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Capsular Polysaccharides and Lipopolysaccharides from Two *Bacteroides fragilis* Reference Strains: Chemical and Immunochemical Characterization

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Fermentor growth of *Bacteroides fragilis* under controlled conditions in a complex medium containing 1% glucose and 10% fetal calf serum resulted in high yields of bacteria. After hot phenol-water extraction of the organisms, capsular polysaccharide was isolated from the aqueous phase and purified by Sephacryl S-300 chromatography in a buffer with 3% sodium deoxycholate. Lipopolysaccharide was isolated by phenol-chloroform-light petroleum ether extraction. The capsular polysaccharide from *B. fragilis* strain NCTC 9343 contained six sugars: L-fucose, D-galactose, D- and L-quinovosamine, D-glucosamine, and galacturonic acid. The capsule of strain ATCC 23745 also contained D-glucose, L-fucosamine, L-rhamnosamine, and a 3-amino-3,6-dideoxyhexose but lacked D-quinovosamine. The latter capsule also contained alanine (4%). The capsular polysaccharides were different immunochemically by ELISA inhibition. The lipopolysaccharide of both strains contained the same sugars (L-rhamnose, D-glucose, D-galactose, and D-glucosamine) and fatty acids (13-methyl-tetradecanoic and 3-hydroxy-hexadecanoic and 3-hydroxy-15 methyl-hexadecanoic as major constituents) and were identical by ELISA inhibition.

Bacterioides fragilis is the predominant organism isolated from cases of intraabdominal sepsis and is the most common blood culture isolate in patients with anaerobic bacteremia (5, 21). This contrasts with the composition of the normal colonic flora, in which this organism is outnumbered by other *Bacteroides* species such as *B. vulgatus* and *B. thetaiotaomicron* (20). *B. fragilis* is an organism which lives symbiotically with the host and can cause serious infection only under certain circumstances. Such circumstances include (i) fecal contamination of the peritoneum, (ii) abscesses in any of several organ systems, and (iii) soft tissue infections (5). This clinical information suggested that a search for a virulence factor would be of value.

Studies during the 1970s detected two interesting surface polysaccharides (10, 11, 16). One component was a capsular antigen, reported to be unique for *B. fragilis*, and the other was a lipopolysaccharide (LPS) which was chemically different from endotoxins of aerobic gram-negative bacteria and biologically impotent (6, 10). The capsular polysaccharide had been shown to be an important virulence factor in the pathogenesis of experimental abscesses (13, 22). The LPS demonstrated some of the same virulence properties at higher doses, but incomplete separation of the two antigens was thought to be a possible explanation for this phenomenon.

The chemical composition and the structures of the capsular polysaccharide and LPS from *B. fragilis* have been investigated, but results have varied (6, 10, 11, 14). One reason for disparate results has been difficulty in obtaining sufficient quantities of purified antigen for chemical analysis and structural studies. Another reason is the decrease in the quantity of extractable capsular polysaccharide with serial in vitro passage of *B. fragilis* (14), although it has been shown that animal passage maintains capsule production. The final difficulty in chemical analyses has been inability to determine the degree of separation of capsule from LPS. This was in part because of the lack of any of the readily identifiable carbohydrates unique to aerobic bacterial endotoxins in the *B. fragilis* LPS (6, 10).

In the present communication, we describe growth conditions for *B. fragilis* resulting in both high yields of the organism and abundant production of capsular polysaccharide. Extraction and separation procedures for obtaining capsu-

lar polysaccharide and LPS in essentially pure form are reported, as are qualitative and quantitative chemical and immunochemical analyses.

MATERIALS AND METHODS

Bacterial strains. *B. fragilis* ATCC 23745 was from the American Type Culture Collection, Rockville, Md. *B. fragilis* NCTC 9343 was from the National Collection of Type Cultures, London, England. Samples of both strains were maintained in peptone-yeast broth at −70°C. To enhance production of the capsular polysaccharide and minimize glycogen content in extracts (15), both strains were serially passed five times through mouse spleens. Initially, 10^8 organisms were inoculated intraperitoneally into a mouse and recovered 24 h later by dispersing the spleen. The organisms were then inoculated intraperitoneally into another mouse, and the recovery process was repeated. Frozen cultures were thawed, inoculated onto blood agar plates, and incubated in a anaerobic jar (GasPak; BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 24 h. All colony variants were identified as *B. fragilis* according to established procedures (7).

