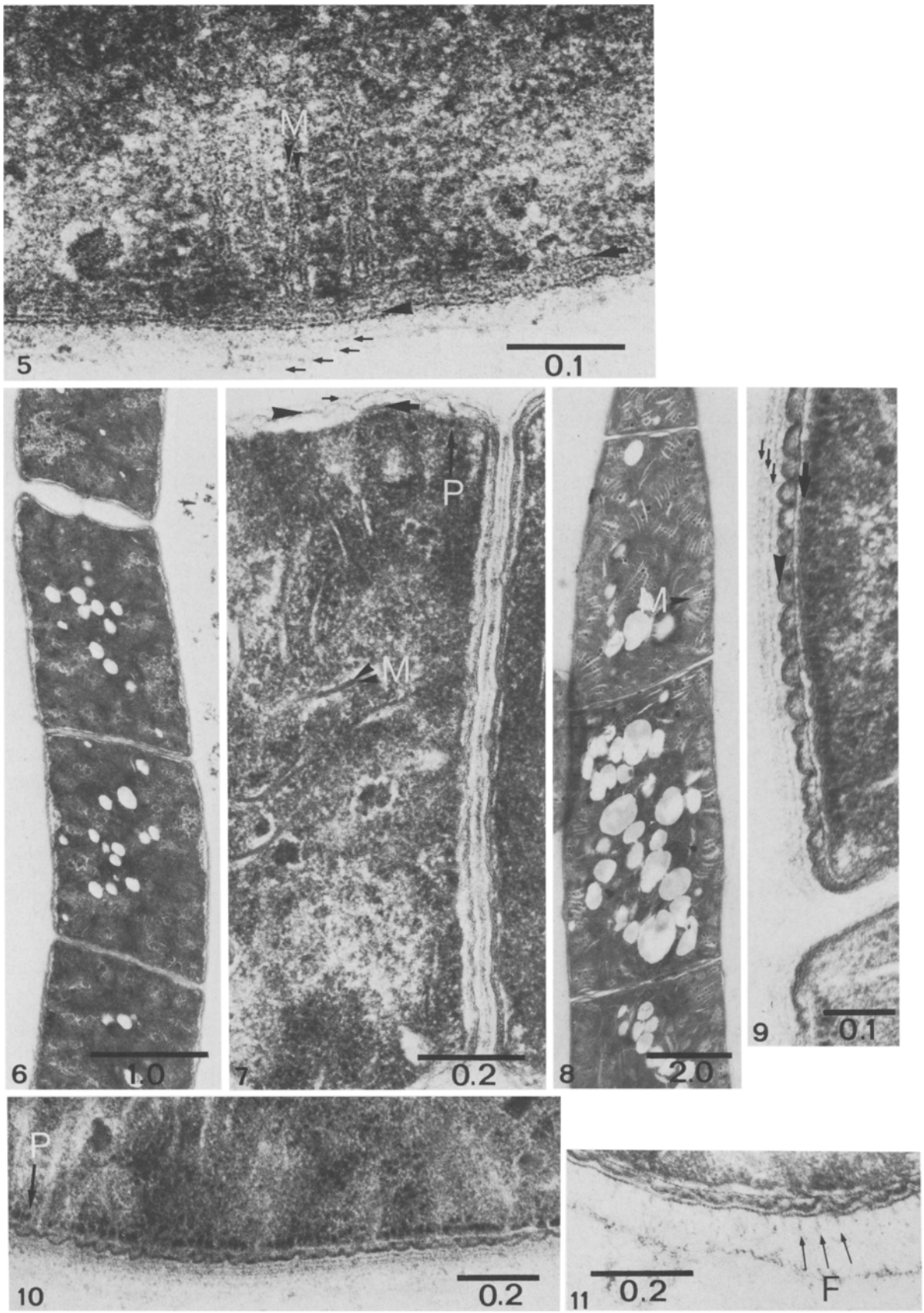
Maintenance

Stock cultures of strain 5ac10 were maintained on acetate plus the growth-promoting organic acids in medium with aluminum phosphate as sediment; the grown bacteria were stored at 2–4° C and transferred every 3 months into new medium. In contrast, strain 4be13 died off within some days at such low temperatures; instead, it remained viable for 3–5 months when kept in viscous agar medium with benzoate at 20° C.

