Competitiveness of Different Polysaccharide Utilization Mutants of *Bacteroides thetaiotaomicron* in the Intestinal Tracts of Germfree Mice

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*Bacteroides thetaiotaomicron*, an obligate anaerobe found in high numbers in human colons, can utilize a variety of polysaccharides. To determine which type of polysaccharide contributes most to the nutrition of *B. thetaiotaomicron* in vivo, we isolated and characterized transposon-generated mutants deficient in the ability to use different polysaccharides. Some mutants were deficient in polysaccharide utilization because of the inability to utilize a component monosaccharide. These mutants included a mutant that was unable to utilize L-fucose (a component of goblet cell mucin), a mutant that was unable to utilize D-galactose (a component of raffinose, stachyose, arabinogalactan, and goblet cell mucin), and a mutant that was unable to utilize either glucuronic acid (a component of mucopolysaccharides) or galacturonic acid (a component of pectin). Other mutants were unable to use the polysaccharide but could use the component sugars. These included four mutants that were unable to utilize starch and one mutant that was unable to utilize galacturonic acid. The mutants were tested for the ability to compete with the wild type for colonization of the intestinal tracts of germfree mice. The only mutants against which the wild type competed successfully in the intestinal tracts of germfree mice were a galactose-negative mutant and a uronic acid-negative mutant. These mutations differed from the others tested in that they affected utilization of more than one type of polysaccharide. This result is consistent with the hypothesis that *B. thetaiotaomicron* is a scavenger which is highly adapted for utilization of small amounts of a variety of carbohydrates and is thus able to sustain loss of the ability to utilize a single type of polysaccharide without significant lowering of its ability to survive in intestinal tracts.

*Bacteroides* spp. are gram-negative obligate anaerobes found in high numbers in the resident microflora of human colons (7). Some species of human colonic *Bacteroides* spp. are notable for the ability to ferment a variety of polysaccharides, including not only polysaccharides from the host’s diet but also polysaccharides produced by the host itself (10). Presumably this versatility contributes to survival in a colon, where a complex mixture of carbohydrates confronts the resident bacteria. But no attempt has been made to test the importance of different polysaccharide utilization pathways in vivo. One approach is to test the abilities of mutants deficient in the utilization of individual polysaccharides to colonize the intestinal tracts of germfree mice.

Recently, we showed that two of three mutants deficient in the utilization of the host mucopolysaccharide chondroitin sulfate were competed against successfully by the wild type when mice were colonized with a mixture of wild-type and mutant bacteria (9). This finding could be interpreted to support the hypothesis that chondroitin sulfate contributes to the survival of *Bacteroides* spp. in intestines. However, there were two reasons to doubt this interpretation. (i) Although one of the chondroitin sulfate-negative mutants was competed against successfully by the wild type, another with a similar phenotype was not. Neither mutation appeared to affect growth on other carbohydrates (9), but we could not rule out the possibility that the chondroitin sulfate-negative mutant which was competed against successfully by the wild type carried a mutation that affected pathways other than that involved in chondroitin sulfate utilization. (ii) The mutant most rapidly outcompeted by the wild type was unable to use chondroitin sulfate because it was unable to use one of the component sugars, N-acetylgalactosamine. Since N-acetylgalactosamine is also a component of glycoprotein mucin secreted by intestinal goblet cells (15), this mutant might also be deficient in the ability to utilize goblet cell mucin.

Before any conclusions can be made about the importance, relative to other polysaccharides, of chondroitin sulfate as a carbon source in intestinal tracts, it is necessary to know how mutations that disrupt other polysaccharide utilization systems of *B. thetaiotaomicron* might affect the survival of the organism in germfree intestinal tracts. *B. thetaiotaomicron* can utilize a number of dietary polysaccharides that might be encountered in animals, including starch, polygalacturonic acid, arabinogalactan, and raffinose. Thus, we wanted to determine whether mutants deficient in the ability to utilize these dietary polysaccharides would be competed against successfully by the wild type in germfree mice.