Media and growth conditions. The basal medium containing 5 g of yeast extract, 20 g of proteose peptone, 5 g of NaCl, and 0.55 g of cysteine-hydrochloride per 1,000 ml was prepared in a prereduced state, dispensed in a 12-liter fermentor (Ultroferm 1601, LKB Produkter Bromma, Sweden), and autoclaved at 121°C for 45 min. Subsequently, a 20% (wt/vol) glucose solution, sterilized by membrane filtration (Millipore 0.22 μm), was added to give a final concentration of 1% (wt/vol). To increase the yield of organisms, filter-sterilized fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) was added to a 10% (vol/vol) final concentration. Overnight cultures (100 ml) of the *B. fragilis* strains were grown in the same medium and were used to inoculate the fermentor. The organisms were then grown to late logarithmic phase at 37°C, with the pH maintained at 7.0. Nitrogen gas supplemented with 3% hydrogen and 5% carbon dioxide was bubbled through the medium during growth. All cultures were checked for purity at the end of the growth cycle under both aerobic and anaerobic conditions. After incubation, the organisms were collected by centrifugation (8,000 × *g*, 4°C, 20 min) and washed with 0.15 M NaCl.

Extraction and purification of the capsular polysaccharides. Pelleted organisms were suspended in water and extracted with phenol-water (29). The aqueous phase was subsequently dialyzed for 4 days against tap water and overnight against distilled water. After lyophilization, the aqueous phase from the phenol-water extract was suspended in a buffer containing 3% sodium deoxycholate, pH 9.5 (0.05 M glycine–0.001 M EDTA–3% sodium deoxycholate). The pH was first raised with 5 M sodium hydroxide to approximately 11.5, and the solution was back titrated with 4 M HCl to pH 9.5. The portion of the crude water phase that was not solubilized by this procedure was removed by centrifugation (5,000 × *g*, 4°C, 15 min), and 20 ml of the clear supernatant was applied to a column of Sephacryl S-300 (5.0 by 90 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated in the buffer described above. The effluent was monitored by a differential refractometer (R403; Waters Associates, Inc., Milford, Mass.). All fractions were also scanned for UV light absorption at 260 nm. The fractions comprising each peak were pooled and precipitated with 4 volumes of ethanol at −20°C. The precipitates collected by centrifugation (5,000 × *g*, 4°C, 20 min) were solubilized in water, dialyzed against distilled water overnight, and lyophilized. All peaks that were contaminated with nucleic acids were treated with RNase and DNase as described earlier (18), dialyzed against distilled water, lyophilized, and used for chemical and immunochemical analyses.

Extraction of the LPS. The LPSs were extracted with phenol-chloroform-light petroleum ether (PCP) (4) from the lyophilized aqueous phase after phenol-water extraction. The LPSs were precipitated out with distilled water from the phenol phase after PCP extraction. The precipitates were washed once with 80% aqueous phenol (wt.vol) and four times with ether. The LPSs were dried, dissolved in distilled water, dialyzed against distilled water for 3 days at 4°C, and lyophilized. The LPS preparations were then chemically and immunochemically analyzed.

Chemical methods. Protein content was determined by the method of Lowry et al. (19), with bovine serum albumin as standard. Amino acids and hexosamines were quanititated by using a Durrum D-500 amino acid analyzer (Dionex, Inc., Sunnyvale, Calif.). The presence of nucleic acids was determined as described earlier (18). Analyses of the monosaccharide constituents were carried out by gas-liquid chromatography (GLC) after hydrolysis in 0.5 M trifluoroacetic acid at 100°C for 18 h, essentially as described by Sawardeker et al. (24). The alditol acetates were analyzed on an SP 1000 wall-coated capillary column (25 m by 0.25 mm), using a Hewlett-Packard 5830A instrument equipped with a flame ionization detector at a 230°C oven temperature, and on 3% OV 225 on Gas-Chrom Q at 190°C, using a Perkin-Elmer 990 instrument with a flame ionization detector. All sugar residues were identified either by cochromatography with authentic standards or with GLC–mass spectrometry of the alditol acetates, using a Varian MAT 311 GLC–mass spectrometer fitted with an OV 225 or SP 1000 column.