Pigments, DNA Base Ratios

In strain 5ac10, c-type cytochromes were found in the cytoplasm and in the membranes; the redox difference spectrum of the residues from acetone-HCl extraction exhibited absorption maxima in alkaline pyridine at 415, 522 and 550 nm. Protoheme indicating the presence of b-type cytochromes was extracted only in minor quantities from the membranes. The cytoplasm of strain 4be13 did not contain remarkable amounts of cytochromes; differentiation by acetone-HCl extraction failed. Significant quantities of both b- and c-type cytochromes were found in the membranes of this strain; the redox difference spectrum of the protoheme in alkaline pyridine had absorption maxima at 419, 526 and 557 nm.

In the cytoplasmic fraction of strain 5ac10, the sulfite reductase desulfoviridin was detected by its absorption maximum at 630 nm (Fig. 12) and red fluorescence when treated with NaOH and exposed to UV light of 365 nm (Postgate 1959). Strain 4be13 did not contain desulfoviridin;



Figs. 5–11. Electron micrographs of ultrathin sections of the filamentous gliding sulfate-reducing bacteria; block staining with uranyl acetate, poststaining with lead citrate. **5–7** *Desulfonema limicola* strain 5ac10; **8–11** *Desulfonema magnum* strain 4be13. (*Small arrows*) outer wall layers; (*large arrows*) cytoplasmic membrane; (*small arrowheads* = *M*) intracytoplasmic membranes; (*large arrowheads*) outer membrane; (*P*) particles; (*F*) radial fibers. Dimensions are given in μm

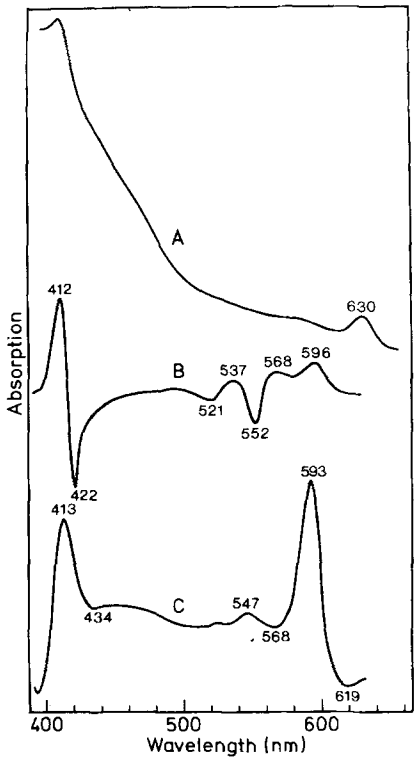


Fig. 12A–C. Absorption spectra measured with the cytoplasmic fractions from the filamentous gliding sulfate reducers. **A** *Desulfonema limicola* strain 5ac10, absolute spectrum (oxidized) showing the absorption maximum of desulfoviridin at 630 nm. **B** *Desulfonema limicola* strain 5ac10, carbon monoxide difference spectrum (dithionite-reduced). **C** *Desulfonema magnum* strain 4be13, carbon monoxide difference spectrum (dithionite-reduced)

instead, its cytoplasm revealed a CO difference spectrum (Fig. 12) similar to that of the sulfite reductase P582 from *Desulfotomaculum nigrificans* (Trudinger 1970; Akagi and Adams 1973). In contrast, the CO difference spectrum obtained from strain 5ac10 (Fig. 12) was characteristic of CO complexes of cytochromes (Weston and Knowles 1973).

The guanine plus cytosine contents of the DNA determined by thermal denaturation are given in Table 1.