In addition, the possibility that goblet cell mucin serves as a substrate needs to be assessed. Mammalian goblet cell mucin is not commercially available. Thus, it is not possible to determine by a direct test whether *B. thetaiotaomicron* can degrade goblet cell mucin. However, *B. thetaiotaomicron* can utilize the four main components of goblet cell mucin, N-acetylgalactosamine, N-acetylgalactosamine, galactose, and fucose (10, 15). Utilization of sialic acid, another component of mucin, has not been tested. One way to determine whether goblet cell mucin is a carbohydrate source for *B. thetaiotaomicron* in intestines would be to obtain a mutant that is unable to utilize L-fucose and test it for the ability to compete with the wild type in the intestinal

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TABLE 1. Characteristics of Tn4351-generated mutants tested in this study

<table>
<thead>
<tr>
<th>Designation</th>
<th>Relevant characteristicsa</th>
<th>Type of insertionb</th>
<th>Source (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fu-1</td>
<td>L-Fuc−, Δ-Ara−, l-Rha−, l-Ara−</td>
<td>IS::R751</td>
<td>This study</td>
</tr>
<tr>
<td>Fu-4</td>
<td>L-Fuc−, Δ-Ara−, l-Rha−, l-Ara−</td>
<td>IS</td>
<td>This study</td>
</tr>
<tr>
<td>MS-1</td>
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<td>IS, Tn4351c</td>
<td>K. Anderson (1)</td>
</tr>
<tr>
<td>MS-2</td>
<td>Amyl−, G4−, Mal+</td>
<td>IS::R751</td>
<td>K. Anderson (1)</td>
</tr>
<tr>
<td>MS-4</td>
<td>Amyl−, G7−, Mal+</td>
<td>IS::R751</td>
<td>K. Anderson (1)</td>
</tr>
<tr>
<td>PG-2</td>
<td>PGA−, GalA−, GlcA−, CS+−</td>
<td>Tn4351::R751</td>
<td>This study</td>
</tr>
<tr>
<td>PG-4</td>
<td>PGA−, GalA−, GlcA−</td>
<td>IS::R751</td>
<td>This study</td>
</tr>
<tr>
<td>AG-4</td>
<td>AG+−/−, Gal−</td>
<td>IS, Tn4351c</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Abbreviations: Amyl, amyllose; G7, maltoheptose; Mal, maltose; PGA, polygalacturonic acid; GalA, galacturonic acid; GlcA, glucuronic acid; CS, chondroitin sulfate; AG, arabinogalactan; Gal, galactose; L-Fuc, l-fucose; Δ-Ara, Δ-arabinose; l-Rha, l-rhamnose; l-Ara, l-arabinose.
b IS, Simple IS4351 insertion obtained by screening Tn4351 mutants for Em+ derivatives; IS::R751, R751 cointegrated with IS4351 and probably flanked with two copies of the IS element; Tn4351::R751, original insertion (an Em+ derivative was not obtained).
c Both the original Tn4351 mutant and the Em+ (IS4351) derivative were tested.

tracts of germfree mice. L-Fucose is the only component besides sialic acid that is uniquely associated with mucin.

MATERIALS AND METHODS

Bacterial strains and growth conditions. B. theta iotaomicron 5482 was obtained from the culture collection of the Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg. For routine cultivation, growth of inocula for the germfree mouse experiments, and transposon mutagenesis, bacteria were grown on trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-glucose (TYG) medium (2). For characterization of the mutants, we used a defined medium (5, 13) to which various carbohydrate sources were added. Glucose, D-arabinose, l-arabinose, l-fucose, l-xylitol, l-rhamnose, D-galactose, D-glucuronic acid, D-galacturonic acid, melibiose, maltose, and maltoheptose (G7) were filter sterilized and added to the medium after being autoclaved. Arabinogalactan, polygalacturonic acid, chondroitin sulfate (type II), amylose, and amylopectin were autoclaved in the medium. All carbohydrates were obtained from Sigma Chemical Co., St. Louis, Mo. The gas phase of liquid medium was 80% nitrogen-20% carbon dioxide. Plates used in the mutagenesis experiments and for isolation of colonies were incubated in GasPak jars (BBL).

