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Growth of Sulfate-Reducing Bacteria with Sulfur as Electron Acceptor

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Abstract. In addition to three new isolates, six strains of representative species of sulfate-reducing bacteria were tested for their capacity to use elemental sulfur as an electron acceptor for growth. There was good growth and sulfide production by strain Norway 4 and the three isolates, two of which had been enriched with sulfur flower and one isolated from a culture with green sulfur bacteria. Slow but definite growth was observed with *Desulfovibrio gigas*. The type strains of *Desulfovibrio desulfuricans*, *D. vulgaris*, and *Desulfotomaculum nigrificans* as well as *Desulfomonas pigra* did not grow with sulfur. The four strains that grew well with sulfur flower were straight, nonsporulating rods and did not contain desulfoviridin.

Key words: Sulfate-reducing bacteria – Growth with elemental sulfur – Desulfoviridin – Morphology – New isolates – *Desulfovibrio* – *Desulfotomaculum* – *Desulfomonas*.

Recently we described a new type of strictly anaerobic bacterium, *Desulfuromonas acetoxidans*, which is able to grow with acetate as sole carbon and energy source. The oxidation of acetate to CO_2 is stoichiometrically linked to the reduction of elemental sulfur or of disulfide compounds (cystine, oxidized glutathione) to sulfide or sulfhydryl compounds, respectively (Pfennig and Biebl, 1976). Sulfate, sulfite or thiosulfate, the electron acceptors of sulfate-reducing bacteria, were not reduced by *Desulfuromonas*. It was therefore interesting to determine the extent of utilization by sulfate-reducing bacteria of elemental sulfur as electron acceptor. The descriptions of sulfate- reducing bacteria (Postgate, 1974; Postgate and Campbell, 1966) do not explicitly mention the use of elemental sulfur. Postgate (1951) was unable to obtain growth with precipitated sulfur using six different *Desulfovibrio* strains; however, some sulfide production was observed with colloidal sulfur. A similar result had been reported by Baars in 1930. Van Gemerden (1967) obtained a temporary acceleration of sulfide production in a sulfate and formate containing *Desulfovibrio* culture when elemental sulfur was added.

From enrichment cultures for sulfur-reducing bacteria of the *Desulfuromonas* type, we obtained several strains of rod-shaped bacteria which proved to be sulfate-reducers. The strains grew well with elemental sulfur instead of sulfate when ethanol or lactate was provided as carbon and energy source. In order to establish whether good growth with sulfur is restricted to the new isolates, a number of established strains of sulfate-reducing bacteria was also tested.

Two of the nine strains were obtained from enrichment cultures with elemental sulfur: strain 4474 originated from an alder swamp near Hamburg (Duvenstedter Brook) and strain 5174 from a forest pond near Braunschweig. Strain 9974 was isolated from a culture designated "*Chloropseudomonas ethylica N2*", kindly provided by M. C. W. Evans, London. The Norway 4 strain of *Desulfovibrio desulfuricans* (NCIB 8310) was sent by J. Le Gall, Marseille, and *Desulfomonas pigra* 11112 by W. E. C. Moore, Blacksburg, Virginia. All other strains (see Table 1) were obtained from the German Collection of Microorganisms, DSM, Göttingen.

The tests were done in 50 ml screw-cap bottles with H_2S-reduced medium containing in grams per liter: ethanol, 1.0; yeast extract, 0.2; KH_2PO_4, 0.4; NH_4Cl, 0.5; $MgCl_2 \cdot 6H_2O$, 0.4; $CaCl_2 \cdot 2H_2O$, 0.05; $NaHCO_3$, 2.0; $Na_2S \cdot 9H_2O$, 0.3; trace element solution of Pfennig and Lippert (1966), 10 ml. Elemental sulfur was added aseptically from wetted, thoroughly ground sulfur flower which was autoclaved for 30 min at 112–115°C. The bottles received a glass bead and were incubated on a rotary shaker at 28°C; thus the sulfur was gradually ground to a fine suspension.

Table 1. Production of sulfide from elemental sulfur and presence of desulfoviridin in different strains of sulfate-reducing bacteria

Species and strains	Final concentration of sulfide produced from sulfur mg S^{2-}/l[a]	Presence of desulfoviridin[b]	Cell shape
Desulfovibrio desulfuricans Essex 6	58	+	slender vibrios or spirilla
Desulfovibrio vulgaris Hildenborough	77	+	
Cf. *Desulfovibrio desulfuricans* Norway 4	434	−	short, straight rods
Sulfate-reducing bacterium 9974	513	−	
Sulfate-reducing bacterium 4474	390	−	
Sulfate-reducing bacterium 5174	460	−	
Desulfovibrio gigas DSM 496	283	+	fat vibrios
Desulfotomaculum nigrificans Delft 74	83	−	long, thin rods
Desulfomonas pigra 11112	23	+	long, fat rods

Medium as described in the text, for *D. nigrificans* lactate was used instead of ethanol

[a] Values obtained after subtraction of the sulfide concentration in a sulfur-free control (about 35 mg S^{2-} per litre)

[b] From published data, except for strains 4474 and 5174 which were tested in this investigation

Control cultures without sulfur flower as well as with sulfate were run in parallel. Sulfide production was followed by the methylene blue method (Trüper and Schlegel, 1964). Tests for c-type cytochromes and desulfoviridin were carried out spectrophotometrically using cell free preparations.

