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NOTES

Encapsulation of *Bacteroides* Species

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Capsules were detected by the India ink method in cultures of *Bacteroides fragilis*, *B. vulgatus*, *B. thetaiotaomicron*, and *B. ovatus*. No capsules were found in the five strains of *B. distasonis* examined.

The *Bacteroides fragilis* group of anaerobes has been the subject of intense research in recent years because its members are frequently encountered in clinical specimens and are part of the intestinal microflora of most persons (9, 11). That *B. fragilis* is associated with clinical specimens more frequently than the other related species implies the existence of particular virulence factors or properties that are associated with pathogenicity. Recently, a group of investigators reported that the possession of a capsule is a unique factor associated with *B. fragilis* and contributes to the pathogenic potential of this strain (8, 10). In the course of investigations on the serological properties of the *B. fragilis* group of organisms, we observed that many of the cultures were encapsulated and that the presence of capsules was not confined to strains of *B. fragilis*. These results have been previously referred to briefly (J. L. Babb and C. S. Cummins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, M215, p. 102). They are presented here again with photomicrographs showing representative results on six strains.

The *Bacteroides* isolates examined were from clinical specimens as well as normal flora and represented five species as defined by their deoxyribonucleic acid homology values (2; J. L. Johnson, personal communication) and phenotypic characteristics (4). Pre-reduced media and anaerobic culture techniques, as outlined in the VPI Anaerobe Laboratory Manual (5), were used. Biochemical characterization of all strains was conducted periodically throughout this investigation to assess purity. Encapsulation of cells grown in broth or on solid media was demonstrated by the India ink wet-mount technique of Duguid (3). In examining these preparations, it is important to use a phase-contrast system, since this enables the bacterial cell to be clearly visualized within the capsule.

When grown at 37°C for 18 h in Trypticase-yeast extract-glucose (TYG) broth medium, most *Bacteroides* strains belonging to the species *B. fragilis*, *B. vulgatus*, *B. thetaiotaomicron*, and *B. ovatus* displayed some degree of encapsulation. Five *B. distasonis* strains examined in this study were not encapsulated. The proportion of encapsulated cells varied among strains but represented approximately 10% or less of the total cell number for most isolates, as estimated from the India ink wet mounts. A few strains, such as *B. ovatus* VPI 0038, were entirely encapsulated.

On solid media, no difference in colonial morphology relative to encapsulation was observed. Furthermore, microscopic examination of isolated colonies revealed both encapsulated and non-encapsulated cells in proportions similar to those observed in broth.

The size of the capsule varied among strains and within a single cell suspension. Although most were one-half to one times the cell diameter, capsules ranging up to four times the cell diameter were observed (Fig. 1). Generally, the capsules were well defined and appeared rigid. However, a few strains possessed copious slime-like layers and could be easily identified by their viscous broth cultures. In general, the capsules were suprisingly resistant to disintegration, especially in Formalin-killed suspensions, which still showed good capsulation even after months of storage at 4°C.

Selected *Bacteroides* strains were grown under different growth conditions to assess the effect on encapsulation. The effect of these parameters was determined by comparing India ink wet mounts of cell suspensions grown under the experimental conditions to those grown in normal TYG medium. Capsule production was not found to vary with culture age, incubation temperature, pH, or several organic and inorganic nutrients (Table 1). However, when strains were grown in a simple glucose-salts medium, or