Isolation and characterization of mutants. All of the mutants used in this study were obtained by mutagenesis of B. theta iotaomicron with Tn4351. The mutagenesis protocol has been described previously (12). The characteristics of the mutants used in this study are listed in Table 1. For all of the Tn4351-generated mutants except one, we were able to obtain Em+ derivatives of the original Em+ transposon insertion by screening several thousand colonies for Em+. Em+ derivatives occur as a result of recombination between the direct-repeat insertion sequence elements that flank Tn4351 (12). With the one exception, PG-2, we were unable to obtain an Em+ version of this mutant, despite several attempts. Accordingly, we used the strain with the full Tn4351 insertion. Since Tn4351 often cointegrates R751, the plasmid which is used to move it into B. theta iotaomicron, we screened each mutant for R751 DNA by colony hybridization, as described previously, with labeled R751 as the probe (8, 9). The strains that had R751 cointegrated in the chromosome are indicated in Table 1 as IS::R751. Mutants were tested for reversion to the wild type as described previously (9).

To ascertain that the mutations did not have a general effect on growth rate, the growth rate of each mutant was determined in glucose defined medium and compared with that of the wild type. If the growth rates were identical by this test, a more sensitive competition assay was used. Equal numbers of mutant and wild-type bacteria were inoculated into glucose defined medium. Once the mixture had grown to an optical density (600 nm) of about 1.0, 0.05 ml was inoculated into a fresh 10-ml tube of glucose defined medium. This process was repeated four times for a total of about 50 generations. Appropriate dilutions of the mixtures were plated, and 80 isolated colonies were screened to ascertain the percentage of wild-type organisms.

The mutants that were unable to use a monosaccharide were tested for sensitivity to the monosaccharide. A carbohydrate other than glucose was used as the growth substrate to avoid glucose-mediated exclusion of the monosaccharide being tested. Galactose-negative mutant AG-4 was inoculated into medium containing polygalacturonic acid (0.5%) and galactose (0.5%), and the growth rate was compared with that of the mutant in medium containing polygalacturonic acid alone. The fucose-negative mutants and the uronic acid-negative mutant PG-2 were tested similarly in medium containing arabinogalactan (0.5%) and either fucose (0.5%) or galacturonic acid (0.5%). Some types of galactose-negative mutants have altered lipopolysaccharide. This often results in heightened susceptibility to antibiotics because of disruption of the outer membrane. Accordingly, we tested the galactose-negative mutant for increased susceptibility to antibiotics by spreading 0.1 ml of a mid-exponential-phase culture of bacteria on a TYG plate and placing disks containing ampicillin, chloramphenicol, and erythromycin on the plate. After incubation at 37°C for 48 h, the diameter of the clear zone surrounding each disk was measured and compared with the zone diameter obtained with the wild type. Zones were considered to be identical if they differed by 2 mm or less.

To determine whether the fucose-negative mutants were deficient in uptake of l-fucose, we tested their abilities to take up l-[6-3H]fucose (70 Ci/mol; Amersham Corp., Arlington Heights, Ill.). Cells were grown in l-rhamnose defined medium. Since l-rhamnose shares part of the catalytic pathway used by l-fucose, it might act as an inducer of l-fucose permease. At the very least, it should not interfere with l-fucose uptake by repressing synthesis of the permease or by exclusion of l-fucose. When the 10-ml cultures
reached an optical density (600 nm) of 0.5 to 0.6, they were harvested by centrifugation (27,000 × g, 25°C) and suspended in 5 ml of 50 mM potassium phosphate buffer, pH 7.0. Suspended cells were equilibrated at 37°C for 5 min, and 1 ml was added to a tube containing 0.1 ml of labeled L-fucose to start uptake. The final concentration of L-fucose was 5 μM (2 μCi/ml). Portions (0.2 ml) of the mixture were taken at intervals, placed on nitrocellulose filters, and washed with 15 ml of phosphate buffer (37°C). The filters were air dried and counted in ACS scintillation fluid (Amer-sham).

Germfree mouse experiments. Germfree BALB/c mice were purchased from the Gnotobiotic Laboratory, University of Wisconsin, Madison. Procedures for maintaining mice and testing them for sterility have been described previously (8, 16). Mice were 7 to 9 weeks old at the start of the experiment. Each test group contained three mice. Each mutant was tested in at least two separate groups of three mice in separate isolators. Cultures of wild-type and mutant bacteria were inoculated into TYG medium and grown to an optical density (650 nm) of 0.5 to 0.6. The cultures were mixed before introduction into the isolator and used to wet sterile food pellets in the isolator. The percentage of wild-type bacteria in each mixture (usually 15 to 25%) was determined by plating appropriate dilutions of the mixture and picking isolated colonies onto defined medium containing a carbohydrate that differentiated wild-type from mutant bacteria. At intervals after colonization (day 0), fecal specimens were taken from each mouse, suspended in 0.5 ml of TYG broth, and plated to obtain isolated colonies. The percentage of wild-type bacteria was obtained by picking at least 80 colonies onto defined medium containing a carbohydrate that differentiated wild-type from mutant bacteria. We used 80 colonies because previous experience had shown that screening a larger number of colonies (up to 200) did not give a different percentage (A. Salyers, unpublished data). In each case, colonies were also picked onto defined medium containing chondroitin sulfate as the carbohydrate source. Very few organisms grow on chondroitin sulfate, so this is an effective test for contaminants. A portion of the fecal suspension was also plated onto TYG plates and incubated aerobically as a check for aerobic contaminants.

