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Fermentation of Mucins and Plant Polysaccharides by Anaerobic Bacteria from the Human Colon

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A total of 154 strains from 22 species of *Bifidobacterium, Peptostreptococcus, Lactobacillus, Ruminococcus, Coprococcus, Eubacterium,* and *Fusobacterium,* which are present in high concentrations in the human colon, were surveyed for their ability to ferment 21 different complex carbohydrates. Plant polysaccharides, including amylose, amylopectin, pectin, polygalacturonate, xylan, laminarin, guar gum, locust bean gum, gum ghatti, gum arabic, and gum tragacanth, were fermented by some strains from *Bifidobacterium, Peptostreptococcus, Ruminococcus,* and *Eubacterium* species. Porcine gastric mucin, which was fermented by some strains of *Ruminococcus torques* and *Bifidobacterium bifidum,* was the only mucin utilized by any of the strains tested.

A variety of complex carbohydrates enter the human colon. Some are ingested in the diet (e.g., plant cell wall polysaccharides, food additives, and meat glycoproteins) or are present in swallowed saliva. Others, such as goblet cell mucin or glycoproteins from sloughed epithelial cells, originate within the intestinal tract itself. Since most of these compounds are not degraded appreciably or absorbed as they pass through the stomach and small intestine, they reach the colon intact. By contrast, simple sugars and disaccharides, which are readily absorbed from the small intestine, do not reach the colon. Thus, complex carbohydrates probably represent a major source of carbon and energy for at least some of the numerous species of saccharolytic bacteria in the colon.

That anaerobic bacteria from the rumen of cattle metabolize a number of plant polysaccharides, including pectin (9), xylan (3, 5, 6), and cellulose (3, 5, 13), has been well documented. However, except for starch digestion (11) and pectin fermentation (11), very little is known about utilization of complex carbohydrates by species of anaerobic bacteria isolated from the human colon. Recently, we reported that some *Bacteroides* species from the human colon ferment a wide range of plant polysaccharides and mucins (18). *Bacteroides* species account for about 20% of the fecal flora, but there are other major genera with saccharolytic species (16). To determine whether some of these saccharolytic species might be capable of fermenting complex carbohydrates, we have surveyed 154 strains from 22 species of *Bifidobacterium, Eubacterium, Peptostreptococcus, Lactobacillus, Fusobacterium* (4), *Coprococcus* (10), and *Ruminococcus* (10, 15, 17) for their ability to ferment plant polysaccharides and mucins. All of these species are present at concentrations of at least 10^9 per g of dry feces, and together they account for about 50% of the normal flora of the human colon (16). Substrates were chosen to resemble components of mucins and plant cell walls ("dietary fiber") that are present in the colon.

MATERIALS AND METHODS

Bacterial species. The bacterial species and the number of strains tested are listed in Table 1. The bacterial cultures used in this survey were isolated from human feces and identified by W. E. C. Moore and L. V. Holdeman (Anaerobe Laboratory, Blacksburg, Va.) according to published procedures (11, 16). Strains of *Gemminger formicilis* (7) did not grow well enough in the inoculum medium to be included in this study. Strains of *R. gnavus* (17) were included, even though this species was not ranked as a major component of the flora (16), because *R. gnavus* is one of the metabolic groups previously included in *P. productus* I (W. E. C. Moore, personal communication).

In their description of the fecal flora, Moore and Holdeman (16) designated subgroups of the species *E. aerofaciens, E. rectale,* and *P. productus.* Since strains from different subgroups within the same species had similar fermentation patterns on the complex carbohydrates tested, we combined subgroups under the appropriate species designation in Tables 1 and 2.

