SEPARATION OF CAPSULATE AND NON-CAPSULATE *BACTEROIDES FRAGILIS* ON A DISCONTINUOUS DENSITY GRADIENT

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SUMMARY. A discontinuous density gradient procedure was used successfully to separate capsulate and non-capsulate cells of strains of *Bacteroides fragilis*. Bacteria from the capsulate fraction retained their capsules on subculture, but non-capsulate cells reverted to the mixed phenotype.

INTRODUCTION

Considerable emphasis has been placed on the importance of the polysaccharide capsule of *Bacteroides fragilis* as a virulence determinant (Kasper *et al.*, 1977). In studies with rats, the polysaccharide capsule has been shown to promote the formation of intra-abdominal abscesses similar to those found in human infection (Onderdonk *et al.*, 1977).

A homogeneous population of bacteria is necessary to investigate the selective advantage of the capsule in pathogenesis. Variation in the proportion of capsulate and non-capsulate cells within individual *Bacteroides* cultures (Babb and Cummins, 1978) and instability of capsule production by *B. thetaiotaomicron* (Burt *et al.*, 1978) have been reported. Booth *et al.* (1979) reported that capsulate *B. fragilis* were resistant to bacteriophage infection and suggested that this might be used to obtain a capsulate population. Percoll density gradients have been used for the separation of prokaryotic cells, for example, to synchronise cell division (Dwek, Kobrin and Grossman, 1980) and to separate *Escherichia coli* possessing colonisation factor antigen 1 (Giesa *et al.*, 1982). In this paper we report the use of a discontinuous Percoll density gradient to separate capsulate and non-capsulate *B. fragilis*.

MATERIALS AND METHODS

Bacterial strains. *B. fragilis* NCTC9343 was supplied by the Department of Bacteriology, University of Edinburgh Medical School and *B. fragilis* ATCC23745 by the American Type Culture Collection, Rockville, MD. *B. fragilis* NCTC10584 was a departmental stock culture.

Culture methods. Bacteria were grown in defined broth (van Tassell and Wilkins, 1978), the basal medium of Deacon, Duerden and Holbrook (1978) or on plates of lysed human-blood agar (LHBA). Cultures were incubated at 37°C in an atmosphere of 90% H₂ and 10% CO₂ in anaerobic jars with two catalyst sachets per jar.

Microscopy. The presence of capsules was determined by light microscopy with Indian ink and eosin-carbol fuchsin negative staining (Cruickshank, 1965). The proportion of capsulate cells was determined by random field counts of eosin-carbol fuchsin stained preparations under a × 100 oil-immersion objective. All determinations were done with late exponential-phase cultures containing approximately 10⁸ cfu/ml.

Density gradient centrifugation. Percoll (a colloidal sol of polyvinylpyrrolidone-coated silica particles; Pharmacia Fine Chemicals Inc., London) is supplied sterile. A stock solution, isosmotic with physiological saline, was prepared by diluting with 1.5 M NaCl in a ratio of 9:1. The pH of this solution was adjusted to 7.0 with 1 M HCl. Solutions of 20, 40, 60 and 80% Percoll were prepared by further dilution with 0.15 M NaCl.

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A 2-ml volume of each of these solutions was layered into a test tube to produce a step gradient with 80% Percoll at the bottom and the 20% solution at the top. A broth culture of the test organism (2.5 ml) was applied to the top of the 20% layer and the gradient centrifuged at 2600 *g* for 20 min in a bench centrifuge. After centrifugation the bands were removed with sterile 2-ml syringes fitted with long needles. Bacteria from the gradients were subcultured without further processing.

RESULTS

Approx. 17% of the cells in the *B. fragilis* ATCC23745 culture produced capsules greater than the width of the cell in size when grown in the defined medium of van Tassel and Wilkins (1978). Capsules of the same dimension were observed in ruthenium red stained preparations by electron microscopy. Fewer than 1% of the cells of *B. fragilis* strains NCTC10584 and NCTC9343 produced capsules of similar size when grown under the same conditions. Capsules produced in the basal medium or on LHBA plates were smaller and difficult to assess because the eosin-carbol fuchsin stain tended to shrink from the edge of the cell to produce a bright halo similar to that of a small capsule.

After centrifugation of cultures in defined medium on a Percoll density gradient, cells with

FIGURE. Photomicrographs of *Bacteroides fragilis* ATCC23745 grown in defined medium: a, culture before density gradient centrifugation; b, cells harvested from top of Percoll gradient after centrifugation; c, cells harvested from interface between 60% and 80% Percoll layers after centrifugation; C = capsulate cell; N = non-capsulate cell.

large capsules remained on top of the 20% layer and non-capsulate cells were concentrated at the 60–80% interface (figure). Most of the cells of *B. fragilis* strains ATCC23745 and NCTC10584 were found at either the 0–20% or the 60–80% interface. However, *B. fragilis* NCTC9343 produced additional bands at the 20–40% and 40–60% interfaces. These bands were formed by bacteria bearing capsules of graded sizes.

In subcultures of bacteria from the top layer of the density gradient, 90% of the cells of *B. fragilis* NCTC10584 and 99% of the cells of *B. fragilis* strains ATCC23745 and NCTC9343 were capsulate. Further subcultures of *B. fragilis* ATCC23745 were examined. Capsulate bacteria represented 95–100% of the total number of cells for the first three subcultures, after which the proportion of capsulate cells gradually diminished. Subculture of the 'non-capsulate' fractions resulted in an immediate return to the initial distribution of capsulate and non-capsulate cells.

DISCUSSION

The method described produced homogeneous suspensions of cells with and without capsules. The capsule fraction of *B. fragilis* strains ATCC23745 and NCTC9343 remained capsulate after subculture into defined broth and could be used in further studies. A proportion of cells of the non-capsulate fraction produced capsules when subcultured. This instability restricts the use of the non-capsulate fraction to investigations in aerobic conditions. In these conditions growth and biosynthesis of the capsule are prevented although viability of the phenotypically non-capsulate population is maintained. This useful technique has allowed us to proceed with in-vitro studies on the interaction of capsulate and non-capsulate *B. fragilis* with granulocytes and serum.

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