RESULTS

Characteristics of mutants. Four separate transposon mutagenesis experiments were done, with approximately 3,000 Em' transconjugants screened in each experiment, to obtain the mutants described in Table 1. All of the mutants reverted to the wild type at frequencies of 10^-7 or less. Each of the mutations was specific for one or two types of polysaccharide. The mutants in Table 1 grew normally on all of the carbohydrates not listed as affected in the column containing relevant characteristics. None of the mutations had a general effect on growth. That is, all were able to compete successfully with the wild type in defined glucose medium.

Both of the fucose-negative mutants were unable to utilize D-arabinose as well as L-fucose but were able to utilize L-rhamnose. This is the phenotype expected for mutations that affect early enzymes in the fucose utilization pathway (3). Since neither mutant was sensitive to fucose, the mutations probably affected steps before phosphorylation. The mutation was not in a permissive gene, because both mutants were able to take up labeled L-fucose as well as the wild type. Thus, the mutation probably inactivated either the L-fucose isomerase gene or the L-fuculose kinase gene. Although the two fucose-negative mutants probably arose from independent events because one contained R751 and the other did not, we cannot rule out the possibility that they are affected in the same gene. Both had identical phenotypes. Nonetheless, both were included in the study in case a difference was detectable in the germfree mouse model system.

Galactose-negative mutant strain AG-4 was not sensitive to galactose. This indicated that phosphorylation of galactose did not occur in the mutant. Susceptibility of AG-4 to antibiotics was the same as that of the wild type, and the colony morphology of the mutant was the same as that of the wild type. Thus, the mutation was probably not in the gene for UDP-galactose epimerase, a gene involved in lipopolysaccharide synthesis (3). AG-4 grew to an optical density of about one-fourth of that of the wild type on arabinogalactan, as expected on the basis of the fact that arabinose accounts for about one-fourth of the total sugars in this polysaccharide. AG-4 also grew to a lower optical density than the wild type on melibiose. This indicates that the mutation was not in a permease gene, because α-galactosidase is cytoplasmic in this organism (K. Anderson, unpublished data). Taken together, these results indicate that the mutation in AG-4 is probably in the galactokinase gene.

The mutation in PG-2 affected utilization of both glucuronic acid and galacturonic acid. This indicates that the mutation is not in a permease gene. The uronic acid-negative mutant PG-2 grew somewhat more slowly in medium containing galacturonic acid plus arabinogalactan than in medium containing arabinogalactan alone, but the degree of sensitivity usually associated with accumulation of the phosphorylated sugar was not observed. Assuming that utilization of uronic acids in B. thetaotaomicron occurs by a pathway similar to that used by Escherichia coli, the most likely site of the mutation is in the gene that codes for the isomerase involved in the catabolism of both glucuronic acid and galacturonic acid (3). PG-2 was not only unable to utilize polygalacturonic acid, but it was also deficient in utilization of chondroitin sulfate. PG-2 reached an optical density on chondroitin sulfate of about half of that attained by the wild type, as expected from the fact that one of the two component sugars of chondroitin sulfate is glucuronic acid.

The four types of starch-negative mutant tested have been described previously (1). MS-3 and MS-4 were unable to utilize amylose, amylopectin, or pullulan but could grow on maltotriose. MS-1 and MS-2 were unable to utilize oligomers of glucose higher than maltotriose. MS-2 and MS-3 may be uptake mutants, because they differ from the wild type only in the ability to bind labeled starch (1). MS-4, like MS-2 and MS-3, is unable to bind starch but also appears to have a lower amylase activity than the wild type. MS-1 appears to have lost a central regulatory gene because it fails to express any of the genes associated with starch utilization (1). MS-1 retained the ability to grow on maltose but had a 4-h lag phase. The other mutants grew normally on maltose.