Stoichiometry of Substrate Oxidation

Table 2 shows acetate consumption and H_2S formation by the filamentous sulfate reducers after transfer from supplemented, well grown precultures into minimal media. H_2S was only formed in the presence of both, acetate and sulfate. If a small part (here less than 10%) of the acetate consumed is subtracted to account for the synthesis of cell material and poly-β-hydroxybutyric acid, the results of Table 2 are consistent with the following equation:

$$CH_3COO^- + SO_4^{2-} \rightarrow 2\,HCO_3^- + HS^-.$$

If strain 4be13 was grown on benzoate at a concentration of 4 mmol per l, between 13 and 14 mmol sulfide were formed per l. Assuming that about 10% of the substrate was assimilated, complete oxidation is obvious also in case of benzoate as electron donor:

$$4\,C_6H_5COO^- + 15\,SO_4^{2-} + 16\,H_2O \rightarrow 28\,HCO_3^- + 15\,HS^- + 9\,H^+.$$

Table 2. Stoichiometric measurements of acetate oxidation by the filamentous gliding sulfate-reducing bacteria strains 5ac10 and 4be13. Tests were carried out in 50 ml batch cultures with defined minimal media containing artificial inorganic sediment to support gliding movement. The data represent the differences of acetate and H_2S concentrations measured directly after inoculation and after 2 weeks of incubation; values are given in mmol/l

	Acetate given	Sulfate given	Acetate consumed	H_2S formed
Strain 5ac10	15.0	20.0	14.6	14.0
	0.0	20.0	0.0	0.22
	15.0	0.0	0.0	0.37
Strain 4be13	15.0	20.0	13.1	12.2
	0.0	20.0	0.0	0.0
	15.0	0.0	0.1	0.26

Discussion

Ecological Aspects

The gliding filamentous sulfate-reducing bacteria described in this paper were able to oxidize a variety of fatty and other organic acids. However, in batch cultures none of the substrates allowed selective enrichment of these organisms; they did not compete successfully with other non-filamentous sulfate reducers that developed in high numbers. Nevertheless, gliding filaments resembling the strains 5ac10 and 4be13 were microscopically detected already in untreated anaerobic sediment samples (Widdel 1983). For understanding the successful competition with other sulfate reducers in nature, two characteristics of the gliding filaments will have to be considered: 1. Gliding motility allows active spreading between sediment particles and into viscous material (Reichenbach 1981); the direction of movement may be influenced by gradients of dissolved substrates. 2. In contrast to smaller unicellular bacteria, the long filaments resisted phagocytosis by protozoa (Widdel 1983). The grazing activity of protozoa selects to the advantage of filamentous bacteria in the activated sludge of sewage treatment plants (Güde 1979). Since certain amoebae and ciliates are capable of thriving in anaerobic sulfide-containing sediments (Fenchel 1969), grazing is not only significant under aerobic conditions.

Ultrastructure and Gliding Movement

The cell wall structure revealed by electron microscopy shows that the newly isolated filamentous sulfate reducers belong to the Gram-negative eubacteria as well as all gliding bacteria known until now (Reichenbach 1981; Reichenbach and Dworkin 1981). The intracytoplasmic membranes detected in the filamentous sulfate-reducing bacteria are not unique for these but have also been discovered by Thauer (1982) in the unicellular acetate oxidizer *Desulfobacter postgatei*.

Mechanistic hypotheses to explain gliding motility have been reviewed by Burchard (1980, 1981). Our findings of a waved outer membrane support the hypothesis of a peristaltic movement as expressed by Humphrey et al. (1979): a wavy motion in the flexible outer membrane would be produced by sequences of "make-and-break" interactions with the rigid peptidoglycan framework visible here as dark middle layer. Waved outer membranes have been shown for different gliding bacteria (Costerton et al. 1961; Strohl 1979; Holt et al. 1979). Other authors supposed all movements in prokaryotes

