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Fermentation of Mucin and Plant Polysaccharides by Strains of *Bacteroides* from the Human Colon

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Ten *Bacteroides* species found in the human colon were surveyed for their ability to ferment mucins and plant polysaccharides ("dietary fiber"). A number of strains fermented mucopolysaccharides (heparin, hyaluronate, and chondroitin sulfate) and ovomucoid. Only 3 of the 188 strains tested fermented beef submaxillary mucin, and none fermented porcine gastric mucin. Many of the *Bacteroides* strains tested were also able to ferment a variety of plant polysaccharides, including amylose, dextran, pectin, gum tragacanth, gum guar, larch arabinogalactan, alginate, and laminarin. Some plant polysaccharides, such as gum arabic, gum karaya, gum ghatti, and fucoidan, were not utilized by any of the strains tested. The ability to utilize mucins and plant polysaccharides varied considerably among the *Bacteroides* species tested.

Bacteroides species account for approximately 20% of the normal flora of the human colon (10, 14). These species are saccharolytic and are known to ferment a variety of simple sugars (11). However, simple sugars are not likely to be available as substrates in the colon, since they are efficiently absorbed in the small intestine. The main sources of fermentable carbohydrate in the colon are probably polysaccharides. Many dietary plant cell wall polysaccharides are not hydrolyzed in the stomach or small intestine and thus reach the colon. These polysaccharides are often referred to as "dietary fiber," and it was suggested that diets high in fiber may be associated with a low incidence of colon cancer (2). Salivary mucins and mucins secreted by the mucosal cells lining the intestine are another possible source of fermentable carbohydrate. Colonic and salivary mucins are glycoproteins. The carbohydrate moiety can be either an acidic mucopolysaccharide, containing uronic acids and hexosamines, or an oligosaccharide, containing L-fucose and sialic acids (9, 12).

Despite the probable importance of polysaccharides as carbon sources for colon bacteria, very little is known about the ability of intestinal *Bacteroides* strains to metabolize these compounds. The breakdown of the acid mucopolysaccharides, heparin, chondroitin sulfate, and hyaluronic acid by an unspeciated strain of *Bacteroides* isolated from human feces has been reported by Gesner and Jenkin (8). More recently, Rudek and Haque (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K122, p. 156) found hyaluronidase and chondroitin sulfatase activity in strains of *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides distasonis*, and *Bacteroides vulgatus*. Strains of *Bacteroides ruminicola* and *Bacteroides succinogenes*, which degrade cellulose, hemicellulose, and pectin, were isolated from the rumen of cattle (1, 4, 5, 20). A number of *Bacteroides* species also ferment starch or inulin (11). However, there has been no systematic study of polysaccharide utilization by *Bacteroides* species from the human intestinal tract. We undertook such a survey, using strains of *B. fragilis*, *Bacteroides ovatus*, *B. distasonis*, *B. thetaiotaomicron*, *B. vulgatus*, *Bacteroides eggerthii*, and several unnamed groups based on deoxyribonucleic acid (DNA) homology data (J. L. Johnson, personal communication). These species and groups were formerly subspecies of *B. fragilis* (3). Substrates for this survey were chosen to reflect the wide variety of linkages and carbohydrate components found in dietary polysaccharides and intestinal mucins.

MATERIALS AND METHODS

Bacterial cultures. Cultures used in this study were obtained from the culture collection of the Anaerobe Laboratory, Virginia Polytechnic Institute and State University. All strains were classified into species according to DNA homology data (J. L. Johnson, personal communication). DNA homology groups that do not have any species designations are listed under the Virginia Polytechnic Institute strain number of the DNA reference strains.

Source of substrates. The following substrates were purchased from Sigma Chemical Co. (St.

