A Sulphate-reducing Bacterium containing Cytochrome c_3 but lacking Desulfoviridin

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SUMMARY

A sulphate-reducing bacterium (strain Norway 4) previously assigned to the species *Desulfovibrio desulfuricans* has now been found to contain cytochrome c_3 but not desulfoviridin. The organism resembles strains of *D. desulfuricans* which contain both pigments, in its morphology, nutrition, metabolism and hydrogenase activity. It is considered to be a mutant which has lost desulfoviridin. This work has not revealed the function of desulfoviridin.

INTRODUCTION

Cytochrome c_3 and desulfoviridin, a soluble porphyroprotein, have been considered (Postgate, 1956*a*, 1959) to be characteristic pigments of the anaerobic sulphate-reducing bacterium *Desulfovibrio desulfuricans*. Neither pigment was detected in six strains of thermophilic sulphate reducers (*Clostridium nigrificans*; Postgate, 1956*b*, 1959) and three strains of *D. orientis* (Postgate, 1959; Adams & Postgate, 1959) examined. Recently a new species of sulphate reducer, *D. gigas*, differing morphologically and biochemically from *D. desulfuricans* though containing cytochrome c_3 and desulfoviridin, was described by Le Gall (1963). The present communication reports the discovery of a sulphate-reducing bacterium which contains cytochrome c_3 and lacks desulfoviridin, but which nevertheless appears to belong to the species *D. desulfuricans*.

RESULTS

Isolation of the strain

The organism, a mesophilic sulphate-reducing bacterium, was originally isolated in 1949 from a sample of salt water from Oslo Harbour, designated *Desulfovibrio desulfuricans* strain Norway 4, and deposited with the National Collection of Industrial Bacteria (NCIB 8310). Until recently the only investigation performed on it was a pyruvate dismutation test (Postgate, 1952). Saleh (1964) and Saunders, Campbell & Postgate (1964) examined this and other strains of sulphate-reducing bacteria for resistance to inhibitors and for DNA base composition respectively, in the hope of detecting differences correlated with taxonomic position. The present authors subjected the 45 strains used in the inhibitor tests to routine examination for desulfoviridin by the method of Postgate (1959). All the 45 strains of *D. desulfuricans* tested gave a positive reaction except strain Norway 4. Microscopic examination of this strain showed a fair degree of pleomorphism, and though the tests for aerobic and anaerobic contaminants (Postgate, 1953) gave negative results

the culture was described by Saleh (1964) as being probably a mixed culture of sulphate reducers. A new culture was grown in Dr J. R. Postgate's (personal communication) modification of medium C (Butlin, Adams & Thomas, 1949) + 0.4 M-NaCl, from a well-isolated colony in a stab culture in Baars's (1980) medium + NaCl + 1 g. yeast extract/l. + agar. This isolate lacked desulfovirdin and grew anaerobically with reduction of sulphate to sulphide; it was used in the work described below. Methods of preparation of media and culture techniques were as described by Macpherson & Miller (1968).

Characterization of the strain

Morphology. The organism was a fat Gram-negative rod, sometimes slightly curved; mean dimensions about $1.6 \times 0.6 \mu$, somewhat longer in old cultures. Motility was progressive and typical of *Desulfovibrio desulfuricans*. No spores were found by conventional staining techniques. Electron microscopy showed many organisms, especially in young cultures, which possessed a single polar flagellum; organisms from 8-day cultures frequently contained a number of granules of electron-dense material.

Media. Vigorous growth occurred in modified medium C + 0.4 M-NaCl and in the chemically defined medium ('standard medium') of Macpherson & Miller (1968) + NaCl, in which the organism has been serially subcultured more than 30 times without noticeable decrease in rate or abundance of growth. Crops of about equiv. 700 μ g. dry wt. bacteria/ml. were obtained in the latter medium. The organism has been adapted to an NaCl-free version of the standard medium. It appeared to grow equally well under an atmosphere of N_2 , or H_2 + 1% (v/v) CO_2 . Acetate was identified chemically and chromatographically among the end-products of growth (Grossman & Postgate, 1955).

Tests of utilization of carbon sources by sulphate reducers are of limited value (see Macpherson & Miller, 1968); growth, especially in media which lack yeast extract, is often scanty or doubtful. A few tests of C sources were made: good growth occurred on pyruvate in lactate-free standard medium + NaCl; fair growth on glucose or choline; no growth on acetate.