Fermentation analysis. Carbohydrate fermentation was determined by using the replicator method

Table 1. *Species of intestinal bacteria surveyed for the ability to ferment plant polysaccharides and mucins*

Species	No. of Strains
Bifidobacterium adolescentis	11
Bifidobacterium bifidum	4
Bifidobacterium breve	5
Bifidobacterium infantis	11
Bifidobacterium longum	10
Coprococcus comes[a]	5
Eubacterium aerofaciens[a]	15
Eubacterium biforme	5
Eubacterium eligens	5
Eubacterium rectale[a]	20
Fusobacterium prausnitzii[a]	5
Fusobacterium russii[a]	5
Lactobacillus acidophilus	6
Peptostreptococcus productus	8
Ruminococcus albus[a]	5
Ruminococcus bromii[a]	8
Ruminococcus gnavus[a]	5
Ruminococcus torques[a]	9
Other anaerobic cocci[b]	12

[a] Rumen fluid (1%) included in the medium.

[b] *Ruminococcus callidus* (1 strain), *Ruminococcus obeum* (2 strains), *Coprococcus catus* (1 strain), *Coprococcus eutactus* (1 strain), and 7 unspeciated strains.

of Wilkins and Walker (22) and Wilkins et al. (23). The Trypticase-yeast extract replicator medium described by Wilkins and Walker (22) was used as the basal medium. Monosaccharides were added to the basal medium as filter-sterilized solutions; polysaccharides were autoclaved in distilled water before addition to the basal medium. Inoculated microtiter plates were incubated for 7 days in an anaerobic chamber (Coy Manufacturing Co., St. Louis, Mo.) under an atmosphere of N_2 (85%), CO_2 (5%), and H_2 (10%). After incubation, the pH of uninoculated medium was 6.9 to 7.1. To improve the growth of strains that required rumen fluid, the basal medium was supplemented with sterile rumen fluid (1% final concentration). Species that were tested on the medium supplemented with rumen fluid are indicated in Table 1. To check that the addition of rumen fluid did not affect fermentation patterns or final pH, two or three strains each of 17 species of intestinal anaerobes, including 6 species of *Bacteroides* previously tested, were grown on basal medium and on medium with added rumen fluid plus a number of different polysaccharides. The *Bacteroides* strains were included because they ferment a variety of polysaccharides (18). No difference in fermentation patterns was observed.

Strains were classified as fermenting a substrate if they lowered the pH of the medium by at least 1 pH unit (from 7.0 to 6.0) as compared with uninoculated control wells and inoculated wells of the basal medium without carbohydrate. The fermentation pattern for each of the strains was determined in duplicate in at least two separate experiments. A pH indicator was included in the medium as a visual check of fermentation, but all pH measurements were made with a pH electrode.

Substrates. Monosaccharides included in the survey were: D-glucose, L-fucose, D-glucosamine, D-galactosamine, D-glucuronate, and D-galacturonate. Glucose was included as one of the substrates to assure that strains were growing well under assay conditions. Strains of all species tested, except *R. bromii, F. prausnitzii,* and *F. russii,* fermented glucose. However, strains of *R. bromii* fermented amylopectin, and strains of *F. prausnitzii* and *F. russii* produced visible growth on the basal medium without carbohydrate. The other monosaccharides were included because they are components of plant polysaccharides and mucins for which fermentation patterns have not been previously reported. Plant polysaccharides included in the study were: amylose, amylopectin, xylan, polygalacturonate, guar gum, locust bean gum, gum ghatti, gum arabic, gum tragacanth, gum karaya, larch arabinogalactan, fucoidan, alginate, and carrageenan. Mucins included in the study were: chondroitin sulfate, hyaluronate, heparin, ovomucoid, porcine gastric mucin, and beef submaxillary mucin. Dextran, a bacterial polysaccharide, was also tested. Structures of these complex carbohydrates may be found in the literature of Whistler (21) or Gottschalk (8).

Commercial sources of the carbohydrates used in this survey and evaluation of their purity have been described (18). Carbohydrate components of the polysaccharides and mucins agreed with published structures. None of the complex carbohydrates contained more than 0.1% monosaccharide.

