SHORT COMMUNICATION

Growth of *Desulfovibrio* species on Hydrogen and Sulphate as Sole Energy Source

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Desulfovibrio vulgaris (Hildenborough), *D. desulfuricans* (Essex 6) and *D. gigas* (holotype) are shown to grow on H₂ and sulphate as sole energy source. Lithotrophic growth on H₂ and sulphate is thus not a unique property of a few newly isolated *Desulfovibrio* strains as previously reported (Badziong *et al.*, 1978).

INTRODUCTION

Desulfovibrio vulgaris (Marburg) can grow on H₂ and sulphate as sole energy source. This property was thought to distinguish it from the type strain *D. vulgaris* (Hildenborough) (Badziong *et al.*, 1978). The DNA relatedness of the two strains has recently been determined by DNA:DNA hybridization and found to interrelate at the 90% binding level (Brandis, 1981). This degree of binding is generally found for very closely related strains. Therefore, the capability of *D. vulgaris* (Hildenborough) and other *Desulfovibrio* species to use H₂ and sulphate was reinvestigated.

METHODS

Bacterial strains. *Desulfovibrio vulgaris* (Marburg) (DSM 2119) is the strain isolated by Badziong *et al.* (1978). *Desulfovibrio vulgaris* strain Hildenborough (DSM 644; NCIB 8303), *Desulfovibrio desulfuricans* strain Essex 6 (DSM 642; NCIB 8307) and *Desulfovibrio gigas* (DSM 496; NCIB 9332) were obtained from the German Collection of Micro-organisms (Göttingen). *Escherichia coli* (NCTC 10537) was from the National Collection of Type Cultures (London, U.K.).

Cultivation of *Desulfovibrio* species. The sulphate-reducing bacteria were grown at 37 °C (34 °C for *D. gigas*) in 500 ml glass fermenters containing 300 ml autoclaved medium, as described previously (Thauer & Badziong, 1981). The medium had the following composition (per litre distilled water): 2.5 g sodium acetate or 5 g 50% (w/w) sodium lactate solution; 6.6 g (NH₄)₂SO₄; 1.8 g NaCl; 0.9 g KH₂PO₄; 0.36 g MgCl₂·6H₂O; 0.26 g CaCl₂·2H₂O; 0.5 ml resazurin solution (2 g l⁻¹); and 12.5 ml trace element solution. The trace element solution, which was autoclaved separately, contained (per litre distilled water): 12.8 g nitrilotriacetic acid brought to pH 6.5 with NaOH; 1 g FeCl₂·4H₂O; 0.5 g MnCl₂·4H₂O; 0.3 g CoCl₂·6H₂O; 0.2 g ZnCl₂; 50 mg Na₂MoO₄·2H₂O; and 20 mg H₃BO₃. Where indicated, filter-sterilized yeast extract solution or vitamin solution was added (10 ml l⁻¹). The vitamin solution contained (per litre distilled water): 2 mg biotin; 2 mg folic acid; 10 mg pyridoxine hydrochloride; 5 mg thiamin hydrochloride; 5 mg riboflavin; 5 mg nicotinic acid; 5 mg DL-pantothenic acid (calcium salt); 0.1 mg vitamin B₁₂; 5 mg *p*-aminobenzoic acid; 5 mg lipoic acid; and 200 mg choline chloride. The pH was adjusted to 7 with 1 M-KOH and the solution kept in the dark at 4 °C under N₂. Before inoculation of the fermenter the medium was gassed with H₂/CO₂ (80:20, by vol.; O₂ concn < 5 p.p.m.) for 15 min at a rate of 250 ml min⁻¹; then the pH was adjusted to 6.2 with autoclaved 1 M-Na₂CO₃ (1–1.2 ml) and the redox potential

was lowered with 0.5–1 ml filter-sterilized 1 M-Na₂S solution. After inoculation [10% (v/v) inoculum] the culture was continuously stirred with a magnetic stirrer at 700 rev. min⁻¹ and gassed with the same H₂/CO₂ mixture as before inoculation, at a rate of 250 ml min⁻¹ (150 ml min⁻¹ for *D. gigas*). Growth was followed by measuring the A_{578} . Samples of the culture were diluted with 100 mM-maleate/NaOH buffer pH 6.0 to give an A_{578} of 0.1–0.3 and a maleate concentration of 50 mM. Cell concentrations (g dry wt l⁻¹) corresponding to the measured A_{578} values are given in Table 1. Each time the A_{578} increased by 0.4 (undiluted culture), 1 ml 3 M-H₂SO₄ or 2 ml 6 M-HCl was added to maintain the pH of the culture below 7. The dissimilation of sulphate and the assimilation of acetate were followed as described earlier (Badziong & Thauer, 1978; Badziong *et al.*, 1979).

Determination of DNA base composition. DNA was isolated by the method of Marmur (1961). The DNA base composition was calculated from the thermal denaturation profile in standard saline citrate buffer. The DNA of *E. coli* B, with a base composition of 52 mol % GC and a T_m of 90.7 °C (Gillis *et al.*, 1970), served as a reference.

RESULTS

Desulfovibrio vulgaris (Hildenborough) is usually grown on lactate and sulphate in a medium heavily supplemented with yeast extract (Postgate, 1979). The first few attempts to grow this sulphate-reducer on H₂ and sulphate failed. After several trials the bacteria were found to grow on H₂ and sulphate provided that they were given time to adapt to the lithotrophic growth conditions. The procedure used was as follows. *Desulfovibrio vulgaris* (Hildenborough) was first grown on a lactate/sulphate/yeast extract medium in a fermenter gassed with H₂/CO₂ (80:20). In successive transfers, lactate was gradually replaced by acetate. Then the yeast extract was omitted. The bacteria now grew on H₂ and sulphate in a mineral salts medium supplemented only with acetate as organic carbon source. Growth was exponential with a doubling time of about 5 h; the growth yield was 9–10 g (mol sulphate)⁻¹. Under the same conditions *D. vulgaris* (Marburg) grew exponentially with a doubling time of about 3.5 h and with a growth yield of 10–11 g (mol sulphate)⁻¹ (Table 1).