The carboxyl groups of uronic acids in the capsular polysaccharides were reduced by the method of Taylor and Conrad (27). The absolute configuration of monosaccharides was determined by GLC of the acetylated (+)-2-octyl-glycosides, as described by Leontein et al. (17).

The ^{13}C nuclear magnetic resonance spectra of the capsular polysaccharides were recorded for solutions in D_2O at 85°C, using a Bruker WT-200 instrument.

The fatty acids were estimated as methyl esters after methanolysis as described by Wollenweber et al. (31) on an SE-30 wall-coated capillary column (25 m by 0.25 mm), using a Hewlett-Packard 5830a instrument at 170°C.

Serological methods. Rabbit antisera against the *B. fragilis* capsular polysaccharides and LPSs were prepared as described previously (11). The enzyme-linked immunosorbent assay (ELISA) was performed as described earlier (18). In ELISA inhibition experiments, serum was preincubated for 1 h with different concentrations of the capsular and LPS preparations assayed. The 50% inhibitory value was recorded as the concentration of inhibitor needed to obtain a 50% lowering of the optical density at 400 nm as compared with control

tubes with no inhibitor added. Double diffusion in agar was done as previously described (11).

RESULTS

The batch culturing in a fermentor of mouse-passed starting cultures of the two *B. fragilis* strains NCTC 9343 and ATCC 23745, under constant conditions in a rich medium supplemented with 1% glucose and 10% fetal calf serum, resulted in recoveries of approximately 3 g of bacteria (dry weight) per liter of culture fluid. Under these conditions, an abundant production of capsular polysaccharide took place. The yield of bacteria and capsular polysaccharide was up to 10 times higher than that obtained when culturing was done in flasks without fetal calf serum.

Pelleted bacteria were extracted with hot phenol-water, and the aqueous phase was lyophilized. The material in the aqueous phase was subsequently chromatographed on Sephacryl S-300, using a 3% sodium deoxycholate buffer (Fig. 1). Three peaks (A, B, and C) were seen with the 9343 extract, and two peaks (A^1 and B^1) were seen with the 23745 extract, using a refractometer to monitor the effluents. All fractions were also sanned for UV light absorption at 260 nm. Peaks A and C from the 9343 extract and peak B^1 from the 23745 extract were heavily contaminated with nucleic acids. The fractions comprising each peak were pooled and precipitated with cold ethanol. The precipitates were collected, dissolved in water, dialyzed against distilled water overnight, and lyophilized. Nucleic acids present in the fractions were removed by nuclease treatment followed by dialysis. The high-molecular-weight material in peak B from the 9343 and in peak A^1 from the 23745 extract will be referred to as the polysaccharide capsules (for analyses, see below). The yield of the capsular polysaccharide extracted and purified by this method was 35% for the 9343 antigen and 48% for the 23745 antigen (calculated from the crude aqueous phase material).

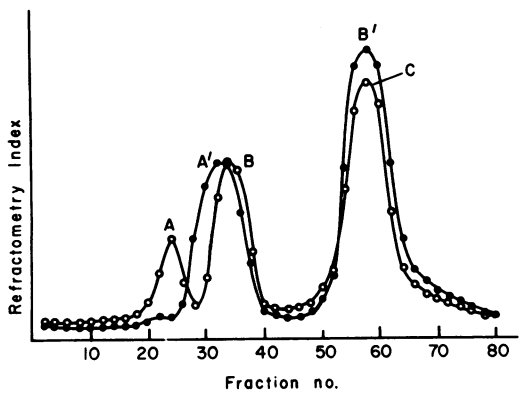

FIG. 1. Elution pattern on Sephacryl S-300 in 3% sodium deoxycholate buffer of crude phenol-water extracts from *B. fragilis* NCTC 9343 (○) and ATCC 23745 (●).