Table 1 shows the concentration of sulfide produced from sulfur by nine strains after 7 and 10 days of incubation. Although none of the strains completely lacked the ability to reduce sulfur, four strains attacked it only to a very limited extent, namely the type strains of *Desulfovibrio desulfuricans* and *D. vulgaris* and the *Desulfomonas pigra* strain 11112 (Moore et al., 1976). Apart from the two sulfur-enriched isolates (4474 and 5174), good sulfur utilization occurred in strain 9974 and in the Norway 4 strain. Growth of these four strains ceased after 2–3 days of incubation, when the growth limiting sulfide concentration was reached.

As the amount of sulfide produced from a given amount of electrons is four times higher with sulfur than with sulfate:

$$8\,[H] + 4\,S^{\circ} \rightarrow 4\,H_2S$$

$$8\,[H] + H_2SO_4 \rightarrow H_2S + 4\,H_2O,$$

the maximal sulfide concentration tolerated by the cells is reached faster and at a lower cell density with sulfur than in sulfate grown cultures. Nontheless growth was clearly established in all cultures that produced significant amounts of sulfide. This was also true for *Desulfovibrio gigas* in which growth and sulfide production was markedly slower.

Two of the four strains growing well with elemental sulfur, Norway 4 and 9974, are known to lack desulfoviridin, the typical pigment of the genus *Desulfovibrio* (Miller and Saleh, 1964; Lee et al., 1973). It appears significant that in the two other sulfur-utilizing strains (4473 and 5174), no desulfoviridin was detected as well. However, all four strains exhibited the absorption maxima characteristic for cytochrome c_3. Lee et al. (1973) showed that strains Norway 4 and 9974 contain desulforubidin which is masked by the cytochrome in the absorption spectra. It remains to be determined whether the two strains 4474 and 5174 also have desulforubidin.

That the capacity to utilize sulfur for growth is not necessarily correlated with the possession of desulforubidin, can be seen in the case of *Desulfovibrio gigas* which contains desulfoviridin. As both pigments are part of the sulfite-reducing enzyme (Le Gall, 1975), none of them should be involved in the reduction of sulfur.

The four strains that grow well with sulfur are morphologically different from *Desulfovibrio desulfuricans* strain Essex 6 and *D. vulgaris* strain Hildenborough. While the cells of these two type strains were distinct vibrios or short spirilla, the cells of the four strains were straight rods (0.6 µm wide, 1.4–2.8 µm long) under all conditions tested. These differences were apparent in our medium as well as in Baars' medium which was proposed as a reference medium for cell shape and size (Postgate and Campbell, 1966). Electron microscopy of negatively stained cells showed a single polar flagellum in strains 4474, 5174 and 9974. In contrast to the original description (Miller and Saleh, 1964), strain Norway 4 exhibited peritrichous flagellation. The classification of this strain with *Desulfovibrio desulfuricans* needs to be reconsidered.

Strains 4474, 5174 and 9974 fermented pyruvate in the absence of sulfate while strain Norway 4 grew very poor under these conditions. Except for strain

4474, malate was used in the presence of sulfate. All strains grew well on ethanol and lactate with sulfate.

In view of the fact that sulfate reduction with acetate had been described (Baars, 1930), all strains listed in the table were tested for their capacity to reduce sulfur or sulfate with acetate. Neither sulfide formation nor growth were observed with any of the strains tested.

The present investigation confirmed for the type strains of *Desulfovibrio desulfuricans* and *D. vulgaris* that elemental sulfur cannot be used as an electron acceptor for growth, although some sulfide is formed. The same is true for the type strain of *Desulfotomaculum nigrificans* and for *Desulfomonas pigra* strain 11112. In view of these results it appears that the four strains of sulfur-utilizing rod-shaped sulfate-reducing bacteria have a broader ecological valence and, therefore, represent a different physiological-ecological group. As enrichment cultures for three of the four strains have shown, these bacteria have a selective advantage in ecological niches where elemental sulfur is available either directly or as a metabolic product of green sulfur bacteria. Further studies will show whether the capacity to use sulfur is important in taxonomy and identification among the sulfate-reducing bacteria.

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