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An unusual strain of *Desulfovibrio* sp. from an Antarctic lake

(*Desulforibrio* sp.; taxonomy; Antarctica)

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1. SUMMARY

An unusual sulphate-reducing bacterium was isolated from Lake Fryxell, Antarctica. Designated strain FESu, it illustrates the difficulty in assigning some isolates of *Desulfovibrio* spp. to a described species. FESu was characterized by its relatively low mol% G + C (45%), its rod-shaped morphology, inability to grow on lactate, pyruvate, malate or choline in the absence of sulphate, and by its lack of desulfoviridin.

2. INTRODUCTION

The genus *Desulfovibrio* consists of 9 species, some of which are distinguished by only a few easily tested characteristics [1]. Difficulty in assigning newly isolated strains to one of the described species has been reported [2,3]. The problems of dividing spectra of strains into species on testable characteristics is discussed by Gordon [4]. In this communication, an unusual strain of *Desulfovibrio* sp. is reported, which exhibits characteristics which seem to place it between some of the presently described species.

3. MATERIALS AND METHODS

3.1. Source of the organism

Strain FESu was isolated from sediment samples from Lake Fryxell, a permanently stratified lake in Ross Dependency, Antarctica. The samples were collected by one of us (C.G.H.) and stored at 4°C once back in New Zealand until enrichments were initiated.

3.2. Media and growth conditions

Medium PCM was Postgate's medium C [5] with the 0.06 $g \cdot l^{-1}$ $MgSO_4 \cdot 7H_2O$ replaced by 1.0 $g \cdot l^{-1}$ $MgCl_2 \cdot 6H_2O$. Media were reduced by the addition of 0.4 $g \cdot l^{-1}$ $Na_2S \cdot 9H_2O$ (final concentration). All carbon sources were prepared as separately sterilized stock solutions and added to the desired final concentration to medium PCM after the medium was autoclaved. Lactate syrup was purchased from Sigma (St. Louis, MO, U.S.A.). When testing for growth on different carbon sources, the yeast extract concentration in medium PCM was reduced to 0.1 $g \cdot l^{-1}$. Sulphate-free growth was tested using Postgate's medium D [5].

Unless otherwise stated, growth was at 25°C and at pH 7.5 to 7.7.

3.3. Characterization of isolates

Electron microscopy, gas chromatography, and determination of the mol% G + C by its melting temperature were all as described by Patel et al.

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[6]. *Escherichia coli* DNA (Sigma) was used as a reference.

Desulfoviridin was tested for by both the method of Postgate [7] and by checking for a 630 nm absorbance peak in cytoplasmic fractions [8]. Cytochromes were detected by the method of Weston and Knowles [9] using a Uvikon 810 double beam spectrophotometer (Kontron, Zurich, Switzerland). A 0.00–0.02 absorbance scale was used. Cultures were checked for spores under different growth conditions by phase contrast microscopy and by staining using the Schaeffer-Fulton method [10].

4. RESULTS

4.1. Enrichment and isolation

Enrichment on 1 $g \cdot l^{-1}$ succinate yielded a culture of non-motile rods as the dominant organism.

Initial attempts to purify using agar shakes with a medium containing the enrichment substrate failed, but use of PCM medium with 4.5 $g \cdot l^{-1}$ 60% lactate syrup allowed growth. Repeated transfer through agar shake cultures using PCM medium with lactate as the carbon source yielded a pure culture of sulphate-reducing bacteria. This strain was designated FESu.

4.2. Morphology

Strain FESu was a rod-shaped bacterium showing a Gram-negative staining reaction. The cells were 1×1–2 μm in size. In liquid culture, FESu grew as a sheet of cells on the culture vessel surface. Although difficult to disperse in old cultures, regular agitation of the culture vessel broke up clumping of cells to give sufficient homogeneity for either subsequent subculture or for measurement of absorbance. Motility was observed in cultures up to 5–6 days old. After this period, motility was rarely seen. Electron micrographs of motile cells showed that FESu possessed a single polar flagellum. No flagella were observed in old non-motile cultures. Division of FESu was by an uneven fission. No spores were observed.

4.3. Growth conditions

Strain FESu showed a magnesium requirement for growth; no growth occured in its complete absence. The absence of sodium in the growth medium did not inhibit or retard growth. Strain FESu grew over a pH range of 6.6–9.0 (the highest tested) with an optimum at 8.0. The upper temperature for growth was 40–42°C with an optimum at 25°C.