Louis, Mo.): D-glucuronate, α-D-galacturonate, D-galactosamine hydrochloride, D-glucosamine hydrochloride, α-L-fucose, α-D-fucose, bovine submaxillary mucin, porcine gastric mucin, ovomucoid, hyaluronate, heparin, chondroitin sulfate, xylan, dextran, amylose, and laminarin. Gum tragacanth, locust bean gum, gum ghatti, gum karaya, and gum arabic were gifts of the Meer Corp. (North Bergen, N.J.). Pectin and polygalacturonate were obtained from Sunkist, Inc. (Ontario, Calif.). Xanthan gum (Keltrol), sodium alginate, and fucoidan were gifts of the Kelco Co. (San Diego, Calif.). Guar gum (Jaguar A-40F) was a gift from Stein Hall Specialty Chemicals (New York, N.Y.). Amylopectin was a gift from the American Maize Products Co. (Hammond, Ind.). Larch arabinogalactan (Stractan) was a gift of the St. Regis Co. (Libby, Mont.).

The neutral sugar composition of the polysaccharides used as substrates was determined by hydrolysis and gas-liquid chromatography of the alditol acetate derivatives (15). The uronic acid composition was estimated by gas-liquid chromatography of the trimethylsilyl ether derivatives (6). The identity of each peak was confirmed by mass spectrometry on a Varian MAT medium resolution instrument. The results of these analyses agreed with those reported in the literature (9, 17). Substrates were also analyzed for the presence of free sugars by subjecting an unhydrolyzed aqueous extract of the substrate to gas-liquid chromatography of the alditol acetate derivatives. All polysaccharides used in this study contained less than 1% free sugars (wt/wt).

Fermentation of substrates. The ability of *Bacteroides* strains to ferment polysaccharide substrates was tested using the replicator method of Wilkins and Walker (18, 19). Microtiter plates containing media were inoculated with 24-h cultures grown in brain heart infusion broth (11). All procedures were carried out in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.). Plates were incubated at 37°C for 7 days in the anaerobic chamber. The plates were then removed from the anaerobic chamber, and the pH of each well in the microtiter plate was measured. A strain was considered to have fermented a given substrate if it decreased the pH of the medium to below 6.0. The initial pH of the medium was 6.9 to 7.1. None of the strains reported in this survey lowered the pH of the basal medium without added carbohydrate below 6.5. All of the strains tested grew on the basal medium with glucose added, dropping the pH to below 5.5.

Replicator medium. The defined medium of Varel and Bryant (16) was modified for use as the basal medium by eliminating the carbonate buffer and adding phenol red and agar. The basal medium contained: $(NH_4)_2SO_4$, 1.0 g/liter; vitamin B_{12}, 5 μg/liter; hemin, 5 mg/liter; K_2HPO_4, 2.26 g/liter; KH_2PO_4, 0.9 g/liter; $FeSO_4 \cdot 7H_2O$, 4 mg/liter; NaCl, 0.9 g/liter; $CaCl_2 \cdot 2H_2O$, 0.027 g/liter; $MgCl_2 \cdot 6H_2O$, 0.02 g/liter; $MnCl_2 \cdot 4H_2O$, 0.01 g/liter; $CoCl_2 \cdot 6H_2O$, 0.01 g/liter; phenol red, 0.1 g/liter; cysteine HCl, 0.5 g/liter; and agar, 15 g/liter. All components of the medium except cysteine and carbohydrate were mixed, the pH was adjusted to 7.6, and the medium was autoclaved. After the medium was autoclaved, filter-sterilized cysteine and sterile solutions of the appropriate carbohydrate for final concentrations of 0.5% were added. A final concentration of 1% was used for ovomucoid, bovine submaxillary mucin, and porcine gastric mucin because of the high protein content of these substrates. Since most of the polysaccharide solutions were too viscous for filter sterilization, polysaccharides in distilled water were autoclaved separately and then added to the sterile media. To determine whether any breakdown of polysaccharides occurred during autoclaving, samples taken before and after autoclaving were tested with a tritiated borohydride assay for reducing ends (13). No significant cleavage (i.e., greater than threefold increase in reducing end concentration) occurred as a result of autoclaving any of the carbohydrates used in this study. The pH was readjusted to approximately 7. The medium was then dispensed into sterile microtiter plates (19).

