Infrared Spectra of some Sulphate-Reducing Bacteria

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SUMMARY

The infrared absorption spectra of forty strains of sulphate-reducing bacteria have been recorded over the range 1800–800 cm.⁻¹. It was possible to identify positively *Desulfovibrio gigas* and the thermophilic *Desulfotomaculum nigrificans*. A broad differentiation was possible between salt-water and fresh-water forms of *Desulfovibrio desulfuricans*. *Desulfotomaculum orientis* and *D. ruminis* showed few characteristic differences from the fresh-water strains of *D. desulfuricans* but there was an indication that, with a more refined technique, at least one of the features otherwise characteristic of the other (thermophilic) desulfotomaculum species might be discerned. The results support to a considerable extent current views on the classification of the sulphate-reducers.

INTRODUCTION

A number of workers (e.g. Thomas & Greenstreet, 1954; Riddle *et al.* 1956; Rideal & Adams, 1957; Goulden & Sharpe, 1958; Scopes, 1962) have considered the use of infrared (i.r.) spectroscopy as an aid to the identification of bacterial species or strains. While i.r. spectra of bacteria are very similar from organism to organism, small differences are detectable both in intensities and in the presence or absence of weak absorption bands. The present work was designed to investigate the i.r. spectra of a range of sulphate-reducing bacteria and to determine whether the organisms could be grouped in a manner consistent with their i.r. spectra, regardless of their current taxonomic classification.

METHODS

Choice and preparation of bacteria. Forty strains of sulphate-reducing bacteria were examined, the choice being determined by availability. These are listed below with the NCIB number in brackets.

Desulfovibrio desulfuricans

Group 1. Fresh-water strains: (Saunders, Campbell & Postgate, 1964 and Dr L. L. Campbell, personal communication). Teddington M (8302), Hildenborough (8303), Wandle (8305), Holland D6 (8311), Beckton (8319), Marseilles (8386), Llanelly (8446), Monticello 2 (9442).
Salt-water strain: Walvis Bay (8397).

- Group 2. Fresh-water strains: Essex 6 (8307), Teddington R (8312), Holland C6 (8372), Berre Eau (8387), Berre Sol (8388), 'Vibrio cholanicus' (9467). Salt-water strains: Norway 4 (8310), Venice 1 (8322), Canet 40 (8363), El Agheila Z (8380), Canet 20 (8391), Canet 32 (8392), Canet 41 (8393), Sylt 3 (9335), Aberdovey (9492).
- Group 3. (Salt-requiring) El Agheila C (8308), California 43:63 (8364), Louisiana 43:11 (8365), Avonmouth (8398), British Guiana (8403).

Desulfovibrio gigas

Unnamed strain (9332).

Desulfotomaculum orientis (formerly *Desulfovibrio orientis*) (Campbell & Postgate, 1965)

Singapore I (8382), Singapore II (8445).

Desulfotomaculum nigrificans (formerly *Clostridium nigrificans* thermophilic)

Teddington garden (8351), Staines G (8353), Holland CT (8356), Delft 3T (8359), Delft 15T (8361), Delft 74T (8395), unnamed strain (8788).

Desulfotomaculum ruminis

Coleman 42 (8452).

The cultures were grown from the freeze-dried preparations at 30° or 55° as appropriate in Professor J. R. Postgate's modification of the medium C of Butlin, Adams & Thomas (1949; see Baker, Papiska & Campbell, 1962). NaCl 25 g./l. was added for salt-water forms. In each case, 1 ml. of stock culture (3–5 days old) was used as inoculum for 100 ml. of the above medium which was then incubated at the appropriate temperature under an atmosphere of nitrogen. The organisms were harvested by centrifugation for 7 min. at 16,000g, resuspended in 0.85% NaCl (w/v) and recentrifuged. The washing procedure was repeated 3 times and the final washed organisms suspended in 0.85% (w/v) NaCl sufficient to ensure a concentration of 10 mg. dry wt. organisms/ml. The spectrum was then determined immediately. Experience showed that no substantial differences were observed in the spectra obtained from 1-, 3- or 6-day cultures; 3 days was therefore taken as a standard incubation time.

Measurement of infrared spectra. The spectra were recorded on a Grubb-Parsons GS3 spectrometer. Programmed slits were used and loop gain and scanning speed were kept constant to minimize differences in response. The films were prepared on plates of the Kodak infrared optical material Irtran 2. This has advantages over the silver chloride plates used by earlier workers: a low background adsorption is obtainable and the use of a compensating plate in the reference beam is consequently unnecessary; in addition the material is quite stable and insensitive to light.

The suspensions of bacteria in 0.85% (w/v) aqueous saline were evaporated on the Irtran plates under infrared lamps. The plates were tilted slightly to form a concentration gradient of organisms across the plates. The plate carrying the dried film was mounted in the spectrometer and rotated until the absorption was $90 \pm 2\%$ at 1650 cm.⁻¹ and 5% at 1800 cm.⁻¹.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Typical infrared absorption spectra for thermophilic sulphate reducers, *Desulfofotomaculum nigrificans*. a, Delft 15 T; b, Staines G; c, Teddington garden; d, unnamed; e, Aberdovey (mesophilic).