Analyses of peaks C and B^1 from the chromatography on Sephacryl S-300 with 3% sodium deoxycholate showed that these fractions also contained LPS (see below). However, pure LPS could be isolated directly from the lyophilized water phase of the crude phenol-water extract by PCP extraction. The washed, dialyzed, and lyophilized LPSs corresponded to about 10% of the starting material.

Chemical analyses of peak A revealed that this peak contained the capsular polysaccharide together with LPS which had not disaggregated. Additionally, there was nucleic acid present in this peak. The material in this pooled fraction was not studied further.

Chemical analyses. The purified capsular and PCP-extracted LPS fractions from *B. fragilis* NCTC 9343 and ATCC 23745 did not contain any detectable amount of nucleic acids (less than 5 μg/mg) as assayed by UV spectra. Neither was any protein demonstrated (less than 5 μg/mg) when the method of Lowry et al. (19) was used.

Amino acid analyses of the capsular polysaccharide and LPS preparations showed that the capsular polysaccharide from the 9343 strain and LPS from both strains contained only trace amounts (less than 10 μg/mg) of various amino acids. The capsular polysaccharide from 23745, however, contained a significant amount (38 μg/mg) of a single amino acid, alanine.

The fatty acids found in the capsular and LPS preparations are listed in Table 1. The fatty acid compositions of the two LPS were identical, and 13-methyl-tetradecanoic, 3-hydroxy-pentadecanoic, 3-hydroxy-hexadecanoic, and 3-hydroxy-15-methyl-hexadecanoic acids were found. In total, the LPS from 9343 contained 29% (wt) fatty acids. The corresponding figure for LPS isolated from the 23745 strain was 27% (wt). The presence of trace amounts of 3-hydroxy-pentadecanoic, 3-hydroxy-hexadecanoic, and 3-hydroxy-15-methyl-hexacedanoic acids (the predominant fatty acids in the LPS) in the capsular polysaccharides from both *B. fragilis* strains indicated that the preparations were still contaminated with approximately 1% LPS. However, some of these fatty acids may have been part of the polysaccharides themselves.

GLC analyses of the monosaccharides of the two LPS preparations demonstrated that both contained the same sugars in the same quantities: L-rhamnose, D-galactose, D-glucose, and D-glucosamine (Table 2).

The sugars found in capsular polysaccharides from the two strains are listed in Table 2. The 9343 capsule contained L-fucose, D-galactose, D-

TABLE 1. Quantitative analyses of total fatty acids present in capsular and LPS preparations from *B. fragilis* NCTC 9343 and ATCC 23745

Fatty acid methyl ester	Amt of fatty acids (μg per mg of sample)			
	9343		23745	
	Capsule	LPS	Capsule	LPS
12:0	<0.1	1.3	<0.1	0.9
14:0	<0.1	2.3	<0.1	1.8
13-Me-14:0	<0.1	41.0	0.7	37.5
12-Me-14:0	<0.1	2.7	<0.1	2.4
15:0	<0.1	5.1	<0.1	3.9
16:0	<0.1	4.4	<0.1	3.3
3-OH-14:0	<0.1	5.6	<0.1	4.2
3-OH-15:0	3.9	45.4	3.6	43.1
3-OH-16:0	5.4	102.3	4.6	97.3
3-OH-15-Me-16:0	3.1	69.2	<0.1	66.4
3-OH-17:0	<0.1	12.3	0.1	11.2

and L-quinovosamine, and D-glucosamine. After carboxyl reduction of the polysaccharide, an increase in the amount of galactose was observed. This finding strongly suggests that the original polysaccharide contained a significant amount of galacturonic acid.

The analysis of alditol acetates of the *B. fragilis* 23745 capsule showed an even more complex array of monosaccharides (Table 2). The polysaccharide contained L-fucose, D-galactose, D-glucose, L-quinovosamine, L-fucosamine, L-rhamnosamine, D-glucosamine, and 3-amino-3,6-dideoxyhexose. This capsular polysaccharide also contained galacturonic acid.