Temperature. The organism was isolated at 30°, and grew readily at 37°. An attempt to 'train' it to grow at 42° failed; on a second attempt, with smaller temperature increments, it was trained to grow at 42.5° but would not grow at 43°.

Pigments. Thick bacterial suspensions showed a strong absorption band at about 554 $m\mu$ in the Hartridge reversion spectroscope, characteristic of cytochrome c_3 , but gave a negative reaction for desulfovirdin. A spectrum of intact organisms was obtained by using a recording spectrophotometer with a reflectance head; a control of *Desulfovibrio desulfuricans* strain Canet 32 (NCIB 8392), a member of the same 'group' (Saunders *et al.* 1964) as the original strain of Norway 4, was used. Each organism showed the three absorption peaks of reduced cytochrome c_3 (419, 525 and 553 $m\mu$; see Fig. 1), and in addition strain Canet 32 showed a strong absorption band at 632 $m\mu$ and a weaker one at 585 $m\mu$ due to desulfovirdin, both absent from the spectrum of strain Norway 4. Postgate (1956*a*) noticed a twofold decrease in desulfovirdin content in *D. desulfuricans* strain El Agheila Z grown by pyruvate dismutation in iron-deficient medium. On the assumption that the minimum iron concentration for initiation of desulfovirdin synthesis in strain Norway 4

may be abnormally high, cultures were grown in modified medium C with up to 20 times the usual iron concentration. No detectable desulfoviridin was synthesized.

Electron acceptors for hydrogenase activity. Washed organisms of strain Norway 4 suspended in non-nutrient buffer were examined at 37° for hydrogenase activity by the procedure of Littlewood & Postgate (1956) except that 15% (w/v) KOH was used as absorbent for H₂S and CO₂. Benzylviologen, thiosulphate, tetrathionate,

Fig. 1. Absorption spectra of *Desulfovibrio desulfuricans* strains Norway 4 and Canet 32, showing the absence of absorption bands corresponding to desulfoviridin in the spectrum of strain Norway 4. The absorption scales for the two spectra are different, since separate adjustments were made according to the bacterial and iron sulphide concentrations of each suspension.

sulphite and dithionite were tested as electron acceptors over the range 5–50 mM; all were utilized. The hydrogen absorption coefficient ($-Q_H$) in each case was 2–4 times that for equimolar sulphate.

Terminal electron acceptors for growth. The organism strain Norway 4 was inoculated into modified medium C + NaCl containing various electron acceptors in place of sulphate: 25 mM-Na₂S₂O₃ or Na₂S₄O₆ gave approximately the same crop, as determined by measurement of optical extinction, as equimolar sulphate; 25 mM-Na₂SO₃ was markedly inhibitory, though 10 mM-Na₂SO₃ gave about the same crop as 10 mM-Na₂SO₄. Dithionite was not tested as an electron acceptor for growth because of its instability.

Dismutation. The organism strain Norway 4 grew in a medium described by Postgate (1952) which lacked sulphate and lactate but contained sodium pyruvate, in keeping with this author's findings with the original strain of Norway 4. Choline was not dismutated.

DISCUSSION

The strain Norway 4, re-isolated by us, appears to be a normal *Desulfovibrio desulfuricans* in respect of its staining reaction, flagellation, ability to grow anaerobically on lactate or pyruvate with sulphate reduction, production of acetate, temperature tolerance and utilization of other sulphur compounds as electron acceptors for growth and hydrogenase activity. It differs from the Hildenborough strain (Postgate, 1951) in showing a considerably lower $-Q_{H_2}$ for sulphate reduction than for reduction of other sulphur compounds, though in our experience little significance can be attached to values for $-Q_{H_2}^{so\frac{1}{2}}$ obtained with *D. desulfuricans* grown in batch culture. Norway 4 resembles other strains belonging to the group II of Saunders *et al.* (1964) in its adaptability to NaCl-free medium and its ability to dismute pyruvate. It contains the cytochrome c_3 unique to *D. desulfuricans* and *D. gigas*, and differs only in lacking desulphoviridin; thus it can probably be regarded as a mutant of *D. desulfuricans* which has lost this pigment. No metabolic abnormality has yet been discovered in this strain which might throw light on the function of this pigment.

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