to be variations of the same kind of rotary motors; in the gliding *Cytophaga johnsonae* and *Flexibacter columnaris*, Pate and Chang (1979) found structures resembling flagellar basal regions. If the dense particles at the inner side of the cytoplasmic membrane in the gliding sulfate reducers shown here are interpreted as components of such structures, this would support the latter hypothesis. Another explanation of gliding movement has been given for *Oscillatoria* filaments where helically arranged fibrils are supposed to act against the substratum by unidirectional waves of bending (Halfen and Castenholz 1971; Castenholz 1973; Halfen 1973). Indeed, strain 4be13 rotated during gliding, and small sediment particles were sometimes seen on the filament surface moving in screw-like curves. Fibrillar structures of unknown composition outside the outer membrane were found by electron microscopy. However, the electron microscopic technique applied to analyse the ultrastructure was not suited to picture helically arranged fibers.

Lapidus and Berg (1982) observed the motion of latex spheres adsorbed to cells of a *Cytophaga* strain. The model given by these authors suggests motion of adsorption sites within the fluid outer membrane along tracks fixed to the rigid peptidoglycan layer. In *Flexibacter*, Burchard (1982a) recently assumed a contractile apparatus to operate for both flexing and gliding.

The trails formed in the artificial sediment (Fig. 3) indicate excretion of slime. The ultrastructure of this slime may be represented by the parallel layers adjacent to the cell envelope. For the role of slime in holding the cells against the substratum and guiding gliding movement see Humphrey et al. (1979) and Burchard (1981, 1982b). Lapidus and Berg (1982), however, pointed out that their observations on gliding motility would be inconsistent with thick intervening layers of slime.

Investigating the influence of chemically different substrata on gliding movement, Arlauskas and Burchard (1982) found that *Flexibacter* did not move until negatively charged polymers were supplied.

Calcium ions have been reported to be necessary for gliding movement (Castenholz 1973; Burchard 1980); but it cannot be explained so far why just only strain 4be13 has a requirement for Ca^{2+} concentrations nearly as high as in sea water.

Considering in conclusion the mechanistic models based on different experimental results and bearing in mind the morphological, physiological and genetic diversity of gliding bacteria, one may assume also multiple mechanisms of translocation within this group.

Taxonomy

The filamentous cellular organization and the gliding motility are particular features that clearly distinguish the strains described in this paper from all other species and genera of sulfate-reducing bacteria known so far. There is a morphological similarity of strains 5ac10 and 4be13 to gliding filamentous organisms drawn and described by Skuja (1956, 1974) as *Achroonema splendens* and *Achroonema macromeres* (family Pelonemataceae; see also Hirsch 1981). However, the purely morphological descriptions of the *Achroonema* species do not allow identification of the presently described sulfate-reducing bacteria with the bacteria observed by Skuja in Swedish freshwater lakes. Strains 5ac10 and 4be13 are clearly marine bacteria and could not be adapted to freshwater media. Since the new isolates are obligate sulfate reducers, we consider it most useful and appropriate for determinative purposes to classify the strains with the physiological-ecological group of sulfate-reducing bacteria. The new genus *Desulfonema* is proposed.

Genus *Desulfonema* gen. nov.

De.sul.fo.ne'ma. L. pref. *de* from; L.n. *sulfur* sulfur; Gr.n. *nema* thread; M.L.neut.n. *Desulfonema* thread-forming sulfate reducer.

Multicellular filaments of sometimes more than 1 mm length with visible cross walls. Granules of poly-β-hydroxybutyric acid may be stored. Filaments are always attached to surfaces. Gliding motility is common, simultaneous rotation may occur. Gram-staining can be positive, but cell walls are characteristic of Gram-negative bacteria. The outer membrane has a waved structure.

Strictly anaerobic chemoorganotrophs or chemolithotrophs, metabolism respiratory. Sulfate and other oxidized sulfur compounds serve as electron acceptors and are reduced to H_2S. Fatty acids and other organic acids are used as electron donors and carbon sources; oxidation is complete and leads to CO_2.