The ^{13}C nuclear magnetic resonance analyses of the two polysaccharides indicated that most of the aminosugars were *N*-acetylated.

Immunochemical analyses. The immunochemical specificities of the capsular strains and the LPS preparations from the 9343 and 23745 strains were investigated in inhibition studies with rabbit antisera raised to the purified preparations. In studies involving inhibition of the interaction between LPS from strain 9343 and its corresponding antiserum, the 9343 and 23745 LPS preparations showed almost identical inhibition curves; the concentrations required for 50% inhibition were 0.3 and 0.4 μg, respectively. Neither of the two capsular preparations showed any inhibition at 100 μg, the highest concentration tested (Fig. 2). Inhibition studies in which the capsular polysaccharide from strain 9343 was used as coating antigen with the anti-9343 capsular antiserum showed that of the four preparations of polysaccharides and LPSs, only the 9343 capsular preparation was active, with 50% inhibition at 0.2 μg (Fig. 3). With 100 μg of the 23745 capsular preparation, only a 20% inhibition was observed. When the 23745 capsular polysaccharide antiserum system was used,

TABLE 2. Carbohydrate analyses of LPS and capsular polysaccharides from *B. fragilis* NCTC 9343 and ATCC 23745

Sugar[a]	Mol%			
	LPS		Capsular polysaccharide	
	9343	23745	9343	23745
L-Fucose	<1	<1	14	3
L-Rhamnose	16	16	<1	<1
D-Galactose	49	51	22	8
D-Glucose	14	13	<1	3
DL-Quinovosamine[b]	<1	<1	42	15
L-Fucosamine	<1	<1	<1	3
L-Rhamnosamine	<1	<1	<1	14
D-Glucosamine[c]	21	20	22	43
3-Amino-3,6-dideoxyhexose	<1	<1	<1	11
Galacturonic acid[d]	<1	<1	+[e]	+

[a] Determined by GLC of the alditol acetates and by GLC of (+)-2-octyl glycoside acetates.
[b] Capsule from 9343 contained both D and L isomers; 23745 capsule contained only L isomer.
[c] Determined by amino acid analysis.
[d] Determined as the increase in galactose after reduction.
[e] Present but not quantitative.

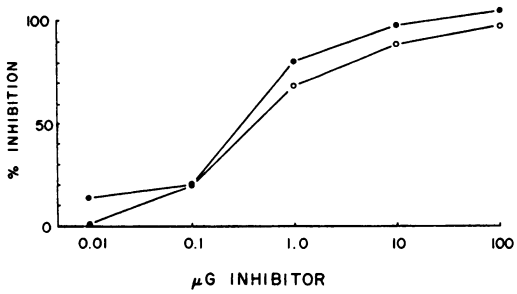

FIG. 2. ELISA inhibition study on *B. fragilis* LPSs. LPS from 9343 strain was used as coating antigen. The antiserum used was directed against 9343 LPS. Inhibitors: 9343 LPS (●); 23745 LPS (○).

the 23745 capsular preparation caused a 50% inhibition at 0.3 μg. A 100-μg concentration of the 9343 capsular preparation gave only 25% inhibition in this system. Neither of the two LPS preparations caused any inhibition of the two capsule-antisera assay systems. By double diffusion in agar, the capsule from strain 23745 gave a line of identity with capsule prepared by previous methods when tested against the 23745 capsular antiserum.

DISCUSSION

Some interesting biological features have been attributed to the cell surface polysaccharides of *B. fragilis*. Earlier studies demonstrated that both the capsular polysaccharide and the LPS of these organisms could promote the formation of abscesses in an animal model of intraabdominal abscess formation. However, on a stoichiometric basis, the capsule was significantly more effective than the LPS in this regard (14, 22). In those studies, it was noted that a number of the carbohydrates found in the capsular polysaccharide were also found in the LPS of these organisms, and complete separation of these two antigens was uncertain (10, 11, 16). Subsequent immunization studies demonstrated that the capsular polysaccharide of *B. fragilis* could induce protective immunity to intraabdominal abscesses formed by *Bacteroides* species. It was suggested that one explanation for cross-protection was contamination of the capsule with LPS (14). More recently, it was demonstrated that the mechanism by which the capsular polysaccharide of *B. fragilis* induces immunity depends on the presence of T cells which have the Ly2+ marker, suggesting a suppressor cell mechanism (23, 26). Similar studies were not conducted with the LPS of these organisms.