Competition experiments. Since the experiments with germfree mice are so expensive and time-consuming, we started with the competition experiments rather than first testing the ability of each mutant to colonize the mice when introduced alone. Previous experience had shown that if the mutant is still detectable at all on days 2 to 5, the mutant alone colonizes the mice at levels indistinguishable from those of the wild type (8, 9). Only if the mutant is not seen even in sample 1 on day 2 is it necessary to test the colonization levels of the individual mutant.

Results of the colonization experiments with the two
fucose-negative mutants are shown in Fig. 1. Neither of these mutants was competed against successfully by the wild type. Similar result were obtained with the starch-negative mutants (Fig. 2 and 3). None of the starch-negative mutants was competed against successfully by the wild type, although in some cases the percentage of wild-type bacteria increased during the first several days of the competition before levelling off. For MS-1, we used the original Tn4351 insertion as well as the Em' derivative in competition experiments to determine whether Em' itself would have any effect on the outcome. The results indicated that the presence of the gene for Em' did not affect the competition (Fig. 2).

Results of competitions involving the two polygalacturonic acid-negative mutants PG-2 and PG-4 are shown in Fig. 4. PG-2 was successfully competed against initially by the wild type, but the mutant began to reappear after about 14 days. This same result was seen in replicate experiments. In the competition involving PG-4, the percentage of wild-type bacteria rose above the initial level but never reached 100%. Thus, the PG-4 mutation was not as deleterious as the PG-2 mutation, although the PG-4 mutation appeared to be somewhat deleterious during the initial competition period.

The most deleterious mutation was the mutation in AG-4. This galactose-negative mutant was successfully competed against by the wild type within 2 to 5 days (Fig. 5). Unlike

PG-2, the mutant was not detected in any samples after the wild type reached 100%. All of the mutants were checked by Southern blot analysis of chromosomal DNA to ascertain that there was only one insertion in the chromosome. In AG-4, Southern analysis revealed that the Em' derivative contained two insertion sequences, whereas a similar analysis of the original transposon insertion showed that there was only one insertion of Tn4351 (data not shown). Accordingly, we did competition experiments using the original Tn4351 insertion as well as the Em' derivative to confirm that the outcome of the competition between the wild-type and Em' strains was not influenced by the extra insertion sequence. The results were the same regardless of which version of the mutant was used (Fig. 5).

DISCUSSION

In two recent studies of polysaccharidase activities in human feces, we attempted to determine whether enzymes that are normally induced during growth of Bacteroides spp. on starch or polygalacturonic acid are produced at fully induced levels in human colons. We found no evidence for induction in vivo of Bacteroides enzymes associated with utilization of either of these polysaccharides (4, 5). These findings indicated that neither starch nor polygalacturonic acid is utilized by bacteria in the human colon at levels high enough to induce the polysaccharidases. One explanation of these findings is that colonic Bacteroides spp. rely mainly on some type of polysaccharide other than starch and polygalacturonic acid for carbon and energy. An alternative expla-
nation is that Bacteroides spp. do not rely primarily on any single polysaccharide when growing in a colon but rather use small amounts of a variety of polysaccharides. When Bacteroides spp. grow in laboratory medium with a single polysaccharide as the sole source of carbon and energy, enzyme specific activities usually rise more than 20-fold above the uninduced levels (4, 5). However, if bacteria in the colon use only small amounts of any particular polysaccharide and utilize more than one polysaccharide at a time, the rise in enzyme specific activities could be relatively small. In our earlier studies of enzyme activities in human colon contents, we would probably not have detected increases in enzyme activity unless they were at least 5- to 10-fold above the uninduced levels. Thus, the results of these studies could not rule out either the hypothesis that Bacteroides spp. utilize small amounts of a variety of polysaccharides in colons or the hypothesis that Bacteroides spp. in colons rely mainly on polysaccharides other than starch and polygalacturonic acid.