The structures of the mucins used in this study may be found in *Glycoproteins* (9). The structures of plant polysaccharides, with the exception of xylan, are given in reference 17. Xylan is a linear polymer consisting of β-D-(1 → 4)-linked xylose residues.

RESULTS

Different DNA homology groups of *Bacteroides* varied considerably in their ability to ferment different mucin polysaccharides (Table 1). Strains of *B. thetaiotaomicron* and *B. ovatus* fermented the widest variety of substrates, including all three mucopolysaccharides (hyaluronate, chondroitin sulfate, and heparin) and the glycoprotein mucin ovomucoid. *Bacteroides '3452A,' Bacteroides '0061-1,' B. fragilis* subsp. *a*, and *B. eggerthii* fermented one or more of the mucopolysaccharides but did not utilize ovomucoid. With the exception of two strains of *B. fragilis* 2393 and one strain of *B. '3452A,'* there was no utilization of bovine submaxillary mucin. None of the strains tested were able to ferment porcine gastric mucin.

Many strains that did not ferment mucin polysaccharides were able to ferment their monosaccharide components (Table 1). The hexuronic acids and glucosamine were fermented by nearly all of the DNA homology groups surveyed. L-fucose was fermented by 6 of the 11 groups surveyed. None of the strains tested fermented D-galactosamine or D-fucose.

Utilization of plant polysaccharides also differed considerably from one DNA homology group to another (Table 2). Some groups, such as *B. ovatus, B. thetaiotaomicron, B. '0061-1,' B. fragilis* subsp. *a*, and *B. vulgatus*, fermented at least six of the polysaccharides surveyed. Other groups, such as *B. fragilis* 2553 and 2393, *B. distasonis*, and *B. 'T4-1,'* were much more restricted in the number of plant polysaccharides they were able to ferment. Although *Bacteroides* strains fermented a wide range of plant polysaccharides, there were some polysacchar-

TABLE 1. *Fermentation of mucin polysaccharides and monosaccharide components by different DNA homology groups of Bacteroides*

Carbohydrate	No. of strains that fermented carbohydrate										
	B. fragilis		*B. thetaiotaomicron* (22)	*B. ovatus* (24)	*Bacteroides* '*3452A*'[b] (19)	*Bacteroides* '*0061-1*'[b] (19)	*B. fragilis* subsp. *a* (17)	*B. eggerthii* (6)	*B. vulgatus* (22)	*B. distasonis* (11)	*Bacteroides* '*T4-1*'[b] (12)
	2553 (23)[a]	2393 (13)									
Polysaccharides											
Hyaluronate	0	0	22	24	19	4	0	0	0	0	0
Heparin	0	0	22	24	0	0	12	6	0	0	0
Chondroitin sulfate	0	0	22	23	19	2	2	0	0	0	0
Ovomucoid	0	0	22	17	0	0	0	0	0	0	0
Bovine submaxillary mucin	0	2	0	0	1	0	0	0	0	0	0
Porcine gastric mucin	0	0	0	0	0	0	0	0	0	0	0
Monosaccharides											
D-Glucuronate	23	13	22	24	19	19	17	6	22	11	12
D-Galacturonate	0	0	22	24	19	14	17	6	22	2	10
D-Glucosamine	23	13	22	24	19	18	17	6	22	11	0
L-Fucose	23	13	22	24	19	0	0	0	22	0	0

[a] The number in parentheses under each group represents the total number of strains tested for that group.
[b] Unnamed DNA homology groups are designated by the number of the reference strain.

TABLE 2. *Fermentation of plant polysaccharides by different DNA homology groups of Bacteroides*