Chemical analyses of the capsular polysaccharide and LPS of *B. fragilis* have been difficult because of the large numbers of sugars found and the technical difficulty of completely separating the capsular polysaccharide from the LPS. Separation difficulties were compounded by the low yield of cells grown in flasks containing thioglycolate broth. Another problem was that the method of extraction of these antigens from cells, involving an outer membrane isolation procedure, was rather inefficient (10, 11, 16).

Fermentor growth of *B. fragilis* strains under controlled conditions with the addition of glucose and fetal calf serum to the medium was a major improvement. These altered growth conditions resulted in yields dry-weight of bacterial mass up to threefold higher than those obtained in flasks without fetal calf serum. Furthermore, the initial extraction of organisms with hot phenol-water instead of the outer membrane isolation procedure resulted in yields of capsular polysaccharide which were two to three times higher. Overall, compared with our earlier growth and isolation methods, the yields of antigen were about 10 times higher. A typical 10-

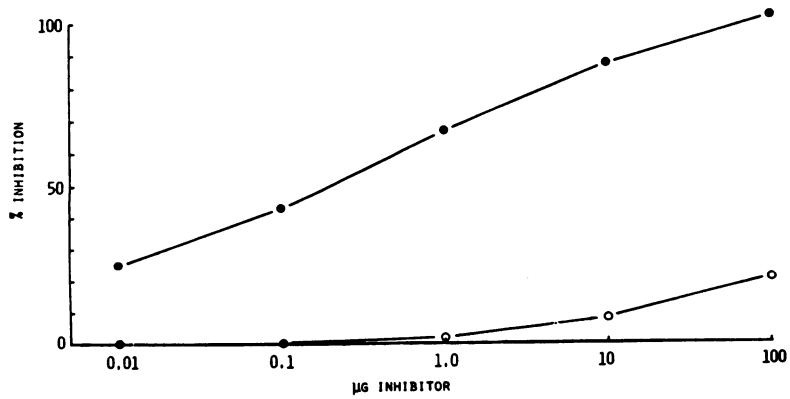

FIG. 3. ELISA inhibition study on *B. fragilis* capsular polysaccharides. Capsular polysaccharide from the 9343 strain was used as coating antigen. Antiserum was directed against the 9343 capsule. Inhibitors: 9343 (●); 23745 capsule (○).

liter batch undergoing these growth and extraction methods resulted in 150 mg of capsular polysaccharide, compared with 15 mg under our earlier methods (10, 11, 16).

Chemical analysis of capsular polysaccharides from two prototype strains of *B. fragilis* revealed that both were essentially free of protein, although the capsule from strain 23745 contained a single amino acid, alanine, in significant amounts. The capsule from strain 9343 contained only trace amounts of amino acids. Amino acids have previously been found as components of polysaccharides (30). Of course, alanine is also found in the bacterial peptidoglycan (25). The location of the alanine residue in the 23745 capsular polysaccharide is still unknown.