Accordingly, in the current study, we used a different approach to determine what polysaccharide sources are important for Bacteroides spp. growing in an intestinal tract. We used germfree mice as a simple model system to assess the consequences of losing the ability to utilize each of the polysaccharides known to be fermented by B. thetaiotaomicron. If B. thetaiotaomicron relies primarily on one or two types of polysaccharide in vivo, mutations that affect utilization of these particular polysaccharides should be deleterious, whereas mutations that affect utilization of other polysaccharides should have no effect. By contrast, if B. thetaiotaomicron uses small amounts of a variety of polysaccharides in germfree intestinal tracts, mutations that interfere with utilization of any single type of polysaccharide might not have a drastic effect on competitiveness because the organism has many alternate carbohydrate sources. Rather, it should be necessary to eliminate utilization of more than one type of polysaccharide before loss of competitiveness was detectable. The results of the experiments reported here support the hypothesis that B. thetaiotaomicron uses small amounts of a variety of polysaccharides in vivo rather than relying primarily on one or two main carbohydrate sources, because the mutations that were most deleterious were those that affected utilization of more than one polysaccharide, i.e., the mutations in PG-2 and AG-4.

Since the mutations in PG-2 and AG-4 affected utilization of a monosaccharide, we could identify in each case the gene that is probably affected by assuming that Bacteroides sp. uses the same type of catabolic strategy as E. coli. Both of the mutations appeared to disrupt genes for enzymes that carry out early steps in the catabolic pathway. Neither mutant exhibited phenotypes associated with accumulation of a phosphorylated sugar, and both mutants grew normally on other carbohydrates. Thus, it is likely that the effect of the mutations in PG-2 and AG-4 was limited to diminished utilization of uronic acid- and galactose-containing polysaccharides, respectively.
The results of competitions involving four different types of starch-negative mutants showed that none of these mutants was competed against successfully by the wild type. Thus, loss of the ability to utilize starch had no detectable effect on competitiveness. This does not rule out the possibility that these mutations would be more deleterious if other organisms besides \textit{B. thetaiotaomicron} had been present, but we can conclude that these mutations were less deleterious than the mutations in AG-4 and PG-2. The relative unimportance of starch as a substrate could reflect the fact that when animals are fed a conventional chow diet, only low levels of starch enter the colon. A diet high in retrograded starch, a form of starch that is not readily degraded in small intestines, might cause starch-negative mutants to be less competitive.

Our finding that both fucose-negative mutants competed equally with the wild type indicates that glycoprotein mucins are not a major source of carbohydrate for bacteria growing in the intestines of mice fed a chow diet. We thought that mucin would be a possible substrate because it is a major component of material entering the colon and appears to be degraded extensively during passage through a colon (14). Some colonic bacteria can ferment hog gastric mucin and bovine submaxillary mucin (6). Hog gastric mucin and bovine submaxillary mucin were not fermented extensively enough by most of the human colonic \textit{Bacteroides} strains to be scored as a growth substrate in a previous survey of polysaccharide utilization by these organisms (10). However, since most colonic \textit{Bacteroides} strains can use all of the monosaccharides and cleave the linkages in goblet cell mucin, we felt that the failure to show extensive degradation of these other mucins did not rule out the possibility that \textit{Bacteroides} spp. obtain some carbohydrate from goblet cell mucin in vivo.

Although human goblet cell mucin is not commercially available and thus cannot be used to test the effect of the fucose-negative mutation directly, we expected that this mutation should affect mucin utilization because fucose accounts for about 20\% of the carbohydrate in goblet cell mucin (15). Goblet cell mucin of rodents is similar in composition to that of humans, and studies of mucin secretion in animals have shown that although the amount of mucin secreted may vary with diet, the composition does not (11, 15). Thus, germfree mice fed a conventional chow diet should produce mucin that resembles the mucin that \textit{B. thetaiotaomicron} would encounter in a human colon. Since the fucose-negative mutant was not competed against successfully by the wild type, it appears that goblet cell mucin is not a major substrate for \textit{B. thetaiotaomicron}. It should be kept in mind, however, that galactose accounts for about 20\% and hexosamines account for about 45\% of the carbohydrate in goblet cell mucin (15). It is interesting that the most deleterious mutations we found, those of galactose-negative mutant AG-4 and a hexosamine-negative mutant tested in an earlier study (CG20; 9), would both be expected to have diminished ability to utilize goblet cell mucins. Thus, we cannot conclusively rule out goblet cell mucin as a carbohydrate source for \textit{B. thetaiotaomicron} in animals.

ACKNOWLEDGMENTS

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