Fig. 2. Typical i.r. absorption spectra for mesophilic salt-water sulphate reducers *Desulfovibrio desulfuricans*. a, British Guiana; b, El Agheila C; c, Louisiana 43:11; d, El Agheila Z; e, Sylt 3; f, Canet 20; g, Norway 4.

Fig. 3. Typical i.r. absorption spectra for mesophilic, fresh-water sulphate reducers *Desulfovibrio desulfuricans*. a, Hildenborough; b, '*Vibrio cholericus*'; c, Essex 6; d, Becton; e, Berre Eau; f, Singapore I.

Fig. 4. Infrared absorption spectra for some other sulphate reducers. a, *Desulfovibrio gigas*; b, *Desulfofotomaculum orientis* Singapore I; c, *D. orientis* Singapore II; d, *D. ruminis*.

RESULTS

Figures 1-4 show examples of the spectra obtained, grouped according to their points of similarity. The spectra have been spread along the ordinate axis to avoid superposition.

Thermophilic organisms

In the 1350-1200 cm^{-1} region, the spectra of nearly all of the sulphate-reducing bacteria examined had a weak band at 1312 cm^{-1} , followed by a plateau leading into the band of relatively moderate intensity at 1240 cm^{-1} . A second, very weak, band was sometimes detectable at 1285 cm^{-1} . In the present series this band appeared convincingly only in the thermophiles, with the single exception of strain Aberdovey. This organism is a mesophilic salt-water form and we were unable to obtain growth at 55°. *Desulfotomaculum ruminis* and the two examples of *D. orientis* showed a trace of the band at 1285 cm^{-1} .

Mesophilic organisms

Salt-water strains. In all the spectra examined, the intense band at 1650 cm^{-1} had a higher frequency shoulder at about 1735 cm^{-1} . This band varied slightly in frequency (it was at 1745 cm^{-1} for strain British Guiana) but more importantly in intensity. In most of the spectra, it varied between 15 and 25 % absorption. In a small number of cases, it was in the range 25-40 % and in these cases the broad weak band at 940 cm^{-1} also had a significantly higher intensity. The salt-water strains all had this characteristic with the exception of Norway 4. This strain is abnormal in other respects also (Miller & Saleh, 1964), specifically in that it lacks the usual pigment desulfoviridin.

Between the bands at 1650 and 1355 cm^{-1} , the absorption generally fell to between 60 and 65 %. However, in three cases, British Guiana, El Agheila C, and Louisiana 43:11 it fell to only 75-80 %. This particular subgroup of the salt-water strains also exhibits different behaviour to inhibitors (Saleh, 1964): all three strains are members of Group 3 of Saunders *et al.* (1964), and are obligate salt-water forms.

Fresh-water strains. The spectra of more than half of all the organisms examined did not show any of the above characteristics. These were all in the group of mesophilic, fresh-water strains and included the organism '*Vibrio cholinus*' (Hayward & Stadtman, 1959) that was reassigned to the species *Desulfovibrio desulfuricans* by Baker *et al.* (1962).

The three organisms *Desulfotomaculum orientis* strain Singapore I and Singapore II and *D. ruminis* (8452) also fell into this group, except that they gave some slight indication of the very weak band at 1285 cm^{-1} which was otherwise a characteristic of the thermophilic group. This subgroup of organisms have other properties distinguishing them from the 'normal' mesophilic, fresh-water sulphate-reducers, for example, they form spores (Adams & Postgate, 1961). The close resemblance between the i.r. spectra of *D. orientis* and the *D. ruminis* is of particular interest, since, at their original classification, all three were assigned to the same species, *Desulfovibrio orientis*.

The most exceptional sulphate-reducing bacterium is *Desulfovibrio gigas*. In the spectrum of this organism, a quite sharp band appears at 1080 cm^{-1} above the contour of the broad band extending from 1140 to 1000 cm^{-1} . In no other spectrum was this band observed.

CONCLUSIONS

The sulphate-reducing bacteria can be divided into several reasonably well defined groups on the basis of their infrared absorption spectra. The groups correspond broadly with the current taxonomic classification of the organisms except that the members of the species of *Desulfovibrio desulfuricans* fall into two groups according to their marine or fresh-water origin, but without distinguishing between the groups of Saunders *et al.* (1964). *D. gigas* and *Desulfotomaculum nigrificans* are clearly distinguishable from other species. The other desulfotomaculum species are difficult to distinguish from the fresh-water desulfovibrio strains but there is some evidence that this might be possible with refined techniques. 'Cleaner' preparations, i.e. less contaminated with ferrous sulphide, and higher resolution spectroscopy are suggestions for refinement.

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