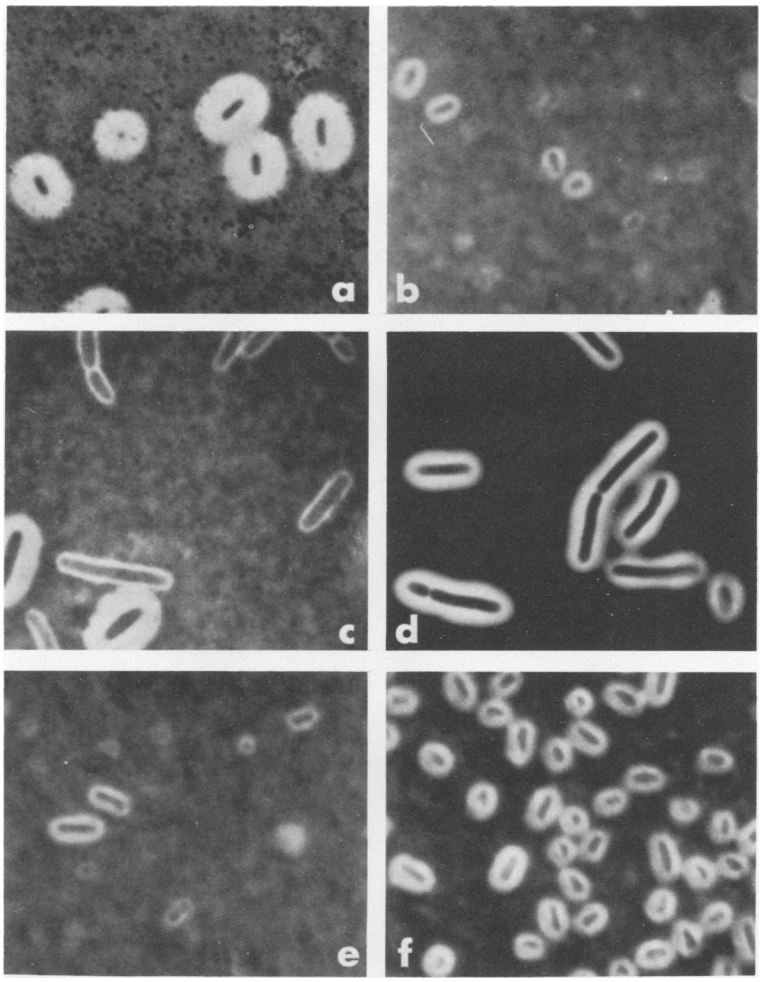

FIG. 1. *Capsulated cells of some Bacteroides species. (a) B. fragilis 2553, ×1,000; (b) B. fragilis 2392, ×1,000; (c) B. vulgatus C7-2, ×1,200; (d) B. vulgatus 4245, ×1,200; (e) B. thetaiotaomicron 5482, ×1,000; (f) B. ovatus 0038, ×1,000. All organisms were grown in TYG medium.*

in media containing one-fourth the normal concentration of yeast extract present in TYG, the proportion of encapsulated cells was slightly elevated to approximately 30% of the total cell number.

The observation that most members of the *B. fragilis* group of miroorganisms displayed some degree of encapsulation is similar to that made earlier by Beerens et al. in their description of the genus *Eggerthella* (*Bacteroides*) (1). Although the reason(s) for the small proportion of encapsulated cells in most cultures is unknown, the results of the present investigation suggest that certain growth conditions influence capsule production.

The relationship between the capsule and virulence is unclear. Onderdonk and co-workers documented a correlation between the presence of a capsule and virulence (10). The presence of a capsule has also been reported to be associated with pathogenicity in a strain of *B. melaninogenicus* (12). Many of the strains examined in the present study had been tested for virulence in an animal model (13). Although the cultures in these virulence studies were not grown under the same conditions as those reported in this investigation, the variability in pathogenicity among strains could not be attributed to the degree of encapsulation displayed by these organisms. *B. distasonis* VPI 4243, an isolate that was not encapsulated, infected 98% of the mice challenged. Alternatively, *B. thetaiotaomicron* VPI 5482 displayed some degree of encapsulation, yet infected only 36% of the mice tested.

Additional studies on the *B. fragilis* capsule have demonstrated that this material is serolog-

TABLE 1. *Factors affecting capsule production of Bacteroides species*[a]

Parameter	Effect on capsulation
Carbohydrate substrate:[b]	
Glucose, 2%	None
Ribose	None
Xylose	None
Cellobiose	None
Mannose	None
Fructose	None
Inorganic nutrients:[c]	
Ca^{2+}, 1 M	None
$MgSO_4$, 10^{-3} M	None
$MnCl_2$, 10^{-3} M	None
$MgSO_4$ % $MnCl_2$, 10^{-3} M	None
Bicarbonate, 10%	None
Rabbit serum supplement[c]	
1%, 5%, 10% (vol/vol)	None
Medium:	
PYG[e]	None
Modified GMB[d]	Slight stimulation (up to 30%)
TYG, 0.25% yeast extract	Slight stimulation (up to 30%)
PYG,[e] 0.25% yeast extract	Slight stimulation (up to 30%)

[a] *Bacteroides* strains examined were *B. fragilis* VPI 2393, 2553, 3390, 3277, 4076, 4255, 6805, *B. distasonis* 4243, *B. vulgatus* 4245, 2277, C7-2, OC-13, and *B. thetaiotamicron* 5482, 2808B, 8651.

[b] TYG medium with 1% specified carbohydrate substrate replacing 1% glucose.

[c] TYG medium plus stated nutrient.

[d] Hemin and vitamin K added, see reference 5.

[e] PYG, Peptone-yeast extract-glucose, see reference 5.

ically active (7, 8; J. L. Babb, Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, 1977) and contains chemical constituents similar to those found in the cell wall polysaccharides (6, 7; Babb and Cummins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, M215, p. 102; Babb, Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, 1977). Therefore, the results of the present investigation suggest that the chemical, serological, and possibly the biological properties of *Bacteroides* strains that have the potential to form capsules may be altered by certain growth conditions.

Our results do not agree with those of previous investigators (6–8, 10) in that we have found that most strains of *Bacteroides*, except those of *B. distasonis*, show capsulated cells in culture. Some of the differences are due to differing ideas as to what constitutes a capsule. We have used the term in the classical sense to refer to structures easily visible in the light microscope that may be up to three or four times the width of the bacterial cell in wet-mount preparations (Fig. 1).

The material shown in sections of ruthenium red preparations (e.g., Fig. 2 of reference 7) seems to be in the form of a thin layer, about 0.2 μm wide, applied to the outer surface of the cell wall. Even allowing for shrinkage during fixation and section cutting, it seems unlikely that the capsules seen in wet india ink preparations, which may be up to four times the cell diameter, could contract to give this appearance. It seems likely therefore that the two methods are visualizing different materials, and it might be preferable to call the ruthenium red-positive material a "micro-capsule" or a "surface-layer."

However, it is obvious that a structure of either kind may be important in determining or modifying pathogenicity if it forms the effective surface of the cell and thereby governs interaction of the parasite with the host.

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