
On the Nutrition of Desulphovibrio desulphuricans: a Correction

By J. R. POSTGATE

Chemical Research Laboratory, Teddington, Middlesex

SUMMARY: High cell densities reached in cultures of Desulphovibrio desulphuricans (Hildenborough strain), grown in a medium of glucose, peptone, yeast extract and salts were due to the presence of a non-sulphate-reducing anaerobic contaminant whose presence was detectable only in special conditions. The relevance of this to previous studies on the Hildenborough strain is discussed and certain quantitative data are corrected.

Studies on the nutrition of a strain of Desulphovibrio desulphuricans (Postgate, 1951a) appeared to indicate that a medium of glucose, salts, peptone and yeast extract permitted growth to about 750 μg. dry wt. cells/ml. Peptone and yeast extract acted synergistically; peptone was the more important constituent since, with high concentrations of peptone, yeast extract could be omitted, but the reverse was not true. Various pure vitamins, amino-acids, etc., were tested for their ability to replace the complex nitrogenous supplements, and a mixture of cysteine, serine, ornithine and isoleucine had some growth-promoting effect. Grossman & Postgate (1958) showed that the cysteine in this mixture could be replaced by Na₂S, indicating that the cysteine functioned through its reducing properties and not as a nutrient.

The present paper reports that the massive growth observed in the complex medium was partly due to the presence of an anaerobic contaminant in the stock strain used. The contaminant was only detectable in special conditions.

METHODS

Organism. A strain of D. desulphuricans called 'Hildenborough' was used throughout; its origin, maintenance and methods of subculture have been described (Postgate, 1951a). A stock was kept in a lyophilized condition, prepared by the procedure described in Chemistry Research (1951).

Media. Medium A consisted of glucose, peptone, yeast extract and sulphates (Postgate, 1951a); medium B consisted principally of sodium lactate, yeast extract and sulphates (Butlin, Adams & Thomas, 1949). When necessary, either medium was supplemented with one or more of the following: (a) agar (British Drug Houses Ltd.), 15 g./l. for solid media; (b) FeSO₄·(NH₄)₂SO₄·6H₂O (0-5 g./l.) when it was desired to detect sulphate-reducing bacteria by blackening due to formation of FeS; (c) freshly sterilized and neutralized l-cysteine hydrochloride (5 × 10⁻³Μ; British Drug Houses Ltd.) when it was desired that all viable organisms should grow (Chemistry Research, 1951; Grossman & Postgate, 1958). Reagents were of 'analytical reagent' grade except where stated.
Rough agar dilutions. A sealed Pasteur pipette was dipped into the culture being tested, and then dipped successively into seven soft-glass test tubes (15×1 cm.) each containing c. 4 ml. molten agar medium (40 ± 1°), supplemented with FeSO₄·(NH₄)₂SO₄·6H₂O. The medium was allowed to set and incubated in air at 30°. This procedure enabled populations to be checked rapidly for anaerobic contaminants, and gave well-separated black colonies of sulphate-reducing bacteria suitable for isolation in the third or fourth dilution. When the medium was supplemented with cysteine well-separated colonies appeared in the sixth or seventh dilution.

RESULTS

A stock of the Hildenborough strain maintained in medium A, which regularly reached high cell densities on subculture, appeared pure when plated out for aerobic and anaerobic contaminants on (1) medium A solidified with agar, (2) medium B solidified with agar, (3) blood agar, (4) glucose peptone agar. On testing by the agar dilution procedure in medium A solidified with agar and containing FeSO₄·(NH₄)₂SO₄·6H₂O white colonies were sometimes observed among the black. The white colonies consisted of Gram-negative non-motile rods. Re-isolation of sulphate-reducing bacteria from the black colonies yielded a strain giving only limited growth in medium A; growth was restored to its former level when a culture derived from a white colony was added. Some properties of the purified and mixed strains are reported below.

The pure Hildenborough strain of D. desulfuricans consisted of typical motile vibrios with no tendency to pleomorphism. Growth in medium A reached a maximum of c. 350 µg. dry wt. cells/ml., but was nevertheless superior to growth in medium B (c. 100 µg./ml.). The cell density in medium A was increased by greater amounts of glucose and sulphate, a maximum of 500 µg./ml. being reached with glucose (80 µmol./ml.) + Na₂SO₄ (40 µmol./ml.). The cell density was not increased by culture in medium A in which the contaminant had been grown and filtered off. Growth of the purified strain in medium A without peptone and yeast extract was stimulated by a mixture of cysteine, serine, ornithine and isoleucine to the same extent as reported for the impure strain.

The hydrogenase activity of the pure strain was greater than the reported hydrogenase activity of the mixed strains; cell suspensions reduced sulphate in hydrogen with \(-Q_H\) values of c. 700 mm.² H₂/mg. dry wt./hr., compared with values of c. 200 for the mixed strain. Sulphite, thiosulphate and tetrathionate were also reduced; thiosulphate and tetrathionate were attacked more slowly than sulphate or sulphite as reported for the mixed strains (Postgate, 1951 b).

The contaminant was an obligate anaerobic, Gram-negative, non-motile rod, often paired, which formed round or disk-shaped colonies with a perceptible yellowish tint in medium A agar + FeSO₄·(NH₄)₂SO₄·6H₂O. It grew very feebly in liquid medium A and frequently died out on subculture; growth was improved by addition of the filtrate of a pure culture of Hildenborough grown in medium A. These observations suggest that there was some nutri-
tional interdependence between the contaminant and the sulphate-reducing bacteria. The cell densities reached in medium A were too low to be measured accurately in a photo-electric turbidimeter; they were of the order of 30 μg. dry wt./ml. The organism did not grow in medium B.