No colonies were observed in agar shakes without paraffin wax seals; sulphate reduction took place only in the reduced depths of such agar shakes. No growth occurred in the upper oxidized parts of the tube.

4.4. Nutritional characteristics

The range of compounds utilized for growth by strain FESu is given in Table 1. Also tested, but not supporting growth were ($g \cdot l^{-1}$); propionate (1), butyrate (0.5), *iso*-butyrate (0.5), 2-methyl butyrate (0.5), 3-methyl butyrate (0.5), hexanoate (0.2), heptanoate (0.2), octanoate (0.2), nonanoate (0.2), decanoate (0.2), dodecanoate (0.2), tetradecanoate (0.2), hexadecanoate (0.2), octadecanoate

Table 1

Compounds utilised by strain FESu for growth with and without sulphate

Compound	Growth of FESu
In the presence of sulphate [a]	
Lactate (3) [b]	++ [c]
Pyruvate (3.5)	++
Malate (1)	−
Acetate (2.5)	−
Formate (1)	−
Succinate (1)	−
Ethanol (1)	+
H_2/CO_2 (80:20 mix)	−
In the absence of sulphate [d]	
Lactate (3)	−
Pyruvate (3.5)	−
Malate (1)	−
Fumarate (1)	−
Choline chloride (1)	−

[a] Tested in medium PCM (see MATERIALS AND METHODS). Other compounds tested but not utilised are given in the text.

[b] Figures in brackets are concentrations in $g \cdot l^{-1}$; organic acids were added as their sodium salts.

[c] ++, Good growth; +, growth; −, no growth.

[d] Tested in medium D (see MATERIALS AND METHODS).

(0.2), fumarate (1), maleate (1), benzoate (1, in the presence of 3 mg · l^{-1} $Na_2SeO_3 \cdot 5H_2O$), glucose (1), cellobiose (1), methanol (1), propanol (1) and butanol (1).

Strain FESu could utilise sulphate, thiosulphate and sulphite as terminal electron acceptors with lactate. Sulphur, malate, fumarate, nitrate and oxygen could not be used as terminal electron acceptors with lactate.

Acetate was produced during growth on lactate with sulphate as the terminal electron acceptor.

4.5. Biochemical characteristics

Redox-difference spectra of membrane fractions of strain FESu showed maxima at 552, 522 and 419 nm. These indicate the presence of cytochrome c_3 [5]. FESu did not possess desulfoviridin.

The mol% G + C of DNA isolated from strain FESu was determined to be 45%. The mol% G + C of the *E. coli* reference DNA was determined to be 50%.

5. DISCUSSION

Oxidation of lactate incompletely to acetate in sulphate-containing medium and the inability to oxidize fatty acids or aromatic compounds places strain FESu in the lactate-utilising sulphate-reducing bacteria, comprising the genera *Desulfovibrio, Desulfotomaculum* and *Desulfomonas.*

Morphology, flagellation, lack of sporulation and lack of cytochrome *b* suggest that FESu is a strain of a *Desulfovibrio* species. The low mol% G + C content excludes it from the genus *Desulfomonas.* Strain FESu has the following in common with *Desulfovibrio baculatus* [1,11]: morphology, flagellation, lack of desulfoviridin, inability to grow without sulphate, and its inability to grow on malate. The features which are different are: FESu lacks cytochrome *b*, can grow on ethanol, has a lower mol% G + C and divides by asymmetrical fission. In many ways, strain FESu behaves like a strain of *Desulfovibrio desulfuricans,* similar to strain Norway 4 [12] which also lacks desulfoviridin. However, the mol% G + C of FESu is some 10% lower than that of strain Norway 4.

Strain FESu illustrates well the difficulty in assigning a newly isolated strain of a sulphate-reducing bacterium to a described species. As there are so few characteristics one can routinely determine, a difference of 2 or 3 characteristics can place an isolate 'in limbo' between the benchmark descriptions of accepted species.

A comparison of the species descriptions given in a recent determinative manual [1] with those given in an older work [13] and an even earlier work [14] show that benchmark descriptions of *Desulfovibrio* species have changed very little over the last 20 years. However, anomalous strains have been reported in the literature over this period [e.g. 2,3,5,12,15–18]. Some reservations on the present system have been expressed by Postgate [1,5]. Species descriptions based on a greater number of strains seem desirable. This may eventually entail amalgamation of existing species within the genus *Desulfoviribo* and possibly formation of new species. The criteria for assigning strains to described species need to be clarified.

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