SHORT COMMUNICATION

Sporulation and Cell Wall Structure of *Clostridium polysaccharolyticum* comb.nov. (Formerly *Fusobacterium polysaccharolyticum*)

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A bacterium named *Fusobacterium polysaccharolyticum* in an earlier paper produced spores and was reclassified as a clostridium. Although it appeared Gram-negative when stained, its cell wall structure was of the Gram-positive type.

INTRODUCTION

In earlier work (Van Gylswyk, 1980) *Fusobacterium polysaccharolyticum* sp.nov. was described as a Gram-negative, non-sporulating anaerobic rod from the sheep rumen that produced butyrate, fermented cellulose and starch, and showed a preference for polysaccharides. This paper reports the ability of this bacterium to produce spores and the Gram-positive characteristics of its cell wall.

METHODS

Bacteria. These were isolates B, E and G described previously (Van Gylswyk, 1980).

Medium. Spores were observed in the growth from slants of medium similar to that used for maintenance slants in the earlier study (Van Gylswyk, 1980) except that ball-milled Whatman no. 1 filter paper cellulose (12 g l⁻¹) replaced cellobiose and Difco Casitone was omitted. This medium was also used to grow bacteria for examination of their cell wall structure.

Examination of cell wall structure. Bacteria were fixed for 1 h in a solution containing glutaraldehyde (3.6 %, w/v), ruthenium red (0.15 %, w/v) and sucrose (4 %, w/v) in cacodylate buffer (0.1 M, pH 7.3) and then washed twice in cacodylate buffer (0.1 M). Post-fixation was done in osmium tetroxide solution (1 %, w/v) at 4 °C before washing once again with the cacodylate buffer. The bacteria were then dehydrated by passage through a graded acetone series followed by two rinses in propylene oxide and embedded in Epon 812. Thin sections were stained with uranyl acetate (30 min) and lead citrate (3 min) before examination in a Siemens Elmiskop 102 microscope at 80 kV.

RESULTS AND DISCUSSION

Isolates B and E produced spores, one per cell, on the cellulose/agar medium. To date, no spores of isolate G have been found. Spores of isolate B are shown in Fig. 1(a); those of isolate E were similar. Sporulation appeared to be preceded by thickening at one end of a cell as evidenced by the presence of plectridial forms. Spores either possessed or did not possess a spore cap and could therefore be either terminal or subterminal. They were

Bar markers represent 10 μm in (a), 250 nm in (b) and (d), and 100 nm in (c).

Fig. 1. *Clostridium polysaccharolyticum* (*Fusobacterium polysaccharolyticum*).

(a) Isolate B. Gram-stained cells with spores.

(b) Isolate B showing Gram-positive cell wall structure. (Transmission electron micrograph).

(c) Isolate B. Part of (b) enlarged to show details of cell wall structure: cytoplasmic membrane (M); densely stained cell wall (W); additional surface layer (S). (Transmission electron micrograph).

(d) Isolate G. Old cell with cell wall structure similar to that of isolate B. (Transmission electron micrograph).

generally oval but occasionally spherical shapes were observed. The mean dimensions of the spores were $1.2 \times 0.8 \mu\text{m}$. Sporulation was previously not observed in liquid media or in solid media containing Difco Casitone (Van Gylswyk, 1980). It is, however, considered premature to ascribe sporulation to particular conditions.

The cell wall structures of isolates B and G were similar (Fig. 1*b, c, d*) despite some minor physiological differences such as the ability to produce extracellular slime (Van Gylswyk, 1980). Although the cytoplasmic membrane was generally clearly resolved, no trilamellar membrane structure, characteristic of Gram-negative bacteria (Costerton *et al.*, 1974), was visible in the surface layer outside the densely stained peptidoglycan layer. The well-developed peptidoglycan layer (14 to 16 nm thick) suggests a Gram-positive type of cell wall. The thickness of the wall is similar to that of *Clostridium perfringens* (Glauert & Thornley, 1969) but less than the normal thickness (30 to 50 nm) of Gram-positive cell walls (Cheng & Costerton, 1977). It has been suggested (Cheng & Costerton, 1977) that a minimum thickness of Gram-positive wall may be required before the stain is retained. These bacteria thus appear to resemble both *Butyrivibrio fibrisolvens* (Cheng & Costerton, 1977) and *Lachnospira multiparus* (Cheng *et al.*, 1979) in that they appear Gram-negative when stained but possess a Gram-positive type of wall morphology.

Bacteria morphologically resembling those described have again been found in high dilutions (10^{-7}) when making counts of cellulolytic bacteria of rumen contents from several sheep fed maize straw diets. The clearings produced in roll bottles containing cellulose/rumen fluid/agar medium often became very large after incubation for 3 or 4 d, clearing more than half of the Astell roll bottles. The edges of the clearings were irregular. No distinct colonies were apparent but on careful examination a band of thin growth could be seen about 5 or 6 mm from the edge of the clearings on the surface of the agar. Viable bacteria could be isolated from this region although partly lysed cells were abundant. It appeared as if the bacteria moved rapidly across the surface of the agar (they were earlier found to possess flagella; Van Gylswyk, 1980) and that they produced readily diffusible cellulases. It is possible that the ability to produce discrete colonies, reported previously, is induced by carrying cultures on cellobiose/rumen fluid/agar medium.

The bacteria lysed readily after an initial period of rapid growth. This often resulted in sterile inocula or long lag periods due to low numbers of viable cells. Sporulation did not always occur or, if it did, the number of spores was often low.

It is suggested that the name of the bacterium be changed to *Clostridium polysaccharolyticum* comb.nov. as it produces spores. It is assumed that isolate G also belongs to this species although spores have not yet been found. No record has as yet been found of a clostridium similar in characteristics to this bacterium both on the basis of G + C mol % (41.6; Van Gylswyk, 1980) and other characteristics (Buchanan & Gibbons, 1974; Van Gylswyk, 1980).

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