Table 1. Growth of purified and contaminated Desulphovibrio desulphuricans (Hildenborough) in various media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Strain</th>
<th>Maximum cell density (μg. dry wt. cells/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Impure Hildenborough</td>
<td>780</td>
</tr>
<tr>
<td>A</td>
<td>Purified Hildenborough</td>
<td>270</td>
</tr>
<tr>
<td>A</td>
<td>Contaminant</td>
<td>c. 30</td>
</tr>
<tr>
<td>A</td>
<td>Purified Hildenborough + contaminant</td>
<td>735</td>
</tr>
<tr>
<td>B</td>
<td>Purified Hildenborough</td>
<td>120</td>
</tr>
<tr>
<td>B</td>
<td>Purified Hildenborough + contaminant</td>
<td>110</td>
</tr>
<tr>
<td>A*</td>
<td>Contaminant</td>
<td>90</td>
</tr>
</tbody>
</table>

* Medium A in which Hildenborough had been grown and filtered off.

The mixed strains (Postgate, 1951a) consisted wholly of Gram-negative organisms which appeared as a mixture of motile vibrios, non-motile rods and intermediate forms. The apparent proportion of rods to vibrios varied during growth, vibrios predominating in the stationary phase. The rods were often elongated and bent, and sometimes paired. This heteromorphic appearance was originally attributed to pleomorphism, since plate tests did not indicate any contaminants, but it is now clear that at least some of the rod-forms were contaminants. Cultures in medium A reached about 750 μg. dry wt. cells/ml., but cultures in medium B only reached cell densities similar to the pure strain in medium B. Moreover, cultures of the mixed strain in medium B appeared to consist wholly of vibrios; few, if any, rod forms could be seen. Nevertheless, even after many subcultures in medium B, the contaminant re-appeared immediately on subculture into medium A. The contaminant was also found by this procedure in a stock that had been maintained in Baars’s (1930) medium for many years.

Other strains. Two other stock strains of D. desulphuricans out of six so far tested were found to be contaminated with an organism similar to that found with Hildenborough. Like Hildenborough, these strains had been checked by plating for aerobic and anaerobic contaminants at the time of their isolation or receipt. The El Agheila Z strain, which has been the subject of communications from this laboratory (Adams, Butlin, Hollands & Postgate, 1951; Postgate, 1952b, c) was pure.

DISCUSSION

The heteromorphic appearance of the Hildenborough strain was originally regarded as ‘typical’, and was attributed to the pleomorphism for which the genus Desulphovibrio is notable. In fact its ‘pleomorphism’ was due to the presence of a contaminant which was detectable culturally only in the special conditions described. The contaminant, like those described by Bunker (1989),
stimulated growth of the sulphate-reducing bacteria in medium A, and its own
growth in this medium was improved by sulphate-reducing bacteria. Moreover,
though it did not grow alone in medium B, it nevertheless survived many
subcultures in this medium provided sulphate-reducing bacteria were present.
Clearly there was an effective syntrophism between the two bacteria; this
made detection of the contaminant difficult.

In view of the presence of a contaminant in the Hildenborough strain of
*D. desulphuricans*, certain quantitative data previously reported require
correction. Fortunately the qualitative information given is unaffected.

The cell densities reached in medium A by *D. desulphuricans*, strain Hilden-
borough, are not as great as originally reported (Postgate, 1951a), but are
nevertheless greater than in medium B. The growth-promoting effect of a
mixture of cysteine, serine, ornithine and isoleucine is confirmed with the
purified strain. This paper reported that, on one occasion, the Hildenborough
strain suddenly lost its ability to give massive growth after more than ninety
passages in medium A. This variation was not attributable to any change in
the medium, cultural conditions or morphology of the strain; a new series
from a stock in Baars's (1980) medium behaved 'normally'. In the light of
the present observations it seems likely that the strain was inadvertently
purified on this occasion, but that the consequent change to a more regular
morphological appearance was overlooked. A return to the stock in Baars's
medium gave the mixed population again.

A further paper (Postgate, 1951b) was concerned with the reduction of
sulphur compounds by cell suspensions in hydrogen. Its general conclusions
remain valid since the contaminant did not have hydrogenase activity, but,
owing to dilution by contaminant, the specific activities of the sulphate-
reducing bacteria in cell suspensions used were presumably greater than those
quoted. The relative rates of attack of substrates by the pure strain were the
same as with mixed population. Errors in growth experiments introduced by
the presence of the contaminant cannot have been great or they would have
been noticed in the appropriate controls.

Inhibition of growth and of hydrogen absorption by various sulphate
analogues and other inhibitors was described, and the nature of the inhibitions
studied (Postgate, 1949, 1952a). The conclusions reached in these papers
remain valid, except that the specific cell activities reported in studies with
suspensions were probably too low owing to dilution by the contaminant.

Postgate (1952c), Grossman & Postgate (1958), in the section entitled 'Fundamental
studies of sulphate-reducing bacteria' in *Chemistry Research* 1952 and
publications subsequent to these, referred to the purified strain. Earlier issues
of *Chemistry Research* described work with the impure strain, though many
of these studies used media in which the effect of the contaminant would be
negligible.

The author is indebted to Dr J. Senez for introducing him to the procedure
described as 'rough agar dilutions'. This paper is published by permission of the
Director, Chemical Research Laboratory.
REFERENCES


(Received 19 May 1953)