RESULTS

The polysaccharides fermented by the largest number of species were the two components of starch, amylose and amylopectin. Strains from 7 of the 22 species surveyed fermented one or both of these substrates (Table 2). Strains of *E. rectale* and some strains of *B. infantis, B. adolescentis, E. aerofaciens,* and *R. bromii* fermented amylopectin, but did not ferment amylose. Amylose differs from amylopectin primarily in that it is a linear $\alpha(1\rightarrow4)$-linked polymer of glucose, whereas amylopectin is a branched polymer of glucose containing linear $\alpha(1\rightarrow4)$ segments linked with $\alpha(1\rightarrow6)$ branches. Amylopectin may have a less compact structure than amylose. Starch contains about 73% amylopectin and 27% amylose.

Xylan was fermented by 8 of 11 strains of *B. adolescentis* and *B. infantis* (Table 2) and by one of eight strains of *P. productus.* Guar gum and locust bean gum, two polysaccharides with similar structures and components, were fermented by one strain of *B. adolescentis* and by

Table 2. *Number of strains of bacterial species from the human colon which ferment monosaccharides and plant polysaccharides*

Substrate	*B. adolescentis* (11)[a]	*B. breve* (5)	*B. infantis* (11)	*B. longum* (10)	*E. aerofaciens* (15)	*E. eligens* (5)	*E. rectale* (20)	*P. productus* (8)	*R. albus* (5)	*R. bromii* (8)
Monosaccharides										
glucosamine[c]	–[b]	–	–	–	7	–	1	4	–	–
L-fucose[d]	–	3	–	–	–	2	1	6	–	–
Polysaccharides										
amylose	7	5	2	–	2	–	–	1	–	6
amylopectin	10	5	9	–	3	–	12	1	–	8
xylan	8	–	8	–	–	–	–	1	–	–
larch arabinogalactan	–	–	–	10	–	–	–	–	–	–
gum guar	1	–	–	–	–	–	–	–	5	–
gum locust bean	1	–	–	–	–	–	–	–	5	–
gum arabic	–	–	–	3	–	–	–	–	–	–
gum ghatti	–	–	–	3	–	–	–	–	–	–
gum tragacanth	–	–	–	6	–	–	–	–	–	–
pectin	–	–	–	–	–	3	–	–	–	–
polygalacturonate	–	–	–	–	–	2	–	–	–	–
laminarin	–	–	–	–	–	–	–	1	–	–

[a] Number of strains tested.

[b] –, substrate not fermented by any of strains tested.

[c] Also fermented by one of four strains of *B. bifidum*, by 4 of 5 strains of *L. acidophilus*, and by 4 of 5 strains of *E. biforme*.

[d] Also fermented by all 5 strains of *R. gnavus*, by 10 of 12 strains of anaerobic cocci, and by 1 of 5 strains of *E. biforme*.

all strains of *R. albus* (Table 2). Unlike *R. albus* strains from the bovine rumen (5), these *R. albus* strains did not ferment xylan. Human *R. albus* strains also differ from rumen *R. albus* strains in their ability to ferment cellulose and may actually belong to different species (1). Pectin and polygalacturonate were fermented by strains of only one species, *E. eligens*. Although these strains consistently fermented polygalacturonate and pectin in several trials, they did not ferment D-galacturonate, the main component of these substrates (Table 2). Laminarin was fermented by one of eight strains of *P. productus* (Table 2).

Strains of *F. prausnitzii, F. russii,* and *C. comes* were the least fermentative of the strains tested. Except for one strain of *F. prausnitzii,* which fermented amylopectin, none of these strains fermented any of the substrates tested. Of the 12 strains of miscellaneous anaerobic cocci, 10 fermented L-fucose, 1 fermented D-glucosamine, and 1 fermented D-glucuronate and D-galacturonate; none fermented polysaccharides. Strains of *L. acidophilus* fermented D-glucuronate (one of four strains) and D-glucosamine (four of five strains), but no polysaccharides.