Carbohydrate	No. of strains that fermented carbohydrate										
	B. fragilis		*B. thetaiotaomicron* (22)	*B. ovatus* (24)	*Bacteroides* '*3452A*'[b] (19)	*Bacteroides* '*0061-1*'[b] (19)	*B. fragilis* subsp. *a* (17)	*B. eggerthii* (6)	*B. vulgatus* (22)	*B. distasonis* (11)	*Bacteroides* '*T4-1*'[b] (12)
	2553 (23)[a]	2393 (13)									
Amylose	23	13	22	16	0	17	17	6	22	0	0
Amylopectin	23	13	22	16	0	18	17	6	22	0	0
Dextran	0	0	22	24	4	17	4	0	1	0	0
Xylan	0	0	0	24	0	0	11	6	6	0	0
Polygalacturonate	0	0	20	20	19	1	17	1	10	0	0
Pectin	0	0	22	23	19	0	17	0	9	0	0
Gum tragacanth	0	0	0	19	0	0	2	0	0	0	0
Locust bean gum	0	0	0	4	0	17	0	0	1	0	0
Guar gum	0	0	0	4	0	17	0	0	1	0	0
Larch arabinogalactan	0	0	22	15	19	13	0	0	16	0	12
Alginate	0	0	0	10	0	0	0	0	0	0	0
Laminarin	1	1	10	1	3	19	0	0	1	11	12

[a] The number in parentheses under each group represents the total number of strains tested for that group.
[b] Unnamed DNA homology groups are designated by the number of the reference strain.

ides that were not utilized by any strains. None of the *Bacteroides* strains tested fermented gum arabic, gum ghatti, gum karaya, gum xanthan, or fucoidan.

DISCUSSION

The *Bacteroides* groups that occur in highest concentrations in the colon are *B. vulgatus*, group '*0061-1*' (previously part of *B. fragilis* subsp. *thetaiotaomicron*), *B. distasonis*, group '3452A' (previously part of *B. fragilis* subsp. *distasonis*), and *B. fragilis* subsp. *a* (10, 14; J. L. Johnson, personal communication). All of these groups except *B. distasonis* fermented a number of plant polysaccharides. In addition, some mucin substrates were fermented by strains of DNA homology groups '*0061-1*,' '*3452A*,' and *B. fragilis* subsp. *a*. Extensive mucin and plant polysaccharide fermentation also occurred in other species such as *B. ovatus* and *B. thetaiotaomicron*, which were isolated from human feces but in lower concentration than species such as *B. vulgatus*. The ability of these organisms to utilize polysaccharides as a source of carbon and energy indicates that mucins and dietary plant polysaccharides could

provide a source of carbohydrate for saccharolytic bacteria in the colon.

Species such as *B. ovatus* and *B. thetaiotaomicron*, which ferment a wide range of polysaccharides, are apparently capable of producing many different glycosidase activities, since they fermented substrates containing $\alpha(1 \rightarrow 4)$, $\alpha(1 \rightarrow 6)$, $\beta(1 \rightarrow 3)$, $\beta(1 \rightarrow 4)$, or $\beta(1 \rightarrow 6)$ glycosidic linkages with different component sugars. These species may also produce sulfatases, since they fermented the sulfated polysaccharides, chondroitin sulfate and heparin, as readily as the unsulfated hyaluronic acid. A number of strains of *Bacteroides* fermented branched polysaccharides such as guar gum, larch arabinogalactan, and amylopectin. These strains may have debranching enzymes as well as enzymes that are active on linear polysaccharides.

A number of the tested substrates (e.g., alginate, guar gum, and larch arabinogalactan) are used as bulking agents and stabilizers in processed food. Although these compounds are normally assumed to be "indigestible," inert substances, our results indicate that the effects of degradation and fermentation of these substances by colon bacteria should be considered in studies of their physiological effects.

The ability of *Bacteroides* strains isolated from the human colon to degrade and ferment both mucins and plant polysaccharides may also have significance in the etiology of some types of colonic disease such as colon cancer. Mucin degradation could impair the protective mucin layer which lines the colon and, thus, increase the vulnerability of the mucosa to harmful substances in the colon contents. Moreover, bacterial metabolism of dietary fiber polysaccharides in the colon may substantially alter their charge and hydration. It was suggested that dietary fiber may protect the colon from bile acids and carcinogens by adsorbing them and promoting rapid elimination (7). The likelihood that the physical properties of dietary fiber polysaccharides may be modified by bacterial action must be taken into account in experiments designed to test this hypothesis. Further investigations of the extent to which these substances are degraded by colon bacteria are being conducted in our laboratory.

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