Media containing a reductant and vitamins are necessary for growth. Marine forms may require brackish or sea water concentrations of NaCl, $MgCl_2$ and $CaCl_2$. Gliding movement and growth is promoted by addition of an artificial light sediment (e.g. aluminum phosphate or agar).

Habitats: anaerobic sulfate-rich sediments with decaying plant material.

Type species: *Desulfonema limicola.*

Desulfonema limicola sp. nov.

li.mi'co.la. L.n. *limus* mud; L.suff., verbal n. *cola* dweller; M.L.masc.n. *limicola* mud dweller.

Gliding filaments of 3 µm diameter; one cell is 2.5–3.5 µm long.

Dissimilatory metabolism dependent on sulfate, sulfite or thiosulfate as electron acceptors. No growth with fumarate, malate or nitrate as electron acceptors. Elemental sulfur is inhibitory. H_2 plus CO_2, formate, acetate, propionate, higher fatty acids up to 14 carbon atoms, lactate, pyruvate, succinate, and fumarate serve as electron donors and carbon sources. On acetate alone, growth is very slow but can be stimulated by mixtures of fatty acids or extracts from anaerobic sludge. Growth with H_2 plus CO_2 or formate does not require additional carbon sources but is promoted by acetate. Not utilized: alcohols, benzoate, sugars. No fermentation of organic compounds. Biotin is required as growth factor.

pH Range: 6.5–8.8, optimum at 7.6. Temperature range: 15–36°C, optimum at 30°C.

The type strain requires at least 12 g NaCl and 2 g $MgCl_2 \cdot 6H_2O$ per l of culture medium for optimum growth and does not develop in freshwater medium. However, also in freshwater sediments morphological similar filaments have been observed.

c-Type cytochromes are present in the membrane and cytoplasmic fraction; in the membranes, also b-type cytochromes have been identified in small amounts by extraction of protoheme. The sulfite reductase desulfoviridin was found in the cytoplasm.

DNA base ratio: 34.5 mol% G + C (thermal denaturation).

Type strain: "Jadebusen", 5ac10, DSM 2076, deposited in Deutsche Sammlung von Mikroorganismen, Göttingen, FRG.

Desulfonema magnum sp. nov.

mag'num. L.adj. *magnus* large, big.

Gliding filaments of 6–8 µm diameter; one cell is 9–13 µm long.

Dissimilatory metabolism dependent on sulfate as electron acceptor. Type strain does not grow with sulfite, thiosulfate, fumarate, malate or nitrate as electron acceptors. Elemental sulfur is inhibitory. Acetate, propionate, butyrate, higher fatty acids up to 10 carbon atoms, succinate, fumarate, malate, benzoate, 4-hydroxybenzoate, hippurate, phenylacetate and 3-phenylpropionate serve as electron donors and carbon sources. On acetate alone, growth is only poor; extracts from anaerobic sludge are stimulatory. Not utilized by the type strain: H_2, lactate, pyruvate, alcohols, 2-hydroxybenzoate, 3-hydroxybenzoate, cyclohexanecarboxylate, sugars. No fermentation of organic compounds. Biotin, 4-aminobenzoic acid and vitamin B_{12} are required as growth factors.

pH Range: 6.6–7.5, optimum at 7.0. Temperature range: 15–37°C, optimum at 32°C. Isolated cultures in the laboratory die off very soon at temperatures lower than 10°C.

Optimum growth requires 20 g NaCl, 5 g $MgCl_2 \cdot 6H_2O$ and at least 1 g $CaCl_2 \cdot 2H_2O$ per l. At lower salt concentrations, growth is retarded, and in freshwater medium, cells are damaged.

In the membrane fraction, b- and c-type cytochromes have been identified; the cytoplasmic fraction contains only traces of cytochromes. Desulfoviridin not present.

DNA base ratio: 41.6 mol% G + C (thermal denaturation).

Type strain: "Montpellier", 4be13, DSM 2077, deposited in Deutsche Sammlung von Mikroorganismen, Göttingen, FRG.

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