B. longum differed from all of the other species tested in that strains from this species fermented three plant gums (gum arabic, gum ghatti, and gum tragacanth) as well as larch arabinogalactan (Table 2). These gums share a common component, arabinose, which is not a major component of any of the other gums tested. Because fermentation of these gums is unusual, we examined strains from two additional species of *Bifidobacterium, B. breve* and *B. bifidum,* which are present in feces in somewhat lower concentrations (10^9 and $<10^7$ per g of dry feces, respectively) than the other *Bifidobacterium* species (16). Neither of these species fermented any of the gums that were fermented by strains of *B. longum.*

The only mucin fermented by any of the strains tested was porcine gastric mucin, which was fermented by six of nine strains of *R. torques* and two of five strains of *B. bifidum.* However, L-fucose and D-glucosamine, which are components of many types of mucin including colonic goblet cell mucin (14), were fermented by species of *Peptostreptococcus, Ruminococcus, Lactobacillus, Eubacterium,* and miscellaneous anaerobic cocci (Table 2).

Substrates not fermented by any of the strains tested were: D-galactosamine, dextran, gum karaya, fucoidan, alginate, carrageenan, chondroitin sulfate, hyaluronate, heparin, ovomucoid, and beef submaxillary mucin.

DISCUSSION

None of the species included in this survey fermented as wide a range of complex carbohydrates as the *Bacteroides* species reported previously (18). Some substances, such as alginate, chondroitin sulfate, hyaluronate, heparin, and ovomucoid, which were fermented by several *Bacteroides* species, were not fermented by any of the species tested in this survey. All of these complex carbohydrates have uronic acid as a major component. In fact, the only uronic acid-containing polysaccharides fermented by any of the strains included in the present survey were pectin and polygalacturonate, and these were fermented by only a few strains from one species (*E. eligens*). This is probably because fermentation of the simple uronic acids, D-glucuronate and D-galacturonate, is uncommon in genera other than *Bacteroides.*

Although *Bacteroides* species from the colon ferment a greater variety of substrates than do species from other major genera in the colon flora, there were substrates fermented by organisms included in the present survey that were not fermented by any *Bacteroides* strains. *B. longum* strains fermented several plant gums, two of which (gum arabic and gum ghatti) were not fermented by any other species we have tested, including species of *Bacteroides.* It should be noted, however, that *Bacteroides* strains that degrade gum arabic have been isolated from sources other than the human colon (2, 20). Porcine gastric mucin was another substrate that was fermented by strains of *R. torques* and *B. bifidum,* but not by *Bacteroides* species.

Except for porcine gastric mucin, no mucin substrates were fermented by any of the strains tested in this survey. A number of the strains of the *Bacteroides* surveyed earlier fermented hyaluronate, heparin, chondroitin sulfate, and ovomucoid, but only a few strains fermented bovine submaxillary mucin. None fermented porcine gastric mucin (18). Of the mucins included in these surveys, ovomucoid, porcine gastric mucin, and beef submaxillary mucin are closest in structure to human colonic mucin. At first glance, the failure of most strains of colon bacteria to ferment porcine gastric and beef submaxillary mucins might seem to indicate that relatively little degradation of mucin occurs in the colon. However, bacterial degradation of blood group antigens in human gut mucin has been reported (12), and results of a recent analysis of high-molecular-weight carbohydrate in the contents of different portions of the human intestinal tract indicate that mucins are degraded in the human colon (J. R. Vercellotti, A. A. Salyers, W. S. Bullard, and T. D. Wilkins, Can. J. Biochem., in press). Many of the strains of colon bacteria tested in this and the preceding survey fermented monosaccharide components

of human mucin, such as fucose and hexosamines. It is possible that the structure of human mucin makes it more susceptible to breakdown by the human colonic flora than porcine gastric mucin or beef submaxillary mucin. Moreover, the breakdown of mucin glycoproteins may require cooperative action by several species of colon bacteria.

In a number of cases, only a few strains from a given species fermented complex carbohydrates. This was also true for some of the *Bacteroides* species reported earlier (18). This diversity within species in their ability to ferment polysaccharides should be taken into account in studies comparing the fecal flora of humans consuming diets containing different fiber levels. Even when distributions of species are the same, the overall metabolic activity could be different, depending on the relative proportion of polysaccharide fermenters. Moreover, since polysaccharide-hydrolyzing enzymes can be inducible (19), the same strain may produce different sets of enzymes, depending on the composition of the material passing through the colon.

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