Growth of *D. vulgaris* (Hildenborough), as of *D. vulgaris* (Marburg), on H₂ and sulphate was dependent on the presence of acetate rather than on the presence of vitamins or yeast extract. By using [¹⁴C]acetate it was found that approximately 70% of the cell carbon was derived from acetate (Table 1) and that the acetate was not oxidized to CO₂. During growth the specific radioactivity of acetate in the culture remained constant. These findings show that acetate was used only for the synthesis of cell components and not as an electron donor for dissimilatory sulphate reduction.

Using the adaptation procedure described for *D. vulgaris* (Hildenborough) it was possible to show that the type strains of *D. desulfuricans* and *D. gigas* could also grow on H₂ and sulphate as sole energy source. Both acetate and CO₂ were required for cell proliferation, approximately 70% of the cell carbon being derived from acetate. Acetate was not oxidized

Table 1. *Characteristics of Desulfovibrio species growing on H₂ and sulphate as sole energy source*

Species	Carbon source	Requirements	Cell carbon derived from acetate (%)*	Mean doubling time (h)	Y _{sulphate} (g dry wt mol ⁻¹)	Cell concn at $A_{578} = 1$ (g dry wt l ⁻¹)
<i>D. vulgaris</i> (Marburg)	Acetate + CO ₂	None	70	3.5	10–11	0.400
<i>D. vulgaris</i> (Hildenborough)	Acetate + CO ₂	None	68	5	9–10	0.396
<i>D. desulfuricans</i> (Essex 6)	Acetate + CO ₂	Vitamins + choline	76	6–7	7–8	0.308
<i>D. gigas</i> (holotype)	Acetate + CO ₂	Yeast extract	70	10–14	7–8	0.298

* Calculated from the amount of [U-¹⁴C]acetate incorporated per mg dry wt cells, and assuming that the dried cells contained 45% carbon (Badziong & Thauer, 1978).

to CO_2 . Unlike the *D. vulgaris* strains, *D. desulfuricans* and *D. gigas* only grew on H_2 /sulphate/acetate/ CO_2 if the medium was supplemented with low concentrations of yeast extract. Approximately 25 mg yeast extract was required to obtain 500–600 mg dry wt cells. Clearly, yeast extract was used only as a supply of vitamins and not as a major carbon or energy source. In the case of *D. desulfuricans* the yeast extract could be replaced by a vitamin solution containing choline (Table 1).

Desulfovibrio vulgaris (Hildenborough) and *D. desulfuricans* (Essex 6) were reidentified after establishment of growth on H_2 and sulphate by the GC content of their DNA (60.5 mol % and 54.3 mol %, respectively; Postgate, 1979). *Desulfovibrio gigas* was identified by its characteristic cell form.

DISCUSSION

Desulfovibrio vulgaris (Hildenborough), *D. desulfuricans* (Essex 6) and *D. gigas* are the sulphate-reducers that have been most thoroughly investigated (Postgate, 1979). The results reported here show that these three strains readily grow on H_2 and sulphate as sole energy source, if the medium is supplemented with acetate and CO_2 as carbon sources, and when they are given time to adapt to the lithotrophic growth conditions. The report of Badziong *et al.* (1978) that *D. vulgaris* (Hildenborough) and *D. desulfuricans* (Essex 6) cannot grow under these conditions must therefore be revised. Sulphate-reducers are known to be particularly prone to lags or periods of adaptation when transferred to new growth conditions. This may be the reason why previous attempts to grow these bacteria on H_2 and sulphate failed.

The first reports on growth of *D. vulgaris* (Hildenborough) (formerly *D. desulfuricans* Hildenborough) on H_2 and sulphate were by Mechals & Rittenberg (1960) and by Postgate (1960). These investigators demonstrated that growth on H_2 and sulphate was dependent on the presence of yeast extract and that only about 10% of the cell carbon was derived from CO_2 . These early experiments excluded the possibility that *D. vulgaris* could grow autotrophically, but they did not convincingly demonstrate that the bacteria can grow on H_2 and sulphate as sole energy source, because five to ten times more yeast extract was added to the medium than cell mass was formed. It was therefore generally not accepted that *D. vulgaris* (Hildenborough) could grow lithotrophically. The results reported in this paper show that *D. vulgaris* (Hildenborough) is capable of growth on H_2 and sulphate provided the medium is supplemented with acetate. In the presence of acetate a requirement for yeast extract was not observed.

Sorokin (1966*a, b, c*) studied growth on H_2 and sulphate of a *D. desulfuricans* strain isolated from stratum hydrogen sulphide water of a petrol deposit. He showed that growth was dependent on the presence of acetate, which was used as a carbon rather than as an energy source. Neither yeast extract nor vitamins were required. Heterotrophic growth of the bacterium was well documented, whereas growth on H_2 and sulphate was not. Neither the extent of growth nor growth yield data were reported. Therefore, it was not possible to evaluate whether growth on H_2 and sulphate was efficient or not.

Pfennig & Widdel (1981) recently reported the isolation of two new sulphate-reducing bacteria which can grow autotrophically on H_2 and sulphate. They also found sulphate-reducers that can use acetate as electron donor for dissimilatory sulphate reduction. The inability to grow autotrophically or to oxidize acetate is thus not a general property of all sulphate-reducing bacteria.

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