The finding of nine monosaccharides in the capsular polysaccharide from strain 23745 and of six in that from strain 9343 is unusual, although some components occur in small amounts, and the structural significance is uncertain. The variety and number of sugars suggest that the primary structure of these polysaccharides is complex. Although this material elutes as a single peak off of molecular sieve and ion-exchange columns (15; unpublished data), there is a possibility that more than one polysaccharide species exists in these preparations. Besides the large number of sugar residues, some of the sugars are unusual in bacterial polysaccharides (Table 2). The capsular polysaccharide of strain 9343 contains D-glucosamine and D- and L-quinovosamine. The 23745 capsular polysaccharide contains D-glucosamine, L-quinovosamine, L-rhamnosamine, L-fucosamine, and a 3-amino-3,6-dideoxyhexose. Quinovosamine has been found in *Vibrio* LPS (9), but the presence of both the D and L isomers of quinovosamine in the same strain is unique. Rhamnosamine has been found in *Escherichia coli* LPS (8), and the D and L isomers of fucosamine have been found in *Pseudomonas aeruginosa* LPS (2). It is evident that the structural elucidation of these polysaccharides is a formidable task. In addition, the nuclear magnetic resonance studies show that some, if not all, of the amino sugars are *N*-acetylated.

The chemical analyses of the LPS preparations from both strains reveal a similar composition of monosaccharides and fatty acids. This result, coupled with the identical immunochemical specificity in ELISA inhibition studies (Fig. 2), suggests that the LPSs of these two strains are identical or share an immunodominant antigenic determinant. The chemical characteristics of the *B. fragilis* LPS have previously made it difficult to determine LPS contamination of capsular preparation. The *B. fragilis* LPS lacks the two carbohydrates unique to aerobic bacterial lipopolysaccharides, 2-keto-3-deoxyoctulsonic acid and L-glycero-D-mannoheptose; therefore, chemical identification of LPS contamination can only be made by lipid analysis. Also, there is no biological screening marker for the LPS because it is not an endotoxin. Recently published studies of the lipids contained in the *B. fragilis* LPS have made it possible to determine LPS contamination of the capsular antigen preparation (31). Using these chemical methods for identification of lipids, we have obtained a relatively pure capsular preparation by solubilizing the aqueous phase of a phenol-water extract in 3% sodium deoxycholate. This is done to disaggregate the LPS so that it can be separated from the capsular polysaccharide by Sephacryl S-300 chromatography. Our earlier studies of the capsular polysaccharide and LPS, using 0.5% sodium deoxycholate buffer, resulted in incomplete separation of the two antigens (10, 11, 16). Even now, despite the improved separation techniques, trace amounts of fatty acids, 1%, were still found in the capsular polysaccharides from both strains, indicating that small amounts of contaminating LPS were present. Although these amounts are insignificant for structural studies, they may cause problems in biological assays. In our hands, pure LPS was most easily obtained by PCP extraction of the crude phenol-water extract. The hydrophobic extraction procedure excluded the coextraction of the capsule. It is interesting to note that PCP extraction of whole, dry *B. fragilis* bacteria failed to release any LPS (6, 10).

Our earlier investigations showed that *B. fragilis* strains have a capsular polysaccharide which can be identified morphologically by electron microscopy (12). Antisera against the earlier preparation of this capsular antigen were found to react with essentially all *B. fragilis* strains tested in an indirect fluorescent-antibody assay, but they failed to react with strains of other *Bacteroides* species (3, 12, 28). As mentioned above, this earlier capsule preparation was found to contain small amounts of contaminating LPS (personal observations). We have now improved upon both the extraction and the separation techniques compared with earlier methods and have better defined the interrelation of these antigens by ELISA inhibition studies. Immunoflourescent studies, using the antisera described in this manuscript, have shown that both capsules and the LPS are surface determinants on *B. fragilis* strains (A. Weintraub, A. A. Lindberg, and D. L. Kasper, J. Infect. Dis., in press). Cross-reactivity between the capsular polysaccharides isolated from each of the two strains was found only at a concentration 10,000 times higher than that of the homologous capsular preparation (Fig. 3). The two reference strains which both stained with the

anticapsular serum reported earlier (unpublished data) have two apparently different capsular polysaccharides, as judged by chemical composition and immunological specificity in the ELISA. However, both strains seem to share LPS with a similar or even identical structure. Because the anticapsular polysaccharide serum used earlier also contained measurable titers of anti-LPS antibodies (unpublished data), it is possible that the LPS rather than the capsule is common to most *B. fragilis* isolates. A survey of the composition and immunochemical specificity of several isolates of *B. fragilis* used in the study by Babb et al. (1) are currently under way and may shed light on this question.

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